Comparative Study on Adenylate Deaminase In The Extracts of Liver of Frog and Rat

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In the memory of Com. Jasbir Singh.

Certificate

The research work embodied in this dissertation has been carried out in the School of Life Sciences, JNU, New Delhi. The work is original and has not been submitted in Part or full for any degree or diploma of any university.

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NEW DELHI KARUNAKAR

C O N T E N T S

INTRODUCTION

Adenylate or AMP deaminase (AMP amino hydrolase, EC 3 5 4.6) catalyses the hydrolytic deamination of AMP to IMP and Ammonia. The enzyme is widely distributed in animal tissues like brain, liver and muscle. It was found that in skeletal muscle AMP deaminase activity is much higher than in other tissues including heart and smooth muscle (Conway and Cooke, 1939). The distribution of the enzyme in skeletal muscle varies greately; white muscle has higher enzyme concentration than red muscle, (Ronca-Testoni, Raggi, and Ronca, 1970). However, the absence of AMP deaminase activity in insect actomyosin preparation, and in other invertebrate actomyosin preparations tested by Maruyama and Tonomura (1957) will be insufficient to prove the absence of this enzyme in all the tissues of invertebrates.

AMP deaminase plays an important role in the regulation of adenylate catabolism in mammalian liver The rate limiting step in the degradation of adenine nucleotides in the mammalian

liver is the conversion of AMP into IMP and ammonia, catalysed by AMP deaminase Vanden Berghe et al, 1977, 1980)

By regulating adenylate catabolism, AMP deaminase seems to help the myokinase reaction in utilising the high energy phosphate of ADP for muscular contraction and in maintaining a high ATP/ADP ratio since myosin ATPase is inhibited by ADP. Infact, muscle myokinase specific for adenine nucleotide, is inhibited by AMP (Ronca-Testoni, Raggi & Ronca. 1970). It also seems to play an important role in stabilising the Adenylate energy charge, when the liver is subjected to metabolic stress thus leading to fall of energy charge (Chapman and Atkinson, 1973).

AMP deaminase was purified from liver of rat, rabbit and rat muscles and from calf brain. The molecular weight of 280 000 daltens which was calculated for liver enzyme by Vanden et al (1976), is in close agreement with the value of 270 000 daltens reported for the muscle

enzyme (Wlfenden etal, 1968). The latter enzyme has been shown to be composed of four subunits (Scopes and Penny, 1971 Boosman etal, 1971), a finding in accordance with the demonstration of a minimum of four binding sites for ATP (Tomozawa and Wolfenden, 1970). The finding of two binding sites for GTP by Tomozawa and Wolfenden (1970) indicates further that the molecular structure of AMP deaminase probably does not fit in a simple model

The kinetic properties of AMP deaminase from brain, liver, erythrocytes and skeletal muscle have been studied extensively. Medicino and Muntz (1958) first demonstrated that the brain enzyme is activated by ATP. The enzyme is also activated by monovalent cations and inhibited by GTP. Setlow and Loaensten, (1967) purified AMP deaminase from Calf brain, and studied some of its regulatory properties. It was found that; the enzyme is activated by ATP and by alkali ions. The most effective alkali ions is that of lithium. Ammonium and tetrmethylammonium ions

have no effect on activity. The activators do not alter the optimum pH of the enzyme, which is about pH 6.2 ATP protects the enzyme against heat inactivation. A plot of the reaction rate with respect to the AMP concentration in absence of activators yields a curve with a pronounced sigmoidal shape. Addition of ATP yields curves which approximate rectangular hyperbolas. The effect of activators is thus to increase the apparent affinity of enzyme for AMP without affecting the maximum reaction velocity. Conversely, increasing the concentration of AMP increases the apparent affinity of the enzyme for ATP. Additon of lithium chloride also increases the apparent affinity of the enzyme for ATP. Bivalent ions at concentrations of 1mM and 5 mM have no pronounced effect on the reaction in the absence of ATP. Most of the ions tested appear to slightly stimulate the reaction in the presence of ATP. The chlorides of Magnesium & Manganese have the same effect as the corresponding sulfates. Nickel and Copper inhibit the reaction, but to some extent. The enzyme from erythrocytes is activated by ATP monovalent cations and inhibited by 2.3

diphosphoglyceric acid (Simley etal, 1967). Activation by ATP has also been, observed in carp muscle enzyme. However in the presence of 0.6 M Kcl, crystalline AMP deaminase from rabbit muscle is inhibited by Pi but not activated by ATP and ADP (Lee, 1968). Smiley and Suelter (1967). Showed that crystalline enzyme from rabbit skeletal muscle is activated by K^+ and Na^+ and by ATP and ADP in the absence of monovalent cations. GTP and GDP inhibit the enzyme. The enzyme from rat skeletal muscle in the presence of 100 mM K^+ is inhibited by ATP, GTP, UTP, CTP, by some organic anions and Pi, whereas ADP removes these inhibitions

The effect of K^+, nucleoside triphosphates, ADP and Pi on skeletal muscle AMP deaminase from frog, pigeon guinea pig, rabbit and rat was investigated by Ronca - Testoni, Raggi and Ronca, (1969). It was found that; K^+ activates the enzyme from all the sources, however, the complete activation of rat and pigeon enzymes is observed at Kcl concentrations lower than those required for

a complete activation of the enzyme from the other sources. At pH 6.5 and at Kcl concentrations higher than 100 mM, ATP, GTP, Creatincposphate, ITP, and Pi inhibit the enzyme from all the species examined. Whereas at 50-100 mM Kcl, ATP activate the enzyme from guinea pig and rabbit. At pH 7.1 and 50-200 mM Kcl, ATP inhibits all the enzymes. AMP deaminase is inhibited to a limited extent by nucleoside triphosphates, although the degree of inhibition depends on the pH and the source. ADP at low Kcl concentrations strongly activates the enzyme, while at high Kcl concentrations the activation is weaker or absent Besides this effect ADP removes the inhibition by nucleoside triphosphate and Pi

Smith and Kizer (1969) have purified AMP deaminase and have studied some of its properties from rat liver. The purified enzyme had a optimum pH between 6.0 and 6.2. Sigmoidicity persisted with the enzyme in the presence of AMP. Addition of ATP alone or with Licl to the enzyme yielded curves that were essentially hyperbolic in shape and lowered the apparent affinity

constant by more than 25%. GTP also appeared to be a modifier of AMP deaminase, but the kind of modificatin is dependent upon substrate concentration and the presence of other modifiers. At low substrate concentration. GTP activated the enzyme, but at higher concentration activation not seen. ATP activation was strongly inhibited by GTP at concentrations approximately 1/10 of those of ATP. When 100-250 ug of purified enzyme was subjected to Disc gel electrophoresis for 30-35 minutes at 5 mA, eight stained bands were readily visible; six of these bands were in the upper (Cathode) half of the gel, while two were in the lower (Anode) half. Attempts to determine which protein bands had enzyme activity were unsuccessful, since the enzyme lost catalytic activity during electrophoresis. The antigen-antibody reaction resulted in destruction of enzyme activity below detectable levels, suggesting that the antiserum contained antibodies to the enzyme. After disc electrophoresis of the enzyme, gel columns were embedded in agar and subjected to double diffusion against antisera.

Under these conditions, two precipitin bands were observed in the lower (anode) region of the gel. Since enzyme activity was lost upon incubation with antibody, it was possible that one or both of precipitin bands contained enzyme-antibody, but, loss of catalytic activity during electrophoresis precluded experiments to investigate this possibility

The enzyme from rat liver differed from the enzyme of calf brain in atleast two instances. a) Na^+ and K^+ were less efficient activators than Li^+ for brain AMP deaminase in the absence of other modifiers, whereas the enzyme from rat liver was readily activated by all three alkali ions, and b) GTP inhibited calf brain AMP deaminase throughout a substrate range from 0.50 mM, while on the other hand, GTP stimulated activity of the rat liver enzyme at lower substrate concentrations and inhibition was not observed until the substrate concentration exceeded 8-10 mM.

The Kinetic properties of this enzyme have been reinvestigated (Vanden Berghe etal,

1977) from rat liver by means of a radio chemical method which permits measurement of enzymatic activity at low (0 02mM) concentration of AMP. In presence of 3mM ATP, the affinity for AMP was greatly increased and kinetics were changed from sigmoidal to hyperbolic. Another effect of a physiological concentration of ATP was to abolish the well known inhibitory effect of Pi. At substrate concentrations up to 1 mM, including the physiological range, there was no effect of Pi in the presence of 3mM ATP, but 0.5 mM GTP was inhibitory. On addition of all three modulators, the enzyme was profoundly inhibited, its activity being only slightly higher than in the absence of effectors.

The hepatic concentration of several nucleotides and metabilites was measured during the first few minuts after an intravenous load of fructose to mice. There was temporary burst of adenine nucleotide breakdown and increase of rate of uric acid excretion. The first changes observed at 30s, were a decrease in the concen-

tration of Pi and simultanious accumulation of fructose-1-phosphate. The decrease in the concentration of ATP and GTP proceded more slowly. An increase in the concentration of IMP was detected only after one minute of load of fructose to mice.

