SOME BIOLOGICAL AND KINETIC PROPERTIES OF 5'-ADENYLIC ACID DEAMINASE

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ABSTRACT

Some Biological and Kinetic Properties
of 5'-Adenylic Acid Deaminase

by Arnold J. Berry

5'-Adenylic acid deaminase is an enzyme of wide biological distribution which catalyzes the conversion of 5'-AMP to IMP. It is isolated as a complex with myosin from rabbit skeletal muscle and several studies indicate that it may be involved in muscular contraction.

This work shows that rabbit skeletal muscle is the best source of enzyme of the animals studied and the enzyme level increases with age in young rabbits. In chicken and pigeon the level in breast muscle is about six fold greater than in leg muscle. The level in heart muscle was very low in all eight species tested.

The effect of divalent cations and some organic acids on crude enzyme preparations is reported. An absolute requirement for a monovalent cation is shown and the activation constants for the alkali cations are estimated.

5'-Adenylic acid deaminase is shown to be allosteric for AMP and this property is not altered by variation of the KCl concentration or heating the enzyme to 50°C.

An apparent K_m of 1.8×10^{-5}M was calculated from the Hill equation.

Activation by ATP is demonstrated for this enzyme. This was not shown by all preparations and more work must be done in this area before any real conclusions can be drawn; however, a scheme rationalizing this activation is presented.

Some Biological and Kinetic Properties of 5'-Adenylic Acid Deaminase

by

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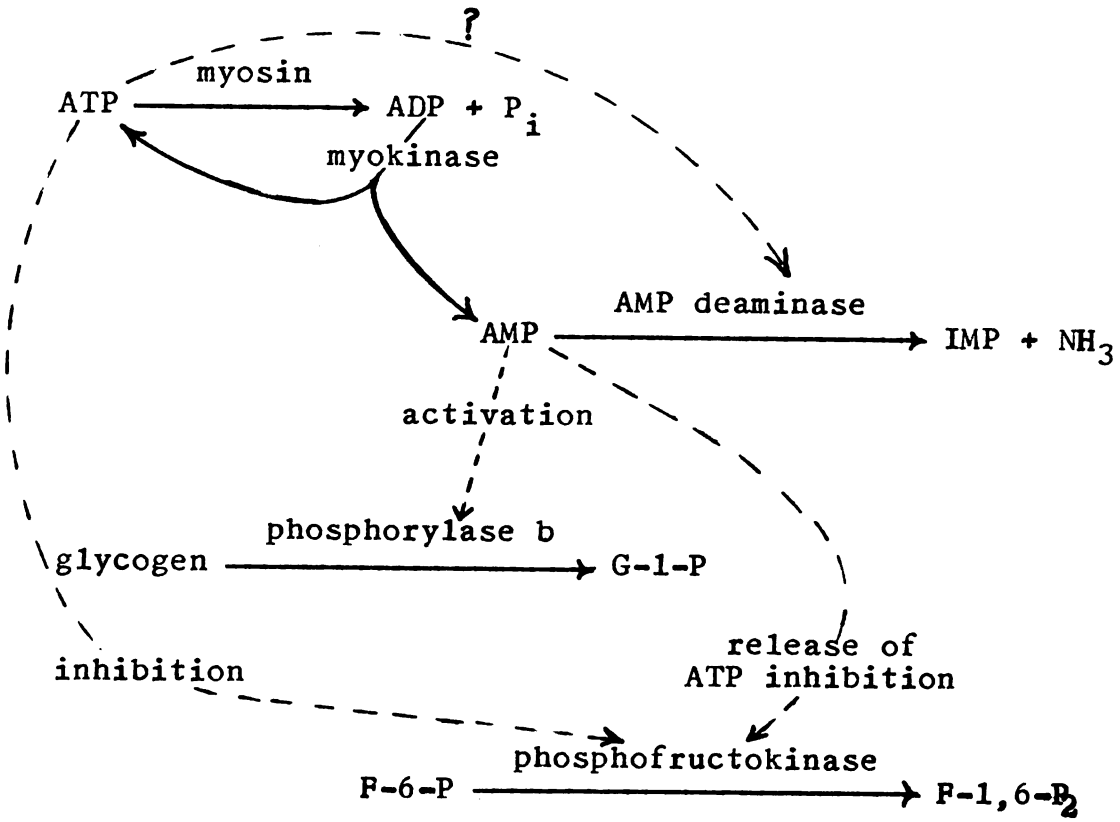
INTRODUCTION

Although 5'-adenylic acid deaminase (5'-AMP deaminase) is an enzyme of wide biological distribution, its physiological function has not been elucidated. When extracted from muscle the enzyme is obtained as a complex with myosin (Herman and Josepovits, 1949) and it was speculated that 5'-AMP deaminase is involved in muscular contraction (Parnas, et al., 1927). Wajzer, et al. (1956) reported that changes in ultraviolet absorption indicated that deamination of 5'-AMP accompanied a single muscular contraction. Nechiporenko and Ferdman (1953) showed that the amount of 5'-AMP deaminase which is complexed to myosin is decreased in myosin extracted from atrophic muscle. Pennington (1961) showed that mice with muscular dystrophy have only one-third the 5'-AMP deaminase level of normal mice. All of these reports seem to indicate a close relationship between 5'-AMP deaminase and muscular contraction.

5'-AMP has been shown to regulate phosphofructokinase (Stone and Mansour, 1966) and phosphorylase b (Cori, et al., 1943). The importance of 5'-AMP deaminase becomes apparent when it is considered that it could be a general control over the metabolic reactions involving 5'-AMP. A scheme showing the metabolic involvement of 5'-AMP is illustrated in Figure 1 on page 2. 5'-AMP activates phosphorylase b and releases ATP inhibition

FIGURE 1

Some metabolic reactions involving 5'-AMP



of phosphofructokinase, both of which stimulate glycolysis. Glycolysis increases the ATP level which inhibits phosphofructokinase and, through muscular contraction, forms more ADP. If the myokinase reaction is important in the conversion of ADP to ATP and AMP, activation of AMP deaminase by ATP would close a regulatory circuit. When muscular contraction causes the ATP level to drop (myosin ATPase) and this deactivates AMP deaminase, the resulting AMP level increase (myokinase) will stimulate glycolysis increasing the ATP level again. During muscular inactivity the concomitantly high ATP level could activate AMP deaminase and the resulting lower AMP level would no longer stimulate glycolysis so that ATP production is decreased.

This work deals with some aspects of 5'-AMP deaminase which may someday help to establish its real function.

HISTORICAL

The first description of the reaction catalyzed by 5'-AMP deaminase was given by Schmidt in 1928. He showed that 5'-AMP deaminase from rabbit muscle could be separated from adenosine deaminase by adsorbing the latter on alumina gel. He also studied the substrate specificity of the enzyme and showed that adenine, adenosine, 3'-AMP, guanine, guanosine, and GMP were not attacked. Indeed, this was part of the evidence used by Embden and Schmidt (1929) to show the non-identity of 5'-AMP (muscle AMP) and 3'-AMP (yeast AMP). Schmidt showed the reaction products to be ammonia and IMP by isolating them, giving the chemical reaction:

$$AMP + H_2O \longrightarrow IMP + NH_3$$

No published attempts to purify 5'-AMP deaminase were given until Kalckar (1947) showed two methods of purification and a very convenient method of assay. At 265 millimicrons the decrease in extinction with deamination of AMP to IMP is 60 per cent. Thus, a spectrophotometric assay is possible making the lengthy ammonia determinations unnecessary.

In 1947, Herman and Josepovits showed that 5'-AMP deaminase was bound to myosin as strongly as ATPase and concluded that both activities were due to the same protein since they could not be separated. Englehardt, et al. (1952) were successful in separating the ATPase

and deaminase by thermal treatment. When the myosin (ATPase) - deaminase complex is heated the myosin is preferentially denatured.

In 1957, Lee reported the crystallization of 5'-AMP deaminase from rabbit muscle with a specific activity of 17.2 and characterized this preparation very extensively. He found that the protein moved in a single peak in electrophoresis (with a variety of ionic strengths, protein concentrations and pH's), in sedimentation and in calcium phosphate gel chromatography. He also determined the isoelectric point to be at pH 5.6, a sedimentation constant of 12.29×10^{-13} sec, a diffusion coefficient of 3.76×10^{-7} $cm^2 sec^{-1}$, and a calculated molecular weight of 320,000. An Arrhenius plot gave an apparent energy of activation of 10,500 calories. The enzyme exibited a sharp pH optimum at 6.4 in 0.10 M succinate buffer and pH 6.1 in 0.10 M citrate buffer. The Michaelis-Menton constant (K_m) was determined to be 1.4×10^{-3}M (0.1M succinate buffer, pH 6.4, 30°C, 2.7×10^{-5} to 2.0×10^{-4}M AMP range). Lee reported that orthophosphate, pyrophosphate and fluoride strongly inhibited the enzyme. No evidence of a metal requirement could be demonstrated by treatment of the enzyme with cyanide, versene or cysteine. The following metal cation effects were reported for the crystalline enzyme (see page 6). The protein was found to be free of the following enzyme activities which exist in the crude actomyosin complex:

adenosine diphosphate deaminase, myokinase, nucleotide triphosphatase, and adenosine triphosphate-creatine transphosphorylase. Furthermore, no nucleotide pyrophosphatase, nucleotidases, adenine, cytosine, or guanosine deaminase activities were detectable.

Ions	Effects
Fe^{+3}, Zn^{+2}, Cu^{+2}, Ag^{+}	Strongly inhibitory
Cd^{+2}, Ni^{+2}	Slightly inhibitory
Mg^{+2}, Ca^{+2}	Very slightly inhibitory
Ba^{+2}, Co^{+2}	No significant effects

Kaldor (1962) studied 5'-AMP deaminase of rabbit myofibrills (the deaminase of myofibrillar preparations). He found that ATP,ADP,ITP and citrate activated the deaminase in succinate buffer. The nucleotide activation was not observed in citrate buffer. Lyubimova and Matlina (1954) also reported that ATP and ADP activated their deaminase preparation from rabbit skeletal muscle. Recently, Cunningham and Lowenstein (1965) showed that calf brain 5'-AMP deaminase is regulated by ATP. The purified enzyme did not show an absolute dependence on ATP and a plot of substrate versus rate gave a sigmoidal curve which became hyperbolic in the presence of ATP.

Askari and Franklin (1965), working with purified preparations of AMP deaminase from human erythrocytes, showed an absolute requirement for a monovalent cation. The enzyme could be activated by K^{+} or NH_4^{+} but not by Na^{+}, Li^{+}, Rb^{+}, or Cs^{+}. In the presence of ATP, which

was shown to be chemically unaltered, the enzyme could be activated by K^+, NH_4^+, Na^+, Li^+, and Rb^+ with the activation decreasing in that order. In the case of the enzyme from cat and dog erythrocytes, only ATP showed an activation and the alkali cations had no effect in the presence or absence of ATP.