Vanden Berghe, etal, (1980) have studied the influence of Coformycin, a nucleoside antibiotic on AMP deaminase activity. The concentration of Coformycin required for maximal inhibition of AMP deaminase in a high speed supernatant of rat liver was more than two orders of magnitude higher than the concentration needed for a similar inhibtion of adenosine deaminase in the same preparation. The inhibitory effect of coformycin on AMP deaminase was not affected by the presence of the physiological inhibitors i.e. Pi and GTP. It was also confirmed that the addition of 50 uM conformycin did not influence the activities of other enzymes of the purine catabolic pathway. It was repeadtedly verified that the addition

of 0.1 uM coformycin to suspensions of isolated rat hepatocytes did not influence the production of unlabelled as well as radioactive allantoin. In contrast, the addition of the inhibitor at a concentration of 50 uM inhibited completely the formation of, radioactive allantion; whereas the rate of production of unlabelled product was decreased by 85%. The influence of fructose on control hepatocytes, is compared with effect of the ketose on cells that had been preincubated with 50 uM coformycin. The rapid degradation of ATP as well as accumulation of fructose-1-phosphate were not modified by the addition of coformycin. The inhibitor provoked, however, a marked elevation in the concentration of AMP, which increased 14 fold above the value at zero min, and to a lesser degree of ADP. As a consequence depletion of the adenine nucleotides pool preceded much more slowly. Prelabelling of the adenine nucleotides pool of the isolated hepatocytes with C^{14} adenine and determination of the radio activity in these catobolites of the adenine nucleotides in the presence of 50 uM coformycin

demonstrated its near absence in IMP, and a marked decrease in allontoin production. Coformycin is a nucleoside antibiotic {3BD ribofuranosy 6,7,8 tri hydroimidazol [4,5d] [2,3] diazepin 8(R) 01} (Sawa et al 1967). It is an inosin analogue is a potent inhibitor of adenosine deaminase from various sources (Snyder & Henderson, 1973; Agarwal etal, 1975). It also inhibits AMP deaminase from muscle (Agarwal and Parks, 1977). From their studies, it appears that hepatic adenosine deaminase can be maximally inhibited by 0.1 uM coformycin, where as AMP deaminase requires 50 uM of the nucleoside antibiotic. Since none of the other hepatic enzymes of purine catabolism was influenced by the maximal concentration of coformycin used in the experiments of Vanden Berghee etal (1980) with isolated hepatocytes, it can be reasonably assumed that only the deaminations of adenosine and of AMP were affected in this system. From the studies of same group, it can be observed that the basal production of allantoin was not influenced by a concentration

of coformycin that maximally inhibited adenosine deaminase in a liver extract constitutes a strong indication that the catabolism of the adenine nucleotides does not occur through adenosine deaminase The quantitative argument between the inhibitory effects of different concentrations on the hepatic AMP deaminase activity and on the production of allantoin, in view of the complete penetration of the inhibitor, suggest that the AMP deaminase constitutes the rate limiting step in the catabolism of the adenine nucleotides in the liver. The incomplete inhibition by 50 uM coformycin of the basal production of allantoin is most probably explained by the fact that the inhibitor does not affect the catabolism of other purines, mainly Guanine derivatives. These conclusions can be reinforced by the analysis of the influence of coformycin on the fructose induced catabolism of the adenine nuceotides. Though coformycin at the concentrations of 0.1 uM did not modify the effect of fructose observed in the absence of inhibitor, in the presence of 50 uM coformycin changes has become substantial.

In the presence of 50 uM inhibitor, though the depletion of ATP as well as the accumulation of fructose - 1 - phosphate is uneffected, there was elevation in the concentrations of ADP and AMP, the latter with increase of fourteen fold. Moreover, the transient accumulation of IMP, which is observed after administration of a fructose load (Woods etal, 1970; Vanden Berghee etal, 1977) was not recorded any more, indicating the inhibition of AMP deaminase.

Experiments were done on developmental changes of chicken liver AMP deaminase and investigated the appearance of multiple forms of AMP deaminase in the developing chicken liver (Spychala, Kaletha and Kakarewicz, 1985). The kinetic properties of the two forms of AMP deaminase from adult hen liver indicated that in the absence of added effectors, the substrate saturation curve was hyperbolic for enzyme form II and sigmoidal for enzyme form I. Both enzyme forms were activated by ATP and inhibited by Pi. In the presence of both ATP and Pi the two enzyme forms displayed hyperhbolic kinetics. The optimum pH

for form II was 6.75, whereas higher activity of form I was observed at pH 6.5. More than one form of AMP deaminase was found in the liver of fetal rat only, where as in the liver of adult rat and man a single form of this enzyme was detected (Ogasawara etal 1978, 1982; Smith etal, 1969, Spychala etal, 1983).

MATERIAL AND METHODS

Material

Adult male albino rats (aged 2-3 months) weighing 140-160 g, were procured from the animal house and maintained in the air conditioned animal house. All the animals were given standard diet and water ad libitum

Adult frogs (Rana hexadactyla) were used throughout our experiments. They were maintained in glass tanks at 25°C. They were regularly fed live Cockroaches. Frogs were starved for two days before the commencement of experiments. For all experiments, frogs weighing 100-125 gms were utilized.

Preparation of the enzyme extract

The animals were anesthesized with anesthetic ether and the liver was chilled immediately after removal. The tissue was homogenized in medium containing 0 05 M Tris buffer (pH 6.5), 0.18 M Kcl, and 2 mM 2-Mercaptoethanol in 1:2 ratio (W/v). The homogenate was centrifuged at 18000 §

for 30 mts. The Supernatant was dialysed in 0.1M Tris buffer (pH.6.5) for 24 hrs with 2 changes every 12 hrs. All the steps were done at 0-4°C. After the dialysis, protein was estimated by Lowry method (Lowry etal., 1951). The protein concentration was adjusted to 30 mg per ml of the extract with Tris buffer (pH 6.5) for all the experiments to keep the enzyme activity constant, inorder to evolve the effects of various modulators. These extracts are used for the studies of AMP deaminase from Frog and Rat livers.

Conditions of Assay

1. 0 1 M solution of 5' AMP (free acid, purchased from Sigma company) is prepared and neutralised to pH 7 with Tris base.

2. 0.1 M solution of 5' ATP (free acid, purchased from Sigma company) is prepared and neutralised to pH 7 with Tris base.

The reaction mixture contained 0.2ml of 0.1M 5'AMP, 0.2 ml of 0.1M 5'ATP, 0 4ml of 0.1M Tris-Hcl buffer (pH 6.3) and the enzyme extract to give a final

volume of 2 ml. The final concentration of AMP was 10 mM, ATP was 10 mM and Tris Hcl buffer was 20 mM in a final volume of 2 ml reaction mixture. The reaction mixture without enzyme extract was Prewarmed at 37°C and the reaction was started by the additioin of enzyme extract. The reaction mixture was incubated at 37°C for 30 minuts. The reaction was stopped by the addition of one ml of 10% trichloracetic acid and deproteinated by high speed centrifugation. Simultaniously reaction blanks were prepared by adding the TCA before the addition of enzyme extract. The enzyme activity is measured by the estimation of IMP spectrophotometrically This spectrophotometric method is based on the increase of absorption at 240 nM due to the formation of IMP in 30 minutes (IMP has absorption maximam at UV-range i.e., at 240 nM.) The amount of IMP formed is calculated from the IMP standard calibration graph. The enzyme activity is expressed as n moles of IMP formed per hour per ml of enzyme extract. Results are expressed as Mean ± SD, and all the experiments were carried out in triplicates.

IMP Calibration Curve

IMP, was purchased from Sigma company IMP standard stock solution was prepared with final concentration of 0.04 mg of IMP per ml. Different concentrations were used (as given in Table No.1), for the calibration curve and the absorbance was read at 240 nM.

Effect of ATP on the Activity of Frog and Rat Liver AMP deaminase at different AMP Concentrations:

The study was carried out by taking different concentrations of ATP at a different concentrations of substrate AMP, as given in the Tables 2-7. Reaction mixture contains 0.2 ml AMP of different concentrations, 0.2 ml of ATP of different concentrations, (as given in tables 2-7), 0.4 ml of Tris-Hcl buffer (pH 6.3) and enzyme of total volume of 2 ml, was incubated at 37°C for 30 mts. and IMP was measured as stated in before.

Effect of Hydrogen Ion concentration on Activity of AMP Deaminase

The effect of hydrogen ion concentration on the activity AMP deaminase was studied in presence and absence of ATP. The Reaction mixture containing 0.2 ml of 0.1M AMP, 0.2ml of 0.1M ATP, 0.4 ml of 0.1M Tris-Hcl buffer and enzyme extract of total volume 2 ml was incubated at 37°C for 30 mts in buffer of different pH values of 4,5,6, 6.2, 6.4, 6.6, 6.8, 7, and 8. and IMP was measured as stated in before.

Effect of ATP on the nature of Frog and Rat liver AMP deaminase activity curve :

The experiment carried out to know the nature of the enzyme activity curve, when it is plotted against different time intervals, in presence and absence of ATP. The incubation mixture consists of 0.1M AMP, 0.1M ATP, 0.1M Tris-Hcl buffer (pH 6.3) and enzyme of total volume 2 ml and reaction was stopped with 1 ml 10% TCA at different time intervals as given Figure No II & III and IMP was measured as stated in before

Effect of Alkali Ions on the activity of Frog and Rat Liver AMP Deaminase :

The alkali ions used for this study are lithium, sodium and potassium. The salts Licl, Nacl & Kcl were dissolved in Tris-Hcl buffer (pH 6.3) to get desired concentrations as given in the Figure No. VI to VII. The studies were carried out separately in the presence and absence of ATP. Reaction mixture contains 0.1M, AMP, 0.1M ATP or water, 0.1M Tris-Hcl buffer (pH 6.3) with dissolved alkali ions of various concentration as given in tables and enzyme of total volume of 2 ml. The mixture was incubated for 30 mts at 37°C. Controls were run simultaniously. To study the reaction in the absence of ATP, controls were run without ATP and alkali ions, whereas to study the reaction in the presence of ATP controls were run only without alkali salts.

Effect of Pi on the Activity of Rat and Frog Liver AMP Deaminase :

Potassium phosphate buffer was used for this study. The activity was measured in terms

of formation of ug of IMP per 30 mts, in the presence and in the absence of ATP. Different concentrations of phosphate buffer was used as given in the table, to deduce its effect on enzyme activity. For reference, enzymatic activity was measured with Tris-Hcl simultaneously in the presence and absence of ATP. Reaction mixture contains 0.1M AMP, 0.1M ATP or water, buffer (pH 6.3) and enzyme of total volume of 2 ml, incubated for 30 mts at 37°C as given in Table No. 14 & 15.

RESULT

IMP Calibration Curve

S.No.	Con. of IMP nmoles	ABS at 240 nM Mean ± SD
1.	51	0.17 ± 0.007
2.	102	0.33 ± 0.0
3.	153	0.49 ± 0.004
4.	204	0.65 ± 0.008
5.	255	0.81 ± 0.006

Table No. 1 IMP standard calibration.