5'-AMP deaminase has a very wide biological distribution. The existence of this activity has been shown in microorganisms (Angarwala,et al,1954; Saruno, et al,1955; Aida,et al, 1965), snail, eel, viper and pigeon (Umiastowski,1964), man, dog, and cat erythrocytes (Askari,et al,1965), rat, rabbit, guinea pig, and ox (Kutscher,et al,1948), fish (Nara,et al,1959,1962), and even pea seeds (Turner,et al,1961).

This deaminase activity has been shown in a variety of tissues including heart, smooth muscle, brain, peripheral nerve, liver, kidney, spleen and lung (Nechiporenko,et al,1949; Eidelman,1953; Kutscher,et al,1948; Sata,1954) but with much less activity than in skeletal muscle. Kutscher (1948) studied the relative levels of deaminase in white skeletal muscle of various species and obtained the following results: guinea pig 200, man 165, rabbit 123, cat 104, chicken 100, rat 70, and ox 42.

The vast distribution of 5'-AMP deaminase in biological systems, including plants, animals, and microorganisms indicates the possibility of a very significant physiological role for this enzyme.

MATERIALS AND METHODS

5'-Adenylic acid (sigma grade) was purchased from the Sigma Biochemical Corporation. All other reagents were of reagent grade. Tetramethylammonium (TMA) ion was used to avoid alkali cations.

All animals were sacrificed by suffocation in CO_2. The desired tissue was immediately excised and chilled in ice.

The spectrophotometric assay used was a modification of that of Kalckar (1947) based on the sixty per cent decrease in absorption which occurs at 265 millimicrons when AMP is converted to IMP. The standard spectrophotometric assay used in this work was: 0.1 M K succinate at pH 6.5, 5×10^{-5} M AMP and 30°C. These measurements were made on a Beckman DU spectrophotometer.

The rates which were measured with the pH stat (Radiometer TTT-1/-SBR2/SBU1/TTA31) were run with no buffer since the liberated ammonia was titrated with hydrochloric acid. This method was especially useful since no AMP concentration limitation existed which was a disadvantage of the spectrophotometric assay due to the high extinction of AMP. Specific activity was defined as the micromoles of AMP deaminated per minute per mg of protein.

The crude deaminase extract which was used for some of the experiments was prepared as follows. Each gram

of tissue was ground with 7 volumes of cold Guba-Straub (Szent-Györgyi, 1951) solution (0.3 M KCl, 0.09 M KH_2PO_4, 0.06 M K_2HPO_4, pH 6.5) and stirred for an hour in the cold. The mixture was centrifuged and the supernatant solution was called the crude AMP deaminase extract.

The crude actomyosin extract was prepared by grinding each gram of tissue in 3 ml of cold Weber-Edsall (Szent-Györgyi,1951) solution (0.6 M KCl, 0.01 M Na_2CO_3, 0.04 M $NaHCO_3$, pH 9.0). The homogenate was allowed to stand overnight in the cold and centrifuged. The supernatant solution was called the crude actomyosin extract.

A low salt treatment was defined as the process of diluting a protein solution to 0.05 M KCl at which point the myosin and deaminase precipitate. The precipitate was then dissolved in 0.5 M KCl again. This separates the deaminase from the low salt soluble proteins.

The enzyme of specific activity 25 was prepared by Dr. Eugene Byrnes (unpublished) and that of specific activity 50 was prepared by Dr. Karl Smiley (unpublished).

The paper chromatography solvent system used to separate nucleotides was; saturated $(NH_4)_2SO_4$: 0.2 M Na acetate (pH 5.9) : isopropanol, 79:19:2 (v/v).

EXPERIMENTAL

A study of the 5'-AMP deaminase levels of several tissues of a variety of animals is of interest for several reasons. This type of study will reveal the best source of enzyme of the animals studied and, the results of species and tissue studies may give some insight as to the physiological function of the enzyme. The data of such a study are given in TABLE I. The level of AMP deaminase is seen to be very low in heart. The standard assay ($5x10^{-5}$M AMP) failed to show any activity; however, higher substrate concentrations demonstrated activity in pigeon and rabbit hearts. As further evidence for the existence of the enzyme in heart tissue, a paper chromatographic study showed that spots which have the same R_f as IMP and AMP appeared from a sample of the reaction mixture: 10^{-2}M AMP, 0.10 M K succinate (pH 6.5) and heart deaminase extract. No IMP appeared in the controls lacking the heart extract or the AMP. The data in TABLE I also show that the enzyme level is much greater (about six fold) in breast than in leg muscle in both chicken and pigeon. The significance of this is not known.

Rabbit muscle is seen to be the best source of enzyme of the tissues studied. The enzyme level increases with age in young rabbits and the data show that mature rabbits have about eight times the level of very young rabbits.

TABLE I

The AMP deaminase content
of some species and tissues of animals

tissue	Activity, units per gram: crude deaminase extract	crude actomyosin extract
* frozen dog heart	0.0	0.0
* frozen mouse heart	0.0	0.0
* frozen rat heart	0.0	0.0
* frozen guinea pig heart	0.0	0.0
* refrigerated beef heart	0.0	0.0
* fresh bullhead fillet	8.6	6.5
* fresh chicken breast	11.2	10.6
* fresh chicken leg	1.6	2.4
+ fresh chicken heart	2.1	1.8
* fresh pigeon breast	9.7	9.6
* fresh pigeon leg	1.7	2.2
+ fresh pigeon heart	1.9	3.3
+ fresh honey bee muscle	0.4	1.0
fresh rabbit heart	#7.3	+4.0
fresh rabbit skeletal muscle:		
* 9 day old rabbit	2.5	-
*16 day old rabbit	9.8	-
*72 week old rabbit	24.3	-
*94 week old rabbit	18.8	-

* standard assay

+ 10^{-2}M AMP, pH stat assay

2×10^{-2}M AMP, pH stat assay

The importance of Ca^{+2} in muscular contraction and the occasional observation of divalent cation activation of AMP deaminase preparations led to a study of the effects of some of these cations on rabbit muscle AMP deaminase at different stages of purity, different AMP concentrations, and various ionic strengths. The effect of some organic acids were also studied since conflicting reports on these effects appear in the literature. TABLE II shows these effects on low salt treated deaminase extract at 10^{-2}M AMP and various ionic strengths (adjusted with KCl). Divalent cations and ionic strength have no effect. Citrate, succinate and lactate activate slightly with lactate being the best of the three (134% of non-activated rate). TABLE III and TABLE IV compare the effects of some of the same reagents at two AMP levels on crude deaminase extract and low salt treated deaminase extract, respectively. These data show that only citrate and lactate have any significant effect and both activate slightly at 10^{-2}M AMP with both the crude and the low salt treated deaminase extract. At 10^{-3}M AMP, lactate still activates slightly; however, citrate activation is greatly increased (236% of the non-activated rate with crude extract and 145% with low salt treated extract). Since the activation is greater on the crude extract than on the low salt treated extract the effect of citrate on AMP deaminase is probably not a direct effect.

TABLE II

Effects of some reagents on low salt treated AMP deaminase at 10^{-2}M AMP and various ionic strengths

reagent(M)	ionic strength	specific activity*
KCl(.10)	0.10	6.18
$CaCl_2$(.01),KCl(.o7)	0.13	6.94
$MgCl_2$(.01),KCl(.07)	0.13	6.37
$MnCl_2$(.01),KCl(.07)	0.13	ppt. formed
$MgCl_2(10^{-2})$,KCl(.10)	0.16	6.54
$MgCl_2(10^{-3})$,KCl(.10)	0.11	7.12
$MgCl_2(10^{-4})$,KCl(.10)	0.10	6.43
Kcitrate(.01),KCl(.04)	0.10	7.50
Kcitrate(.01),KCl(.14)	0.20	8.13
Kcitrate(.01),KCl(.24)	0.30	7.55
Klactate(.01),KCl(.09)	0.10	8.29
Klactate(.01),KCl(.19)	0.20	8.29
Klactate(.01),KCl(.29)	0.30	7.36
Ksucc.(.01),KCl(.07)	0.10	6.89
Ksucc.(.01),KCl(.17)	0.20	7.07
Ksucc.(.01),KCl(.27)	0.30	7.37

* determined with the Radiometer pH stat at 10^{-2}M AMP
abbreviations: succ.(succinate)