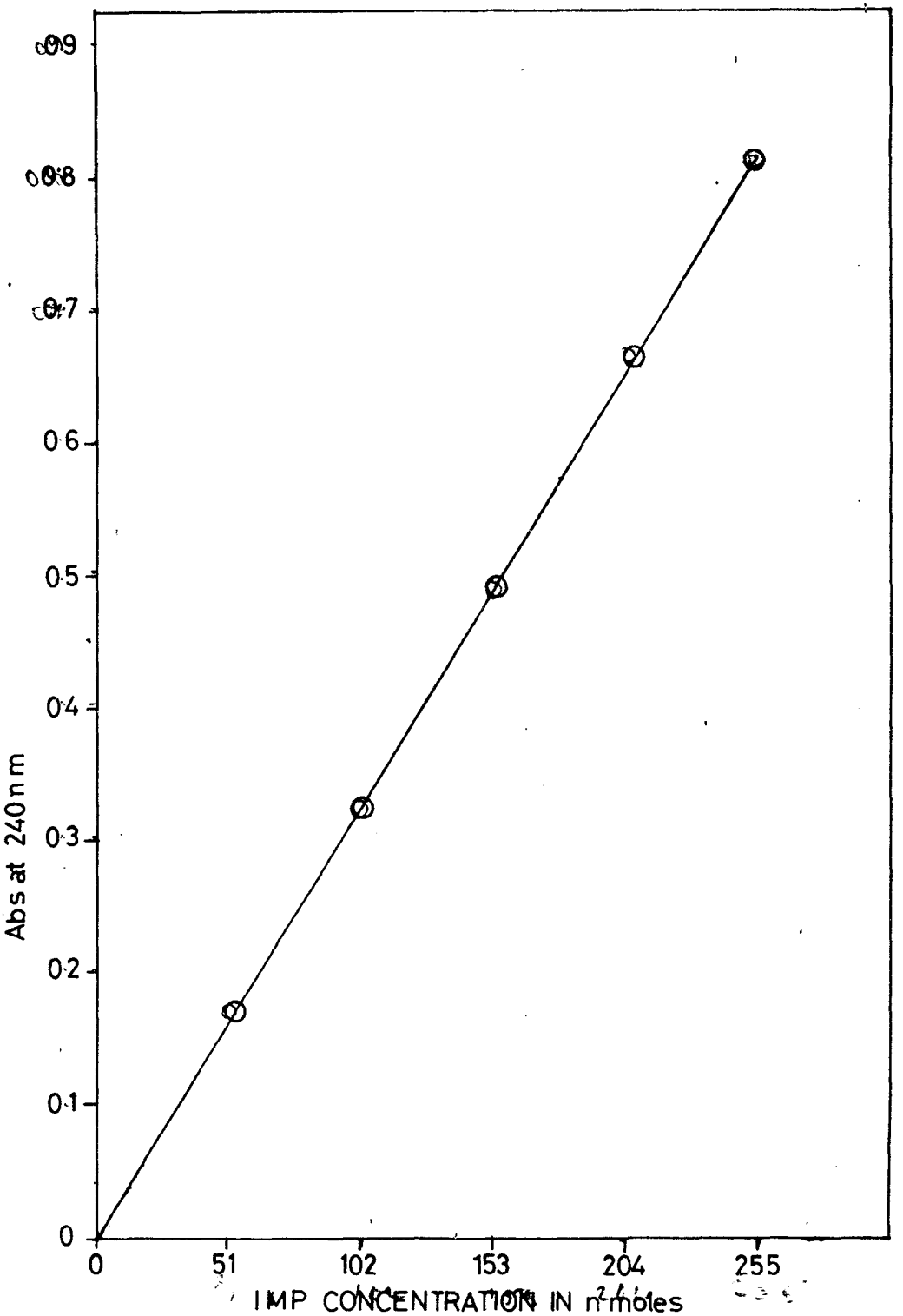


Fig. No 1 IMP Calibration Curve

Effect of ATP on the Activity of AMP Deaminase:

Frog liver enzyme at the substrate concentration 0.1M AMP, shows maximum reaction velocity at 0.08M Conc. ATP. Whereas the rat liver enzyme at the same substrate concentration shows maximum reaction velocity at 0.06M ATP Conc. In both cases it is shown that with the decrease of substrate concentrations, the concentration of ATP required to get maximum activity also decreases.

See Table No 2-7.

S.NO.	Con. of ATP (M)	nmoles of IMP/hr/ ml of Enz. ext. Mean ± SD
1.	0.02	6.41 ± 0.53
2.	0.04	12.44 ± 0.41
3.	0 06	14.70 ± 0
4.	0.08	16.60 ± 0.39
5	0.1	16.96 ± 0
6	0 12	17.34 ± 0.48

Table No.2 Frog Liver AMP deaminase activity at different ATP concentrations when the substrate AMP con. is 0.1M. The reaction mixture contained 0.4 ml of Tris Hcl buffer (pH 6.3) along with 0.2 ml of AMP and ATP of Concentrations metioned above and Enzyme extract of total volume 2 ml.

S.No.	Con of ATP (M)	nmoles IMP/hr/ml of Enz.ext. Mean ± SD
1.	0.02	4.90 ± 0.56
2.	0.04	9.80 ± 1.06
3.	0.06	11.31 ± 0.91
4.	0.08	11.69 ± 0.53
5	0.1	12.06 ± 0
6.	0 12	11.69 ± 0.53

Table No.3 Frog Liver AMP deaminase activity at different ATP concentrations when the substrate AMP con. is 0.05M. The conditions are same as given in Table No.2.

S.No.	Con. of ATP (M)	nmoles IMP/hr/ml enz. ext. Mean ± SD
1.	0.02	4.52 ± 1.04
2.	0.04	5.65 ± 0.59
3.	0.06	6.41 ± 0.65
4.	0.08	7.92 ± 0.61
5.	0.1	6.79 ± 0
6	0.12	8.29 ± 0
7.	0.14	7.92 ± 0.52

Table No.4. Frog Liver AMP deaminase activity at different ATP concentrations when substrate AMP con. is 0.02M. The conditions are same as given in Table No.2.

S.No.	Con. of ATP (M)	nmoles IMP/hr/ml of enz. ext. Mean ± SD
1.	0.02	35.83 ± 0.44
2.	0.04	45.26 ± 0.72
3.	0.06	49.03 ± 0.79
4.	0.08	51.68 ± 0.41
5.	0.1	48.66 ± 0.39
6	0.12	52.81 ± 0.46
7.	0 14	52.80 ± 0.75

Table No.5 Rat Liver AMP deaminase activity at different ATP concentrations when substrate AMP Con. is 0.04 M. The conditions are same as given in Table No. 2

S.No.	Con. of ATP (M)	nmoles IMP/hr/ml of enz. ext. Mean ± SD
1.	0.02	26.83 ± 0.71
2.	0.04	35.08 ± 0.43
3.	0.06	33.57 ± 0.41
4.	0.08	35.08 ± 0.47
5.	0.1	36.58 ± 0.43
6	0.12	34.70 ± 0
7.	0.14	38.10 ± 1.6

Table No.6, Rat Liver AMP deaminase activity at different ATP concentrations when substrate AMP is 0.02M. The conditions are same as given in Table No.2

S.No.	Con. of ATP (M)	nmoles IMP/hr/ml/enz. ext. Mean ± SD
1.	0 02	49.79 ± 0.75
2.	0 04	68.90 ± 0.43
3.	0.06	72.67 ± 0.43
4.	0 08	72.17 ± 0.51
5.	0.1	72.92 ± 0.69
6	0.12	73.43 ± 0.41

Table No. 7 Rat Liver AMP deaminase activity at different ATP concentrations when substrate AMP con. is 0.1M. The conditions are same as given in Table No.2

Effect of Hydrogen ion Concentration on Activity

The activity curve of the enzyme at different pH values shows a broad opltimum plateau between pH 6.2 to 6 6 for frog liver enzyme, whereas it is pH 6.0 to 6 3 for rat liver enzyme. Frog liver enzyme shows highest activity at pH 6.4 in presence of ATP and at pH 6 6 in the absence of ATP. Whereas rat liver enzyme shows highest activity at pH 6.2 irrespective of the presence of ATP. ATP increases the enzyme activity but does not alter the optimum pH in both the cases.

See Table No 8 & 9.

S.No.	pH of Buffer	IMP (nmoles/hr/ml of ENZ extract) Mean ± SD	
		I	II
1.	4	4.52 ± 1.06	16.22 ± 0.53
2.	5	5.65 ± 0.53	23.72 ± 0.49
3.	6	6.78 ± 0	26.40 ± 0
4.	6.2	7.54 ± 1.08	27.54 ± 1.06
5.	6.4	7.54 ± 0.56	27.53 ± 0.54
6.	6.6	8.29 ± 0.51	27.15 ± 0
7.	6.8	6.79 ± 0	24.14 ± 1.08
8.	7	6.41 ± 0.49	20.93 ± 0.13
9.	8	3.02 ± 1.06	12.07 ± 0

Table No 8 Effect of Hydrogen ion concentration on the AMP deaminase activity of Frog (I) and Rat (II) Liver in absence of ATP. The reaction mixture contained 0.2 ml of 0.1M ATP, 0.2ml of 0.1M AMP, 0.4ml of 0.1M Tris Hcl buffer with different pH values mentioned above and enzyme extract of total vlume 2ml.

S.No.	pH of Buffer	IMP (nmoles/hr/ml of ENZ extract) Mean ± SD	
		I	II
1.	4	7.54 ± 0.75	44.13 ± 0.53
2.	5	11.82 ± 0.45	60.73 ± 0.54
3.	6	15.84 ± 0.72	70.16 ± 0
4.	6.2	16.59 ± 0	73.56 ± 0.48
5.	6.4	16.84 ± 0.41	72.04 ± 0.51
6.	6.6	16.84 ± 0.48	66.79 ± 0.61
7.	6.8	16.60 ± 0.51	63.74 ± 0.42
8.	7	15.08 ± 0.78	60.35 ± 0
9.	8	6.28 ± 1.15	39.61 ± 0.51

Table No. 9 Effect of Hydrogen ion concentration on the AMP deaminase activity of frog (I) and Rat (II) Liver in the presence of ATP. The conditions are same as mentioned in Table No.8

Effect of ATP on the nature of Frog and Rat Liver AMP Deaminase Activity.