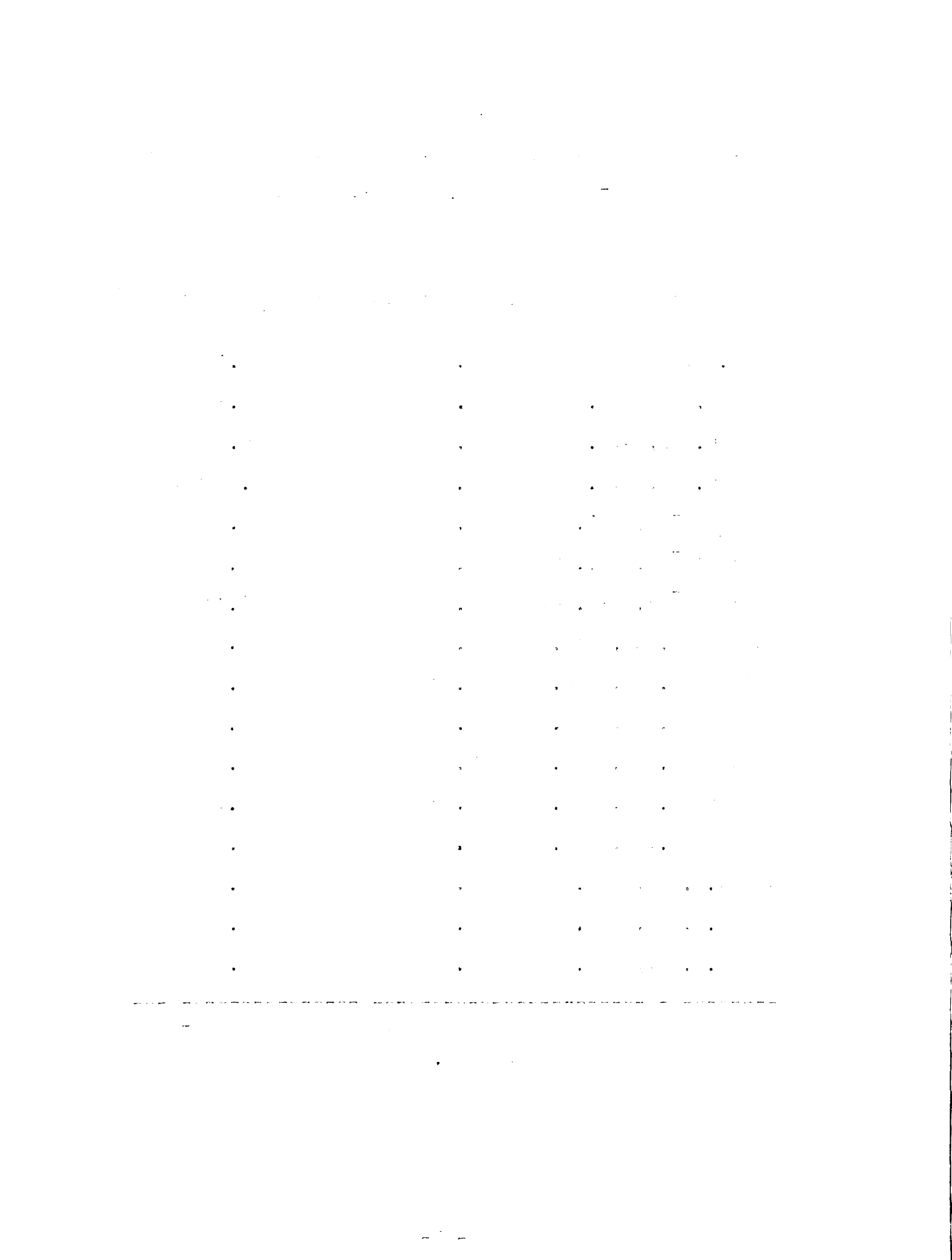


TABLE III

The effects of some reagents on crude deaminase extract at 10^{-2}M and 10^{-3}M AMP

reagent	(M)	AMP conc.,M	specific activity*
none	-	10^{-3}	3.23
$CaCl_2$	10^{-2}	10^{-3}	3.52
$CaCl_2$	10^{-3}	10^{-3}	3.48
$CaCl_2$	10^{-4}	10^{-3}	3.18
K citrate	10^{-2}	10^{-3}	7.62
K lactate	10^{-2}	10^{-3}	3.85
none	-	10^{-2}	8.20
$CaCl_2$	10^{-2}	10^{-2}	7.45
K citrate	10^{-2}	10^{-2}	9.08

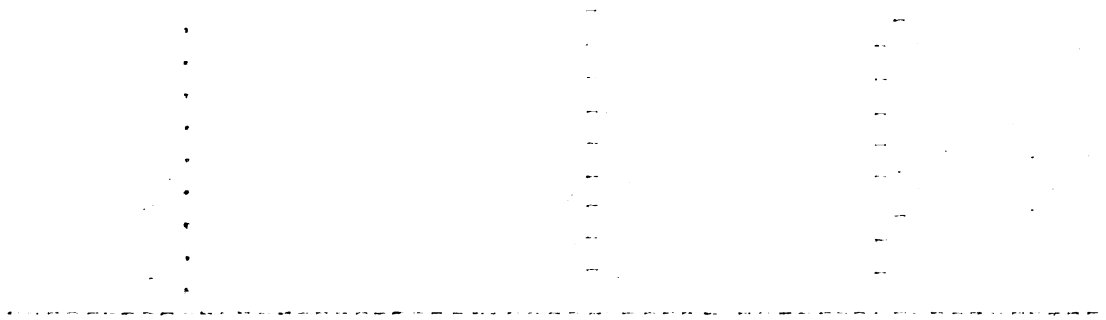
*determined with Radiometer pH stat and each reaction contained 0.10 M KCl

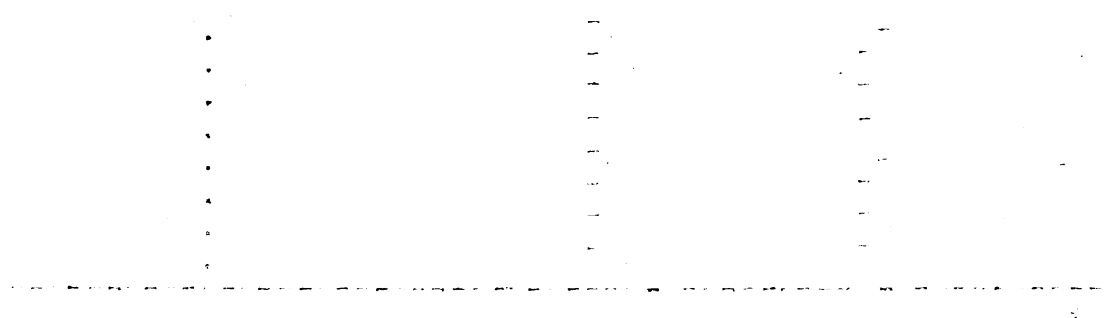
TABLE IV

Effects of some reagents on low salt treated deaminase at 10^{-2}M and 10^{-3}M AMP

reagent	(M)	AMP conc.,M	specific activity*
none	-	10^{-3}	8.85
$CaCl_2$	10^{-2}	10^{-3}	8.02
K citrate	10^{-2}	10^{-3}	11.58
K lactate	10^{-2}	10^{-3}	9.04
none	-	10^{-2}	12.19
$CaCl_2$	10^{-2}	10^{-2}	13.18
K citrate	10^{-2}	10^{-2}	12.77
K lactate	10^{-2}	10^{-2}	13.10

*determined with Radiometer pH stat and each reaction contained 0.10 M KCl





The activity of purified rabbit muscle enzyme (specific activity 25 or 50) always seemed to increase with increasing KCl concentration when this was used to adjust the ionic strength in several experiments. Suspecting that KCl was activating the enzyme, a group of experiments were done to determine the effects of some monovalent cations on AMP deaminase. TABLE V shows the relative activation and concentration of maximal activation for some monovalent cations. K^+ is the best activator and it is seen that AMP deaminase has a definite monovalent cation requirement.

The activation constants (K_a) for the monovalent cations were determined spectrophotometrically. Lineweaver-Burk plots were made and the activation constants were calculated from these plots. A sample is shown in FIGURE 2 for NaCl and the complete data are given in TABLE VI.

Lineweaver-Burk plots for AMP and purified rabbit muscle AMP deaminase (specific activity 50) repeatedly exibited upward curvature. This suggested that the enzyme may be allosteric; thus, Hill plots (Monod, et al., 1963) were determined for AMP and AMP deaminase. The slopes varied between -1.2 and -1.4 with most at -1.3. This slope did not change when the Kcl concentration was varied between 0.05 M and 0.15 M. An attempt was made to desensitize the enzyme (destroy the allosteric nature) by heating to 50°C for 5 minutes which denatured

TABLE V

The relative activation and concentration of maximal activation of some monovalent cations on AMP deaminase

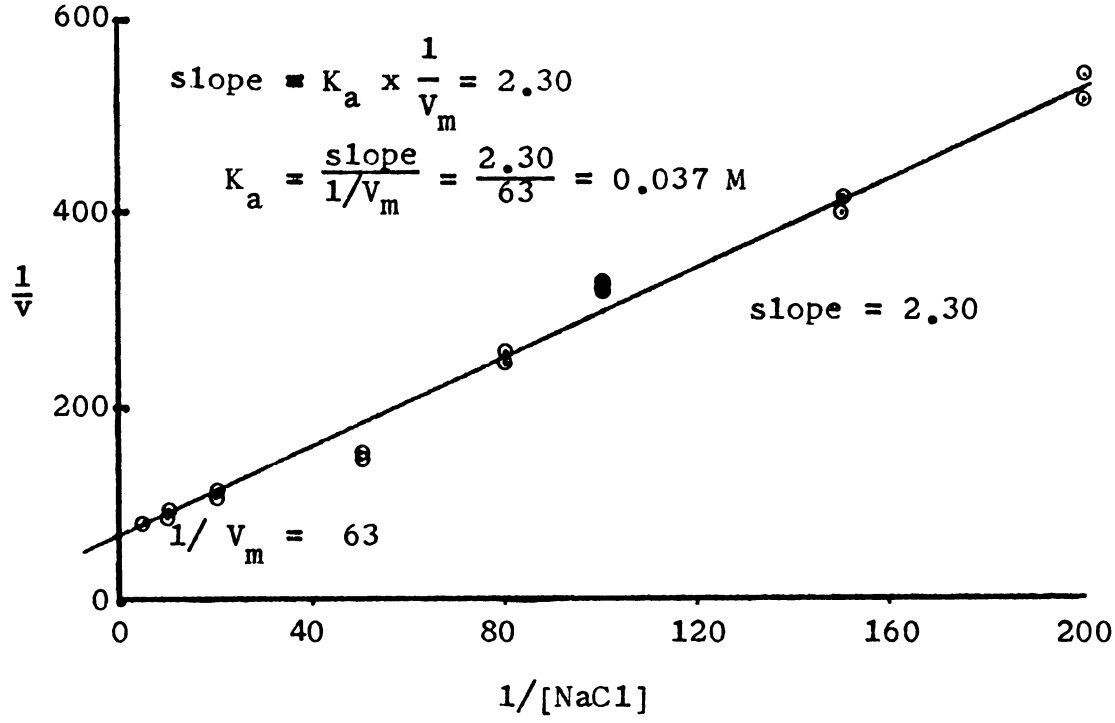
reagent	rel. activation*	conc. of max. activation#, M
KCl	100	0.11 ± .02
NaCl	54	0.12 ± .02
LiCl	26	0.10
NH_4Cl	23	0.10
RbCl	14	-
CsCl	0	-
TMACl	0	-
none	0	-

* Standard assay at 0.15 M cation and enzyme specific activity of 25

\# Standard assay with enzyme specific activity of 25. Based on one determination for LiCl and NH_4Cl.

FIGURE 2

The calculation of K_a for NaCl and AMP deaminase*



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* Each reaction contained 1.25×10^{-4} M AMP in 0.05 M imidazole HCl buffer at pH 6.5. The ionic strength was adjusted with TMACl. Rates were determined spectrophotometrically using enzyme of specific activity 25.

TABLE VI

The activation constants

of monovalent cations and AMP deaminase

reagent	K_a, M*
KCl	0.020 ± .002
NaCl	0.057 ± .018
LiCl	0.061 ± .015
NH_4Cl	0.021
RbCl	0.059
CsCl	no activation
TMACl	no activation