In presence of ATP, the nature of the enzyme activivity curve is profoundely hyperbolic in both Frog and Rat liver. Whereas in the absence of ATP, the curve is sigmoidal. In both the cases the maximum velocity is not altered. In these experiments about 25 mts required for getting maximal velocity in presence of ATP, whereas in its absence about 60 mts are required to get the same final velocity.

See fig No. II & III.

Figure No. II

Graph depicting the effect of ATP on the nature of Frog liver AMP deaminase activity curve. The reaction mixture contained 0.2ml of 0.1M ATP, 0.2ml of 0.1M AMP, 0.4ml of 0.1M Tris Hcl buffer buffer (pH 6.3) and enzyme of final volume 2ml - incubated at 37°C for 30 minutes.

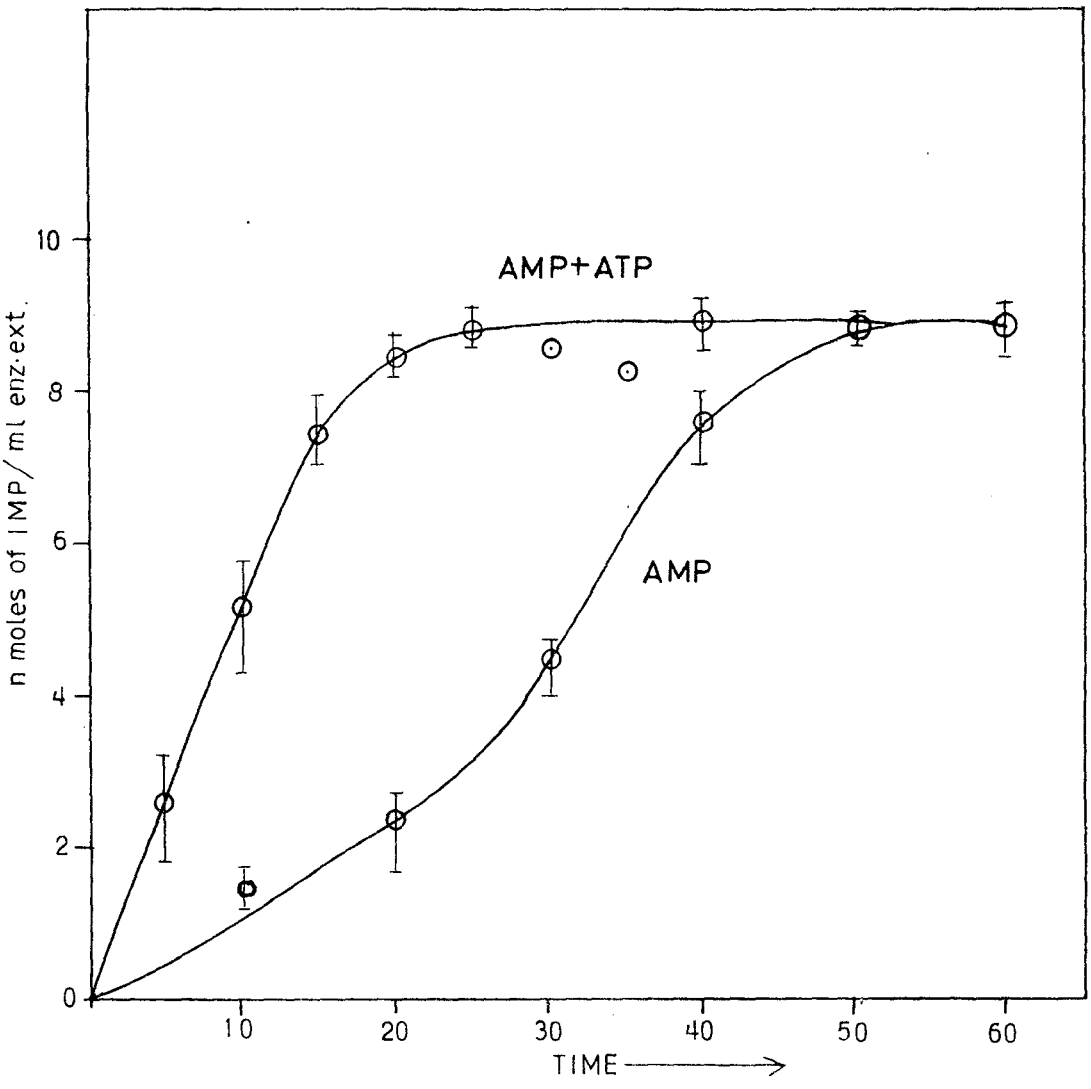
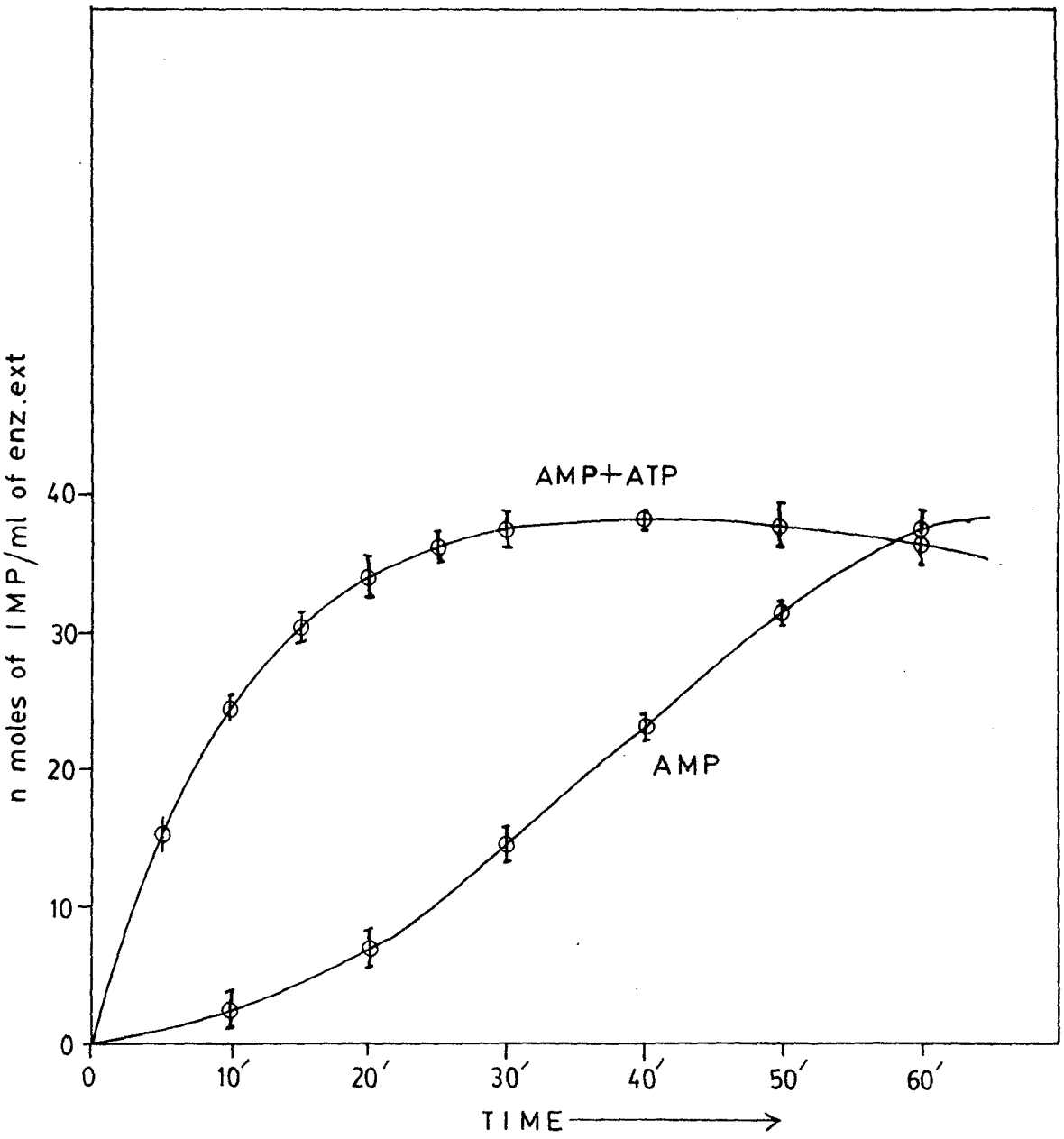

AMP+ATP
AMP
n moles of IMP/ml enz·ext.
TIME
0
2
4
6
8
10
10
20
30
40
50
60

Figure No. III

Graph depicting the effect of ATP on the nature of Rat liver AMP deaminase activity curve. The conditions are same as given in figure No. I.


n moles of IMP/ml of enz.ext
0
10
20
30
40
AMP+ATP
AMP
0
10′
20′
30′
40′
50′
60′
TIME

Effect of Alkali ions on the activity ofFrog and Rat Liver AMP deaminase

Among the three alkali ions, Li^+ shows a profound effect on the activity of Rat and Frog liver AMP deaminase, both in the presence and absence of ATP, whereas the other Na^+ and K^+ show marginal effect. 0.4M Licl in presence of ATP (about 28.6%) stimulation of Enzyme activity. The same concentration shows 50% stimulation in the absence of ATP. Whereas Rat liver enzyme shows 27.6% stimulation at 0.2M Licl in presence of ATP and 107% stimulation at 0.4M Licl in the absence of ATP. AT high concentrations of alkali ions (K^+, Na^+ and K^+) as given in table No. 10-13, the reaction is inhibited both in the presence and absence of ATP. In both cases inhibition by Li^+ in the presence of ATP is greater than in its absence.

See fig No IV to VII.

Figure No. IV

Graph depicting the effect of alkali ions (Na^+, K^+ & Li^+) on the activity of Frog liver AMP deaminase in the presence of ATP. The reaction mixture contained 0.2 ml of 0.1M AMP, 0.2ml of 0.1M ATP, 0.4ml of 0.1M Tris. Hcl buffer (pH 6.3) with required concentration of alkali salt as given in graph and enzyme extract of total volume 2ml - incubated at 37°C for 30 minutes.