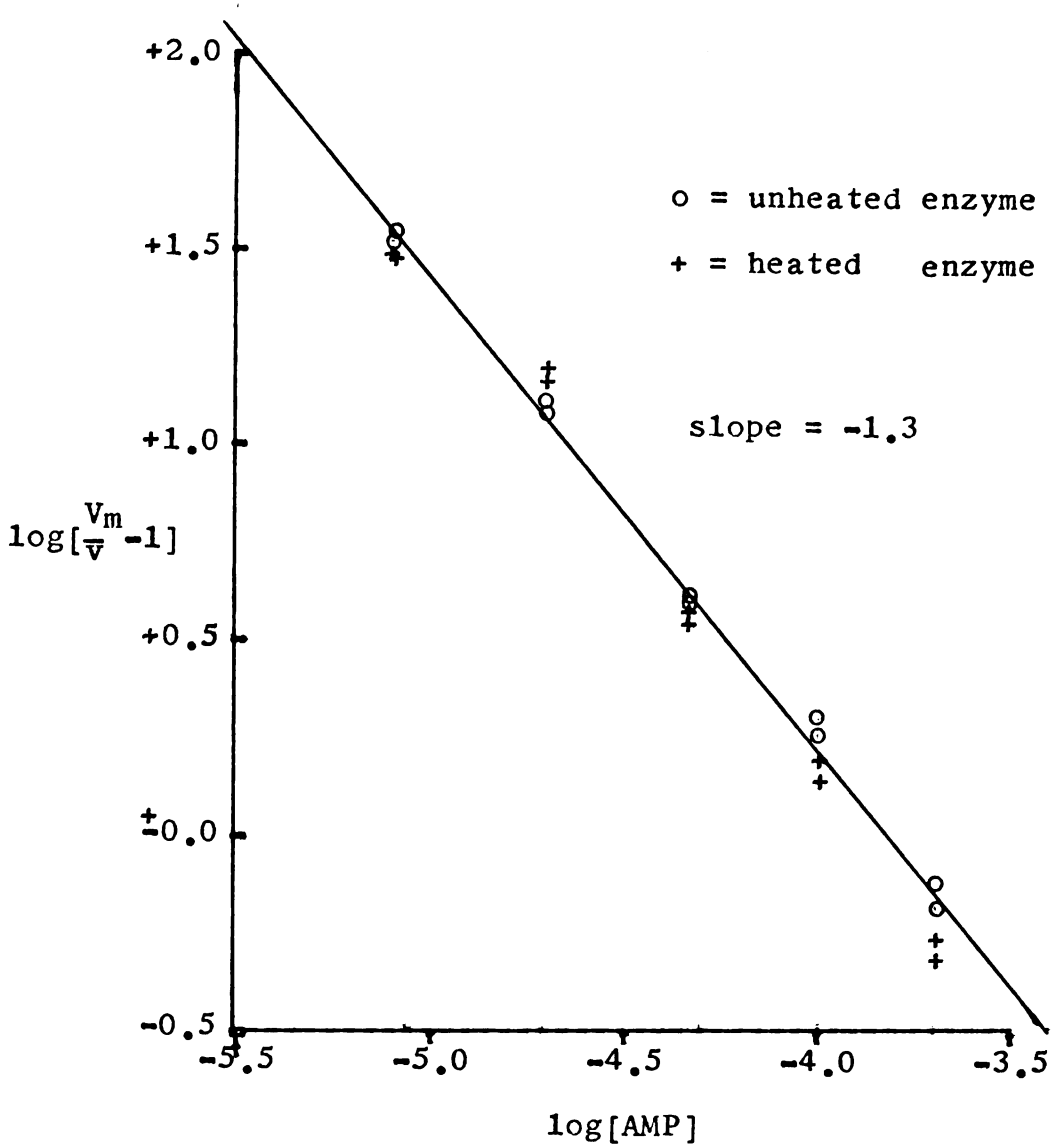
* reaction conditions as in FIGURE 2. Based on one determination for NH_4Cl and RbCl.

about one-half of the protein. Hill plots were determined on an enzyme sample before and after a heat treatment. FIGURE 3 shows that this treatment did not alter the Hill slope and thus, did not desensitize the enzyme.

Conflicting reports on nucleotide activation of rabbit muscle AMP deaminase appear throughout the literature. The effect of ATP on deaminase was superficially investigated and reproducible data have not been obtained. At times ATP has been shown to strongly activate AMP deaminase. TABLE VII shows a typical set of data where activation was obtained. This activation seemed to be a function of the particular enzyme preparation since some prepatations did not show ATP activation under any conditions. It is hoped that the parameters of this phenomenon will be elucidated in the near future.

FIGURE 3

Hill plots of heated and unheated enzyme



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*Spectrophotometric assays were done using enzyme of specific activity 50. The enzyme was heated rapidly to 50°C and cooled rapidly after heating 5 minutes. Each reaction contained 0.10 M KCl, 0.05 M imidazole HCl buffer (pH 6.5) and variable amounts of AMP.

TABLE VII

The effect of ATP on AMP deaminase activity

[AMP], M	[ATP], M	rate, OD/min.*	% activation
5×10^{-5}	0	0.066	-
5×10^{-5}	10^{-4}	0.166	253

* reaction mixture as in FIGURE 3.

DISCUSSION

It may be stated from the data in TABLE I that AMP deaminase increases with age in young rabbits. This was a very superficial study taken from data in which the ages of the rabbits were accurately known. The difference between the activities of the two oldest rabbits may not be significant; however, it may also be due to the deaminase levels dropping in older rabbits. Although not documented in TABLE I due to lack of knowledge of the exact age, the general observation was made that older rabbits have lower deaminase levels. It is now a general practice of this laboratory to use rabbits which are about one year old. Kendrick-Jones and Perry (1965) observed that the deaminase levels in rabbits increase from about one-eighth of the adult level at age nine or ten days to normal adult levels at about fourteen days of age. This is not in exact agreement with the data in TABLE I which says that a sixteen day old rabbit has at most only one-half of adult deaminase levels. A much more detailed study would have to be done to follow the exact chronological appearance of AMP deaminase.

No enzyme activity could be found in heart tissue when the standard assay (5×10^{-5}M AMP) was used; however, activity was detected when the AMP level was raised to 10^{-2}M. Further evidence that AMP deaminase occurs in

heart muscle was given by showing with paper chromatography that IMP is produced from AMP by heart extracts.

An interesting observation in both chicken and pigeon is that the activity is much higher (about six fold) in breast than in leg muscle. The possibility that white muscle is generally higher in activity than red muscle is ruled out since pigeon breast is red muscle.

In studying the effects of some cations and anions on AMP deaminase (TABLES II, III, and IV) it was observed that citrate strongly activates the enzyme in crude extracts and slightly activates low salt treated preparations. This suggests that the activation is not direct but is caused by some factor produced by citrate and the low salt soluble fraction of the crude extract.

A monovalent cation requirement for muscle AMP deaminase has never been reported before. TABLE V shows a definite requirement and that K^+ fulfills this best. Other workers have always used a buffer with a high monovalent cation concentration and this is probably the reason the requirement has never been seen before.