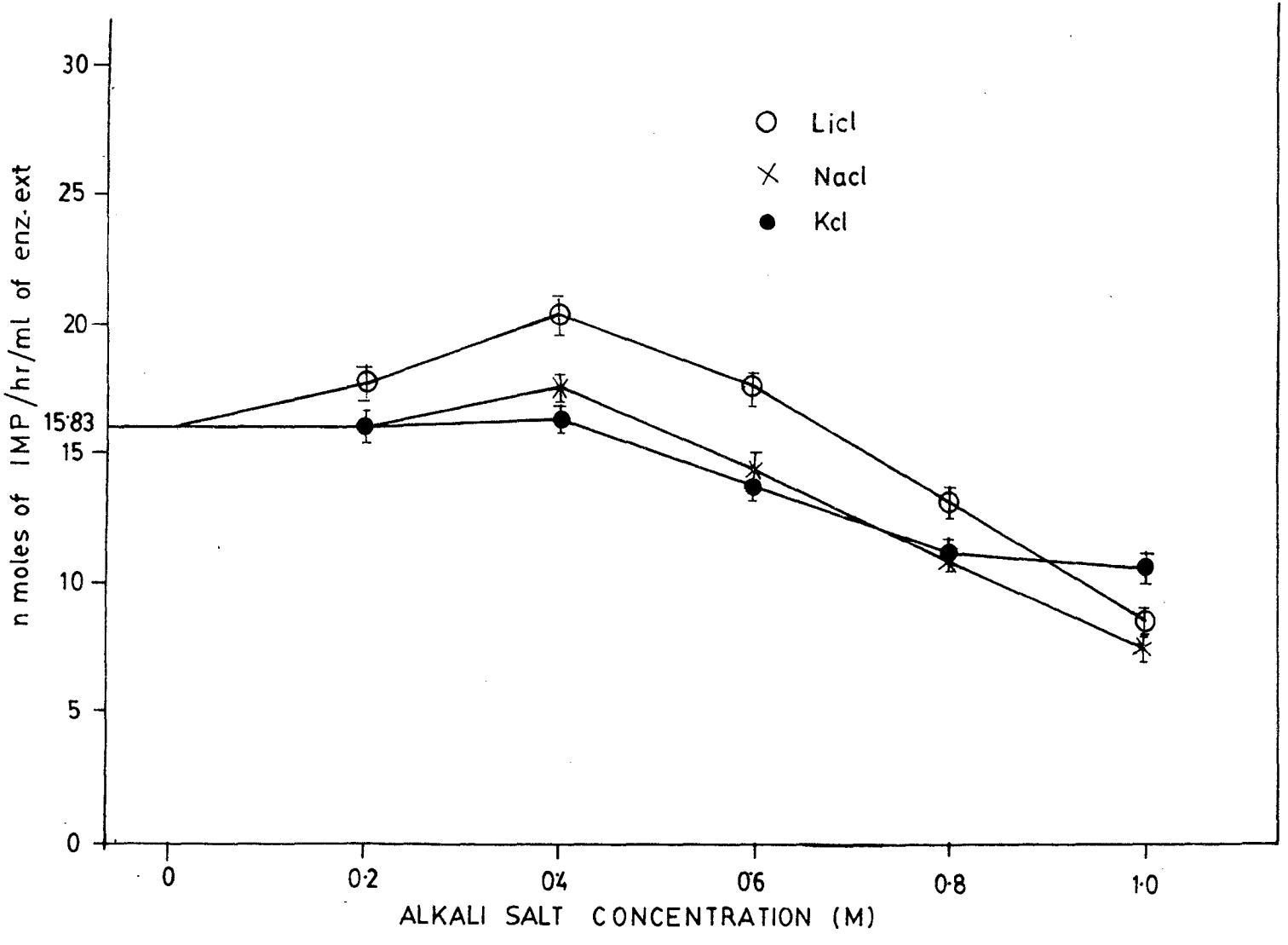

30
25
20
15·83
15
10
5
0
n moles of IMP /hr/ml of enz. ext
Licl
Nacl
Kcl
0
0·2
0·4
0·6
0·8
1·0
ALKALI SALT CONCENTRATION (M)

Figure No. V

Graph depicting the effect of alkali ions (Na^+ , K^+, & Li^+) on the activity of Frog liver AMP deaminase in the absence of ATP. The conditions are same as given in Figure No. III.

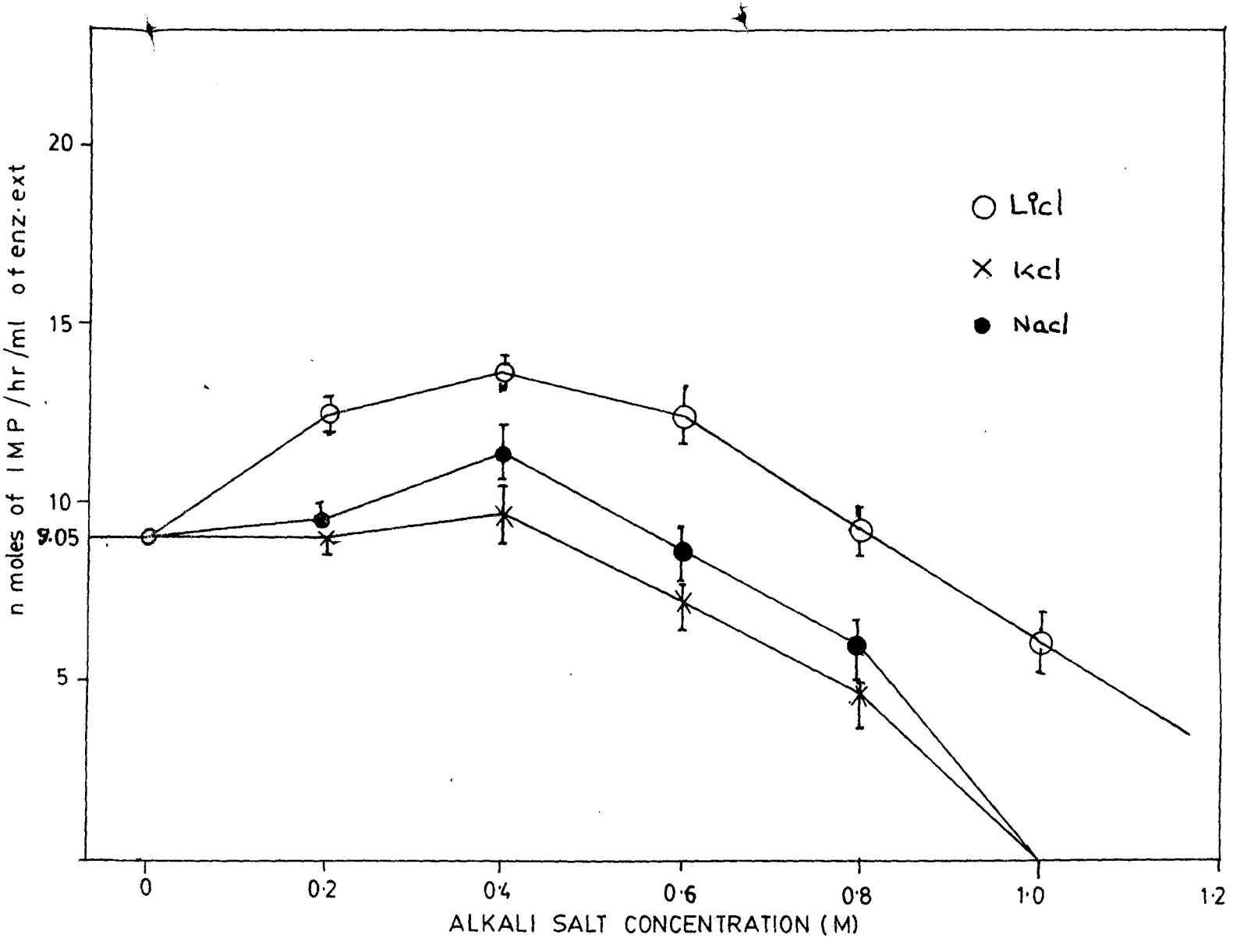

n moles of IMP /hr /ml of enz·ext
20
15
10
5
0·05
0
0·2
0·4
0·6
0·8
1·0
1·2
ALKALI SALT CONCENTRATION (M)
O LiCl
× KCl
● NaCl

Figure No. VI

Graph depicting the effect of alkali ions (Na^+, K^+ & Li^+) on the activity of Rat liver AMP deaminase in the presence of ATP. The conditions are same as given in figure No. III.

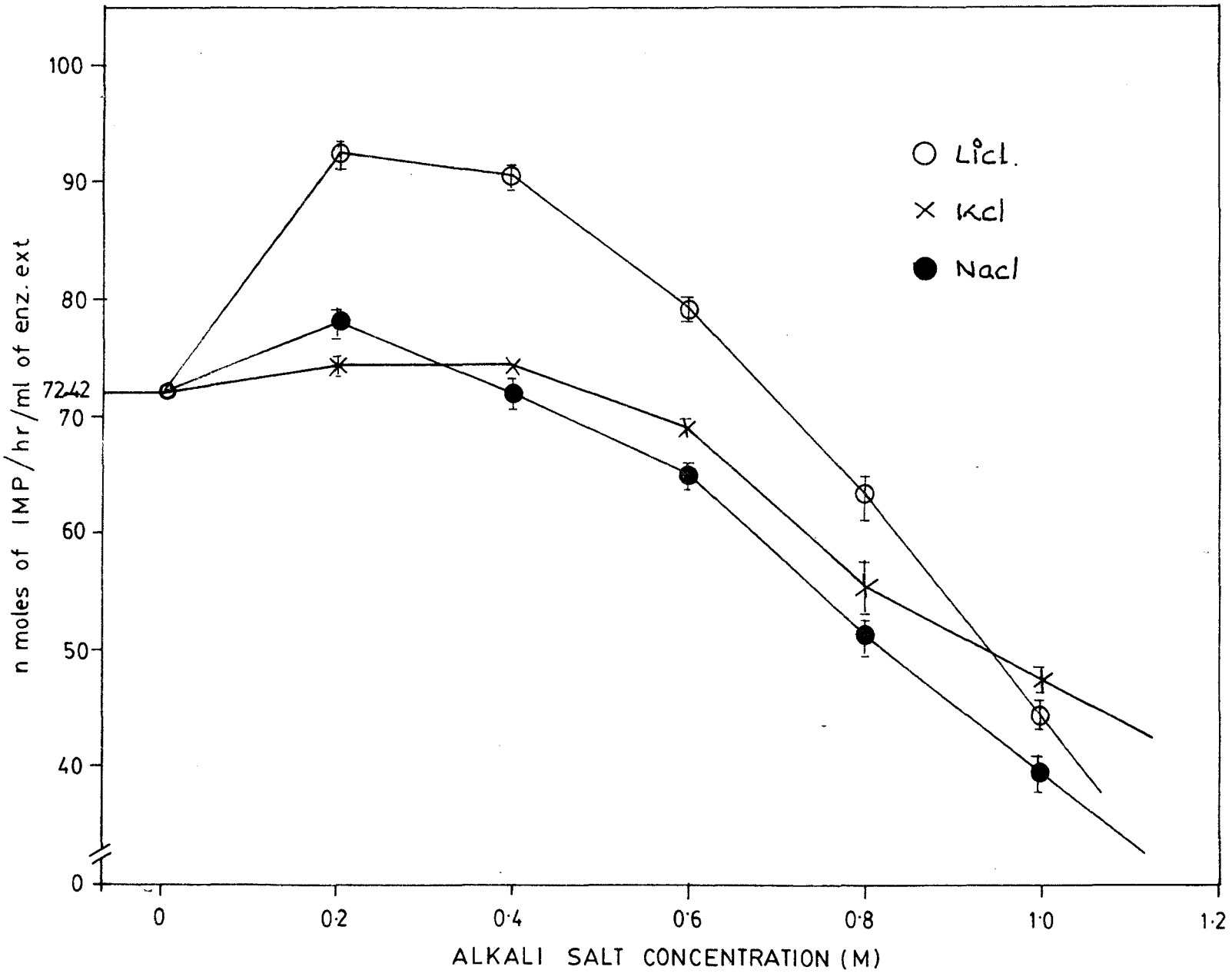
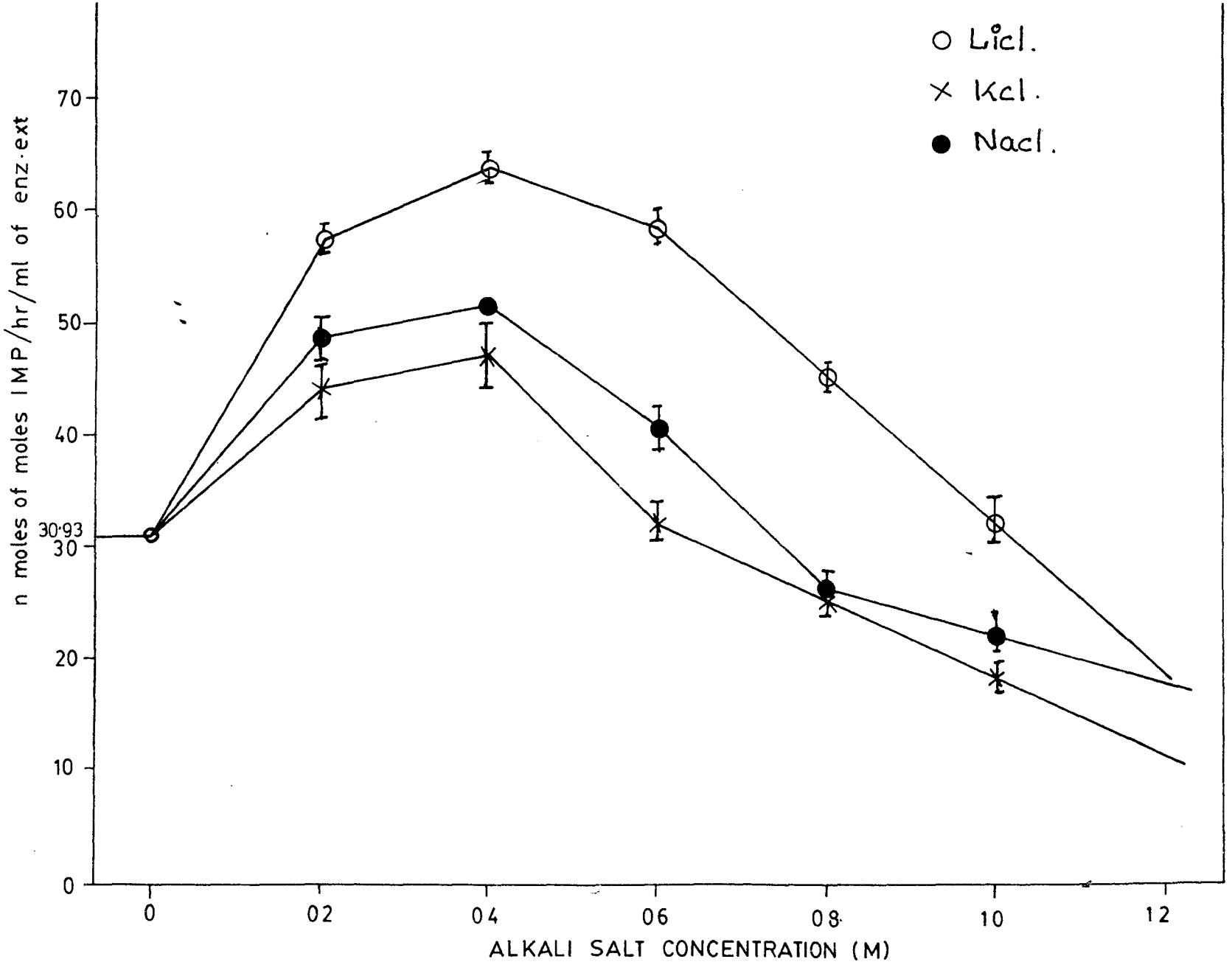

100
90
80
72.42
70
60
50
40
0
n moles of IMP/hr/ml of enz. ext
0
0·2
0·4
0·6
0·8
1·0
1·2
ALKALI SALT CONCENTRATION (M)
O LiCl.
× KCl
● NaCl

Figure No. VII

Graph depicting the effect of alkali ions (Na^+, K^+ & Li^+) on the activity of Rat liver AMP deaminase in the absence of ATP. The conditions are same as given in Figure No.III.


○ Licl.
× Kcl.
● Nacl.
n moles of moles IMP/hr/ml of enz·ext
70
60
50
40
30·93
30
20
10
0
0
02
04
06
08
10
12
ALKALI SALT CONCENTRATION (M)

Alkali Salts	Concentration in (M)	% Stimulation	% Inhibition
Licl	0.2	11.94	-
	0.4	28.61	-
	0.6	11.93	--
	0.8	-	16.67
	1.0	-	45.23
Nacl	0.2	2.4	-
	0.4	9.54	-
	0.6	7.07	-
	0.8	-	28.55
	1.0	-	52.36
Kcl	0.2	2.4	-
	0.4	7.14	-
	0.6	-	9.47
	0.8	-	26.15
	1.0	-	33.29

Table No. 10 percentage of stimulation and inhibition by Alkali ions on activity of Frog liver AMP deaminase with respect to Control in presence of ATP. The reaction mixture contained 0.2 ml of 0.1M AMP, 0.2ml of 0.1M ATP. 0.4 ml of Tris Hcl buffer (pH 6.3) with different concentrations of Alkali salt as mentioned above and enzyme extract of total volume 2 ml.

Alkali Salt	Concentration in molar (M)	% Stimulation	% Inhibition
Licl	0.2	37.45	-
	0.4	51.93	-
	0.6	38.89	-
	0.8	-	4.19
	1.0	-	25.08
Nacl	0.2	8.28	-
	0.4	24.9	-
	0.6	-	3.75
	0.8	-	33.37
	1.0	-	100
Kcl	0.2	4.19	-
	0.4	8.39	-
	0.6	-	16.68
	0.8	-	45.85
	1.0	-	100

Table No. 11 shows percentage of stimulation and inhibition by Alkali ions on the activity of Frog liver AMP deaminase with respect to control value in the absence of ATP. Conditions are same as given Table No. 10.

Alkali salt	Concentration in (M)	% Stimulation	% Inhibition
Licl	0.2	27.62	-
	0.4	25.53	-
	0.6	9.91	-
	0.8	-	12.49
	1.0	-	39.06
Nacl	0.2	4.69	-
	0.4	0.52	-
	0.6	-	9.88
	0.8	-	29.68
	1.0	-	45.83
Kcl	0.2	3.12	-
	0.4	2.6	-
	0.6	-	3.64
	0.8	-	22.91
	1.0	-	33.84

Table No. 12 shows percentage of stimulation and inhibition by alkali ions on the activity of Rat liver AMP deaminase with respect to control value in the presence of ATP conditions are same as given in Table No.10.

Alkali salt	Concentration (M)	% Stimulation	% Inhibition
Licl	0.2	86.58	-
	0.4	107.34	-
	0.6	89.03	-
	0.8	43.90	-
	1.0	2.42	-
Nacl	0.2	58.55	-
	0.4	67.08	-
	0 6	31.72	-
	0.8	-	15.85
	1.0	-	29.26
Kcl	0.2	43.91	-
	0.4	53.67	-
	0.6	6.11	-
	0.8	-	19.50
	1.0	-	39.02

Table No. 13 shows percentage of stimulation and inhibition by Alkali ions on the activity of Rat liver AMP deaminase with respect to control value in the absence of ATP. The conditions are same as given in Table No. 10

Effect of Pi on the Activity of Rat and Frog liver AMP deaminase:

0.05M phosphate buffer completely inhibits the enzyme activity in the absence of ATP, where as 0.1M phosphate buffer has not shown any substantial effect on enzyme activity in the presence of ATP. in both Rat and Frog liver extracts. Presence of 0.01M phosphate buffer shows an Inhibition of 72.7% and 74.6% inhibition on Rat liver enzyme in the absence of ATP.