FIGURE 3 shows that Hill slopes for AMP and AMP deaminase are -1.3. Hill slopes between one and two indicate a cooperative interaction between two or more binding sites (Monod, et al, 1963). The apparent affinity constant ("K") calculated from FIGURE 3 is 1.8×10^{-5}M. The Lineweaver-Burk plots made by Lee (1957) showed no such

allosteric nature and it was thought that the rather severe heat treatment in Lee's preparation may have desensitized the enzyme. FIGURE 3 shows that the enzyme heated under the same conditions as those used in Lee's preparation did not desensitize the protein since the Hill slope did not change.

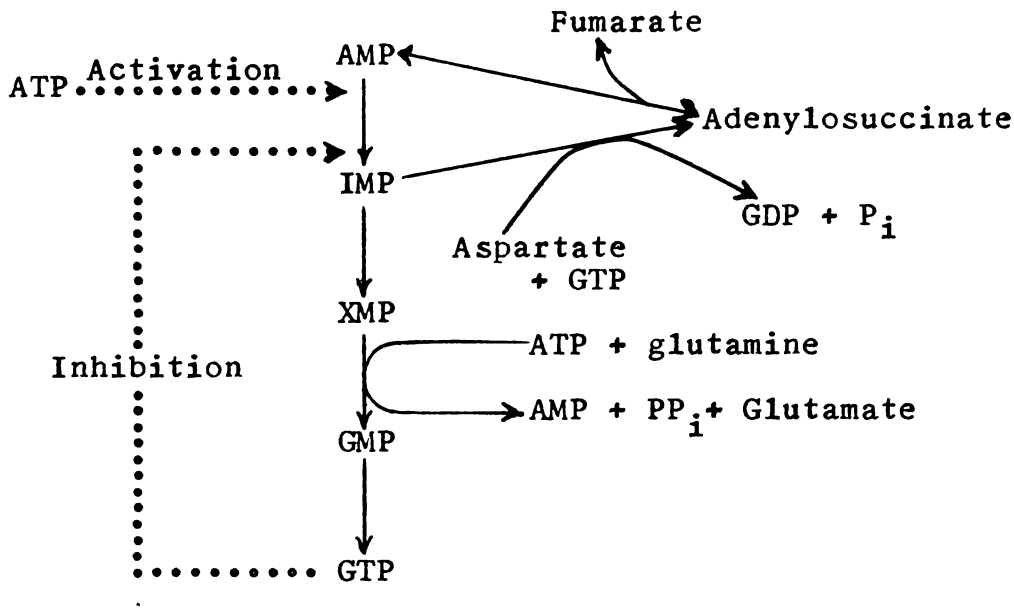
It is believed that the enzyme crystallized by Lee was significantly different than the protein used for this study. Lee showed that his enzyme was homogeneous in a variety of ways and yet the specific activity (17.2) was much lower than some of the preparations used in this work (specific activity 50).

Lee also reported that ATP (10^{-4}M) did not affect the reaction rate and the data in TABLE VII show that ATP more than doubles the rate. It must be pointed out that the ATP activation was not always observed and seemed to depend on the particular enzyme preparation used. It is hoped that this problem will be solved in the near future.

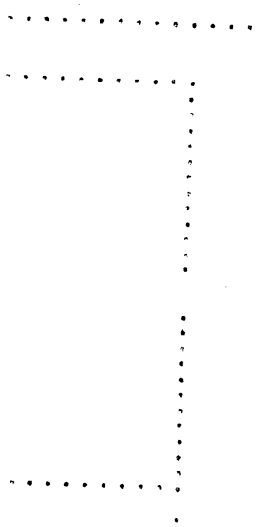
AMP deaminase preparations from seven tissues other than skeletal muscle have been shown to be regulated by ATP (Lowenstein, et al, 1966). This group of workers also reported that AMP deaminase from muscle is activated by ATP (personal communication) but they too, have problems reproducing the data.

Lowenstein (1966) proposed that AMP deaminase is important in the regulation of the interconversion of

purine nucleotides in mammals. This proposal was invoked to explain several observations; 1) ATP activates the conversion of AMP to IMP and participates in the conversion of XMP to GMP. 2) GMP inhibits the conversion of AMP to IMP and participates in the conversion of IMP to AMP via adenylosuccinate. Schematically:



This scheme rationalizes the nucleoside triphosphate specificities in the conversion of IMP to adenylosuccinate and XMP to GMP. FIGURE 1 which deals with the involvement of AMP and ATP in the regulation of glycolysis and in muscular contraction does not contradict the purine nucleotide regulation scheme of Lowenstein. Indeed, they are complementary.



SUMMARY

1. Rabbit skeletal muscle was shown to be the best source of AMP deaminase of a variety of animals and tissues studied. Very young rabbits were seen to have a lower enzyme level than mature rabbits.

2. Citrate was shown to activate crude AMP deaminase preparations; however, some evidence suggested that the activation may not be a direct interaction of citrate and AMP deaminase.

3. A monovalent cation requirement was demonstrated for rabbit muscle AMP deaminase and K^+ was shown to fulfill this best. The relative activation, the concentration of maximal activation, and the activation constants of a group of monovalent cations were reported.

4. Rabbit muscle AMP deaminase was shown to have an allosteric interaction with AMP which exibited a Hill slope of -1.3. Thermal treatment severe enough to denature the enzyme (50°C) or variation of the KCl concentration between 0.05 M and 0.15 M did not alter the Hill slope.

5. ATP was reported to activate some rabbit muscle AMP deaminase preparations although this was not always reproducible. A scheme was presented which rationalizes the ATP activation of the enzyme.

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