See Table No. 14-15.

S.No.		Buffer (pH 6.3)	0 1M ATP	nmoles IMP/hr/ml of enz. ext. Mean ± SD
1.		0 1M Tris Hcl	+	75.07 ± 0.82
2.		0 1M Tris Hcl	-	29.79 ± 0.32
3.		0.1M $Po4^-$ Buffer	+	74.69 ± 0.71
4.	a.	0 01M $Po4^-$ Buffer	-	7.54 ± 0.21
	b.	0 05M $Po4^-$ Buffer	-	0

Table No 14 effect of P_i on the activity of Rat Liver AMP deaminase. '+' indicates ATP presence, '-' indicates ATP absence. The other conditions are 0.2 ml of 0.1M AMP, 0.4 ml of buffer with concentrations mentioned above and enzyme extract of total 2 ml

S.No.	Buffer (pH.6.3)	0 1M ATP	nmoles IMP/hr/ml of enz. ext. Mean ± SD
1.	0 1M Tris Hcl	+	16.84 ± 0.54
2.	0.1M Tris Hcl	-	8.29 ± 0.42
3.	0.1M $Po4^-$ Buffer	+	16.59 ± 0.89
4. a)	0.01M $Po4^-$ Buffer	-	2.26 ± 0.66
b)	0.05M $Po4^-$ Buffer	-	0

Table No 15 Effect of[illegible] on the activity of frog liver AMP deaminase '+' indicates presence of ATP; '-' indicates ATP absence. The other conditions are same as given in Table No. 14.

DISCUSSION

This comparative study shows that Frog and rat liver AMP deaminases have some fundamental common characterstics such as optimum pH range, sigmoidal nature of enzyme activity in presence of ATP, stimulation by ATP, and maximum stimulation by alkali ion lithium, which will suggest a common regulatory mechanism.

However some minor differences are also observed, such as concentrations of ATP required for stimulation and concentrations of alkali ions for stimulation and inhibition.

Apart from other minor differences, the enzyme activity appears to be a major difference between frog and rat liver AMP deaminases and this high activity of rat liver AMP deaminase in the given time can be attributed to the high metabolic rate of the animal.

Effect of pH

The deamination proceds rapidly over a broad pH range by both frog and rat liver AMP deaminases. The activity curve of the enzyme of different pH levels, shows a broad optimum plateau between pH 6.2 to 6.6 for frog liver enzyme, where as pH 6.0 to 6.4 for rat liver enzyme. This is in support of the work of Josef Spychala etal 1985, who investigated the effect of pH on the activity of multiple forms of chick liver AMP deaminases, and highest activity was observed at pH 6.5 for form I and at pH 6.75 for form II. In both case of rat liver and frog liver enzyme. ATP increased the enzyme activity pronouncely in the given time, but does not altered the optimum pH range substantially. That is the addition of ATP to the reaction has a negligable or no effect on the position of pH optimum and which indicates that charged groups at the active sites of AMP deaminase are not affected when the enzyme binds ATP. The optimum pH range is a little more towards the acid value.

Effect of ATP

In the presence of ATP, the nature of the activity curve is profoundly hyperbolic with both frog and rat liver AMP deaminases where as in the absence of ATP, the curve is sigmoidal in the both cases. It is clear from the data that the effect of ATP is thus to increase the apparent affinity of the enzyme for AMP without affecting the maximum reaction velocity. The data shows that about 25 mts are required for getting maximal reaction velocity in presence of ATP, where as in its absence about 60 mts are required to get the same final velocity.. Since the enzyme possessed activity even in the complete absence of ATP, the affector ATP does not appear to be an indispensable cofactor, and this is in agreement with work done on AMP deaminase from other different tissues by different scientists. From the data, it is clear that the highest activation is obtained when ATP and AMP are present in more or less equal concentrations Frog liver AMP deaminase at the substrate AMP concentration 0.1M has shown maximum

activation at the 0.08M con. of ATP. Where as the rat liver enzyme at same substrate concentration shown maximum activation at 0.06M concentration of ATP. In both cases it is clear that with decrease of substrate concentrations, the concentrations of ATP required to get maximum activation also decreases. At a low concentration of AMP maximum activation should therefore be observed at lower ATP concentrations than at a high concentration of AMP.

The exact manner by which ATP stimulates the deamination reaction is not yet clear. The experiments of Hurwitz etal , 1957 c^{14} labeled AMP in the microbial extract and experiments of Medicino, 1957 with p^{32} labelled AMP, using the extract of brain acetone powder, indicate that ATP is not labelled by AMP. Further the products adenine and ionosic acid, reapectively were shown to arise directly from labelled AMP. The activating phenomena would appear to involve some reaction or interaction of ATP with the protein. Medicino etal., 1957,

say that inspite of fact that no inorganic phosphate and no ADP could be detected when the enzyme was incubated with AMP and ATP, it is still possible that a direct phosphorylation of the protein to phospho protein which could be termed in such a system could not be detected by the assay system used.

Effect of Pi :

Pi is an potential inhibitor of AMP deaminase of Frog and Rat liver. 0.05 M phosphate buffer completely inhibits the enzyme activity in the absence of ATP, where as 0.1M phsophate buffer has not shown any substantial effect on activity of AMP deaminase in presence of ATP, in both cases, means that ATP completely removes inhibition by Pi.our results shows presence of 0.1M phosphate buffer shown as effect of 72.7% inhibition of frog liver enzyme activity and 74.6% inhibition of rat liver enzyme activity in the absence of ATP.

Regulation of AMP deaminase:

Thus in general ATP, Pi and other nucleotides play an important role in regulation of the AMP deaminase to cooperate and co-ordinate with the other biological functions in the broader biological systems, like in helping muscular contraction (Ronca-Testono etal., 1969) and in maintaining the Adenine nucleotide pool in the liver (Chapman etal., 1973).

Since ADP is the AMP deaminase activator, in the conditions in which free ADP accumulates in muscle, it removes the inhibition by Pi and the enzyme is activated. On this aspect muscle AMP deaminase seems to help the myokinase reaction in utilising the high energy phophate of ADP for muscle contraction and in maintaining a high ATP/ADP ratio since myosin ATPase is inhibited by ADP. Infact muscle myokinase specific for adenine nucleotides is inhibited by AMP which has a Ki value more than Km for ADP.

Loading of rat liver with fructose either by intravenous injection into living animal or by adding fructose to the medium perfusing the isolated liver causes a rapid loss of hepatic adenine nucleotides, is caused by a decrease in the concentration of both inhibitors Pi and GTP. This temporary burst of adenine nucleotide break down accompanied by a large accumulation of fructose-1-phosphate in the liver, and soon counteracted by the decrease in the conctration of ATP (Woods etal, 1970; G.Vanden Berghe etal, 1977). The hepatic changes on fructose loading can be satisfactorly explained on the basis of the properties of enzymes of fructose metabolism and adenine nucleotide degradation. The primary step as suggested by Maenpaa et al (1968), is the rapid reaction of ATP with fr ctose catalysed by Keto hexokinase. This causes a decrease in the concentration of ATP and Pi, both of which are essential in stablizing AMP and therefore the total adenine nucleotide content of tissue. The continuous generation of high energy phosphate groups, mainly in the form ATP, requires dosely regulated adenine nucleotide

pool in the cell. This pool consists of ATP, ADP and AMP, maintained in equalibrium by adenylate Kinase. It is continuously subjected to catabolism, which can potentially convert AMP into ionisine and ultimately into uric acid or allantoin in the liver. Calculations from the amount of uric acid excreted by man (less than 600 mg/day) and data obtained by cathelerization of the hepatic vein (Grunst etal, 1975) show that as little as 3 - 5 nmol of uric acid is formed per min per g of liver under basal conditions; in contrast, an intravenous load of fructose can increase this rate by one or two orders of magnitude The determination of metabolite concentrations in the liver during the early periods after a load of fructose indicated that, as expected, the accumulation of fructose-1-phosphate and a simultaneous decrease in Pi and ATP were the first changes to occur. From the work of Maenpaa etal (1968) it appears that the decrease in the concentration of the adenine uncleotides was terminated after about 2 mts, when the concentration of ATP reached about 1 mM, where as the

increase in uric acid and allantoin continued for a much longer time in the blood. This has been interpreted either by assuming that at that time a new steady state between nucleotide synthesis and degradation has been reached, or that degradation has been stopped and that uric acid is formed from IMP which was accumulated in liver. Woods etal (1970) have discussed the point that accumulation of fructose-1-phosphate in the liver after a load of fructose cannot be attributed to lower activity of liver aldolase as compared with that of fructokinase Further, they have concluded that the accumulation of IMP, causing an inhibition of aldolase, was the explanation for the build up of the fructose derivative. This is quite contradictory with the finding of Vanden Berghe etal (1977) that the accumulation of IMP occurs only during the second minute after intravenous administration of fructose, when the concentration of fructose-1-phosphate has already reached its maximum. From the work presented by Vanden Berghe (1977), it appears that, in the liver, adenine nucleotides are degraded by the pathway, which starts with hydrolytic

deamination of AMP, by adenylate deaminase, a step regulated by the concentration of ATP, GTP and Pi. The activity of this catabolic pathway can be enormously increased after load of fructose, apparently because of a change in the concentration of these effectors

Significance of sigmoid curve :

From the studies of Chapman and Atkinson, (1973), it can be known that the adenylate Energy charge will be stabilized by the adenylate deaminase reaction. In liver, as in most other tissues and organisms studied, the adenylate energy charge (the mole fraction of ATP plus half the mole fraction of ADP) has been shown to be maintained at a value of approximately 0.90 (Chapman, A.G., etal 1971). When the liver is subjected to metabolic stress, such as the trapping of large amounts of phosphate that results from the presence of abnormally high levels of a phosphoryl aceptor, a relatively small drop in the energy charge value is accompanied by a decrease in the total

concentration of adenine nucleotides (Maenppa , et al 1968; Woods et al 1970). This is followed by the decrease in adenylate pool level by the increased action of AMP deaminase. If this enzyme were to become active when the charge falls, the resulting removal of AMP would tend to buffer or protect the value of the energy charge since removal of AMP must increase the mole fractions of ATP and ADP. This was supported by the fact that the AMP deaminase activity is maximal at a low value of energy charge (0.6 to 0.7) and proceds much more slowly at physiological energy charge (0.9) because the concentration of substrate AMP is limiting under the latter conditions. The properties of liver AMP deaminase that so far investigated may have evolved as part of the system that protects against wide excursions of energy charge under given conditions. This protection would be, at the expense of a decrease in the total adenylate pool and at the same time it was found experimentally that the cell be protected against complete depletion of the adenine nucleotide pool (Raivio et al 1969; Woods et al 1970). From this it seems likely that the response of adenylate deaminase to the concen-

tration of ATP is an evolutionary adaptation that safe guard against excessive depletion of the pool of adenine nucleotides. A sigmoid response to the concentration of substrate AMP, would seem to be sufficient to protect against sharp falls in the value of energy charge, but a response might lead to near total depletion of the pool under conditions of energy stress, since the sigmoid responding nature requires high concentration of substrate AMP to get maximal activity. Since limited amount of adenine nucleotides present in the cell, stabilization of the charge at the expense of pool size cannot be a long term effect; it can protect only against sharp drops incharge during the trasient period while a new steady state, in which use and regeneration of ATP are against equal, is established.

Effect of Alkali ions :

The additon of alkali ions causes a pronounced increase in the rate of reaction in the absence of ATP. Alkali ions Na^+, K^+ have much smaller effect when ATP is present, both on frog and rat liver AMP deaminase activities.

At high concentrations of alkali ions (K^+, Na^+ and Li^+) the reaction is inhibited both in the presence and absence of ATP. The frog liver AMP deaminase has shown maximum stimuation of activity 28.61% at 0.4M Licl in the presence of ATP with respect to control, where as in the absence of ATP, the stimulation is 51.93%. Frog liver AMP deaminase activity is inhibited, which is of 16.67% at 0.8M Licl in presence of ATP; where as the inhibition is of 4.19% in the absence of ATP. In contrast, rat liver AMP deaminase shown maximum stimulation of 27.6% at 0.2M Licl in presence of ATP and 107.34% at 0.4M Licl in the absence of ATP. The enzyme shows inhibition of 12.49% at 0.8M Licl in presence of ATP, where as in its absence at the same concentration of Licl, there is no inhibition at all with respect to control. This clearly shows that, inhibition by Licl in presence of ATP is greater than in its absence. These results are in complete agreement with result of Setlow et al., (1966), on calf brain AMP deaminase. The stimulation of both frog and rat liver enzyme activities is

very less by other alkali salts Nacl and Kcl in presence of ATP. Enzyme from Frog liver shows maximum stimulation by Nacl and Kcl at 0.4M concentrations; where as rat liver enzyme show maximum stimulation at 0.2M concentrations of salts. Enzyme from both livers is inhibited by Nacl and Kcl at concentration of 0.6M, in presence of ATP, and the inhibition by Nacl is more than inhibition by Kcl in both the cases. In contrast, in the absence of ATP, stimulation of enzyme from both livers by Nacl and Kcl is maximum at the salt concentration 0.4M; however inhibition of frog liver enzyme activity started at 0.6M, where as inhibition of rat liver enzyme activity started at 0.8M. The interesting feature is that the frog liver enzyme activity in the absence of ATP is completely inhibited and no activity is found at 1.0M concentrations of Nacl and Kcl.

The interesting difference between frog and rat liver AMP deaminases is that, the inhibition of frog liver enzyme activity by Nacl and Kcl is more in the absence of ATP, where

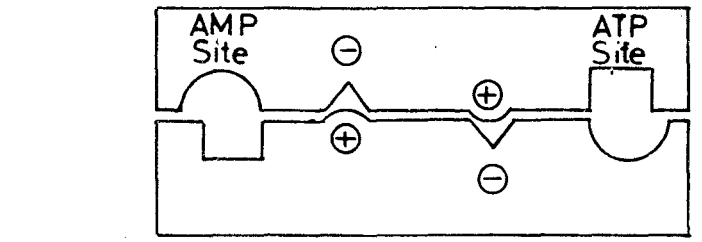
as in case of rat liver enzyme activity the inhibition by Nacl and Kcl is more in presence of ATP! Though no experiment is done here to prove that the inhibition by high concentrations of alkali ions is reversible, it can be concluded as reversible taking the work of B.Setlow etal (1966) into account. In their work, the incubation of the Calf brain AMP deaminase in 0.5M Kcl or Licl at o°C or 25°C for periods upto 24hrs, followed by 100 fold dilution, showed that the inhibition by high concentration of alkali metal salts is fully reversible with respect to both the stimulated and the unstimulated reactions. Among the three alkali metal salts, Lithum salt has greater effect on AMP deaminase activity from both rat and frog liver extracts. One of the possibilities for this pronounced effect of lithum ions can be postulated to be result of a close fitting of this ion compared to others into a negatively charged crevice in the enzyme. These differences in the activity of AMP deaminase of rat and frog liver, may have its own biological basis in order to correlate these difference with the function of biological system as whole, need an further

extensive study at different levels. The nature of inhibition of enzyme activity by high concentrations of alkali ions is still under investigation.

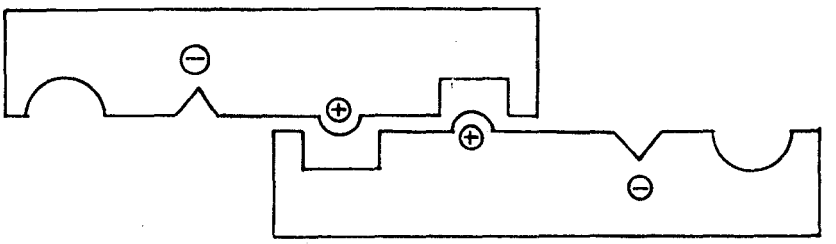
The same lithium ion which has shown profound stimulatory effect on AMP deaminases of Rat and frog liver, plays an important role in inositol metabolism. Lithium ion exerts a profound alteration in inositol metabolism by inhibiting the conversion of myoinositol 1- phosphate into myo-inositol by the enzyme myo-inositol-1-phosphatase, thus it lowers the concentration of myo-inositol and induces a large accumulation of myo-inositol-1-phosphate. This may gradually deplete the membrane content of phosphotidylinositol and this inturn could decrease the sensitivity of these receptors mechanisms in which inosital phospholipids are important components of the transducing mechanism (Berridge et al, 1982).

Various models have been proposed to account for the kinetic behaviour of regulated enzymes. They involve either a change in the

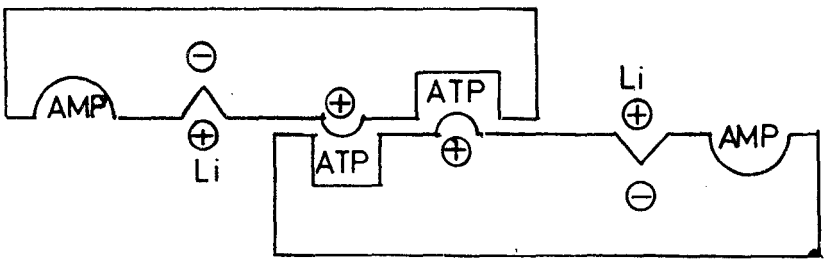
degree of aggregation of sub units, a change in the arrangement of subunits, a change in the comformation of subunits of the enzyme or various combinations of these phenomena in response to a change in the concentrations of substrate or modifier. Setlow and Lowenstein (1966) proposed a model for brain AMP deaminase, seems appropriate for present data. It appears to be at least three binding sites on the enzyme. The first site binds the substrate and other two sites bind ATP and alkali metal ion. According Setlow & Lowenstein model, the enzyme exists as an equilibrium of two forms: an inactive form, A, which can be pictured as having an active site inaccessible to AMP, and an active form B, in which the active site is freely accessible to the substrate, as given in the diagram. The active from B, can be stabilized in any of three ways; by substrate, by activator, or by alkali metal ion. According to this model, the enzyme is predominantly in the inactive form A, when the substrate concentration is low (less than 5 mM AMP) and when ATP and alkali ions are absent.



A



B



C

DIAGRAM

see TEXT for explanation.

When the concentration of AMP is increased, the equilibrium is displaced from B toward C, and hence from A toward B Saturation of the active site with AMP prevents the return to Form A. This is single model shows how a cooperative interaction can occur. A molecular of ATP is shown to combine at a specific site in each subunit in such a way that the enzymatically active arrangement of subunits is stabilized. This could occur, for example, by the alignment with the ATP binding site of a cationic site on the adjacent subunit. The effect of alkali ions is shown as consisting of the breaking up of an ionic interaction between the subunits which stabilizes form A, but other mechanisms are possible The same cationic site that is shown as stabilizing the inactive form of the enzyme in the absence of substrate or activator is also shown as stabilizing the active form in the presence of ATP, but separate sites for these different purpose are clearlypossible. For the sake of simplicity, this is a model in which the change between active and inactive forms occurs by a rearrangement of two symmetrical subunits.

C O N C L U S I O N

The AMP deaminase from frog and rat liver is similar in many respects to theAMP deaminase from calf brain (Sctlow et al, 1967), chick liver (Spychala et al, 1985) and skeleton muscle of frog, pigeon, guinea pig, rabbit and rat (Ronca-Testoni et al, 1969). Our results show a broad optimum plataeu between pH 6.2 to 6.6 for frog liver enzyme, where as pH 6.0 to 6.4 for rat liver enzyme and also shown that ATP does not attened the optimum PH range. In presence of ATP, the nature of the activity curve is hyperbolic with both from and rat liver AMP deaminases and in absence of ATP the curve is sigmoidal. The result shows that ATP increases the apparent affinity of the enzyme for AMP without affecting the maximum reaction velocity. The activity of Rat & Frog liver AMP deaminases is inhibited pi in the absence of ATP & in it's presence, the inhibition by Pi is completely removed. The addition of alkali ions caused a pronounced increase in the rate of reaction in the absence of ATP. Alkali ions Na^+, K^+ when ATP is present as with frog

and rat liver AMP deaminase activities. High concentrations of these alkali ions inhibited the reaction in presence and absence of ATP.

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