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ACTIVATION AND INHIBITION OF AMP DEAMINASE BY GTP AND ATP

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SUMMARY

1. GTP can both activate and inhibit the AMP deaminase activity of muscle homogenate depending upon the assay conditions chosen.
2. Purified AMP deaminase is inhibited at all concentrations of GTP.

In a study of AMP deaminase (AMP aminohydrolase, EC. 3.5.4.6) levels in different tissues of four species we found that while both ATP and GTP caused strong activation of this enzyme in muscle homogenates of all species studied (see Table I) other tissues showed considerable variation in their response to the addition of these substances [7].

TABLE I

COMPARISON OF ATP AND GTP ON ACTIVITY OF AMP DEAMINASE IN MUSCLE HOMOGENATES OF FOUR SPECIES

Psoas muscle was homogenized in 0.25 M sucrose forming a 10% (w/v) homogenate. Enzyme assays were performed by microdiffusion at 37 °C. All assays were performed in triplicate by measuring NH_3 release by microdiffusion.

Activity	Rat		Guinea pig		Rabbit		Mouse	
	μmoles/min g wet tissue	%	μmoles/min g wet tissue	%	μmoles/min g wet tissue	%	μmoles/min g wet tissue	%
O	4.2	100	6.4	100	19.7	100	8.8	100
+ATP	21.0	500	12.6	197	78.2	397	76.0	889
+GTP	13.4	319	8.3	129	49.1	249	45.0	511

However, the inhibitory effect of GTP on AMP deaminase from many sources is so well documented [1–5] that the unexpected activation which we always obtained with muscle homogenate under our conditions was puzzling and clearly called for a re-examination of the effect of GTP on AMP deaminase under different conditions.

A search of the literature on the effect of GTP on AMP deaminase from various sources showed inhibition in all cases, with the single exception of a paper by Razin

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and Mager [8] who reported an activation of rabbit erythrocyte AMP deaminase by GTP, but they added that they could offer "no explanation for the discrepancy between our findings and the observation reported by Setlow et al. [2], on the inhibitory action of GTP on AMP deaminase in a variety of different organs".

A short account of part of this work has been published previously [9].

Enzyme assays

Tissues were prepared by homogenizing 1 g of tissue in 9 ml 0.25 M sucrose in a Potter–Elvehjem homogenizer by the method of Hogeboom [10]. Buffered substrate was prepared by dissolving AMP (free acid) (Schwarz-Mann) in Tris—HCl buffer and adjusting to pH 7.2 with Tris to a concentration of 15 mM. The assay was started by adding 1 ml of suitably diluted enzyme at 37 °C to a mixture of 1 ml of the buffered AMP in 1 ml of 0.05 M Tris–HCl buffer giving a substrate concentration of 5 mM AMP. In the experiments in which ATP or GTP was used 1 ml of 15 mM buffered ATP or GTP replaced the Tris buffer. In these cases the final concentration of substrate and nucleoside triphosphate was 5 mM in each case.

Assays were performed either by measuring NH_3 formation by microdiffusion [11] or spectrophotometrically by following the fall in absorption at 265 nm, or its increase at 285 nm [12] in a Beckman DU spectrophotometer fitted with a Hilger Gilford recording apparatus.

Unit of enzyme activity

This is defined as μmoles of adenylic acid converted to inosinic acid per min at pH 7.2 in 0.05 M Tris buffer. Specific activity is defined as enzyme units per mg of protein. The enzyme activity of tissue homogenates is expressed as enzyme units per gram of wet tissue (units/g).

It is well known that AMP deaminase activity is affected by a large number of different inorganic and organic cations and anions [1–6, 12–14]. When the activation by GTP was encountered first, it became essential to test if it was simply a cation effect caused by the presence of Na^+ or an effect due to nucleoside triphosphate.

This was investigated by comparing the activation produced by Na^+ and $Tris^+$ salts of the nucleoside triphosphates (see Table II). It is evident from this table

TABLE II

COMPARISON OF Na^+ AND TRIS SALTS OF ATP AND GTP ON AMP DEAMINASE ACTIVITY OF MUSCLE HOMOGENATES

Psoas muscle was homogenized in 0.25 M sucrose forming a 10% (w/v) homogenate. Enzyme assays were performed by microdiffusion at 37 °C. Enzyme activity was assayed by microdiffusion in all cases. Control results were arbitrarily expressed as 100; the other results were calculated as a percentage of the control. Enzyme activity expressed as a percentage of the control.

	Rat	Guinea pig	Rabbit	Mouse
Control	100	100	100	100
ATP (sodium salt)	800	210	655	2000
ATP (Tris salt)	660	197	577	1467
GTP (sodium salt)	—	—	511	940
GTP (Tris salt)	190	124	422	873

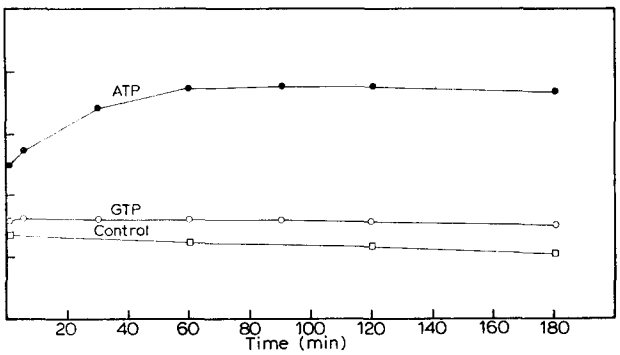

Fig. 1. Effect of incubation with ATP and GTP on activity of AMP deaminase in rabbit psoas muscle homogenate. 10% homogenates in 0.25 M sucrose were incubated at 37 °C in a water bath. Enzyme assays were performed by microdiffusion at 37 °C as described in text. ●—●—●, +ATP ○—○—○, +GTP; □—□—□, control.

that the sodium salts of both ATP and GTP in all cases showed a slightly greater activation effect than the corresponding Tris salts, nevertheless the Tris salts showed a substantial activation effect in their own right, proving that GTP is an activator of AMP deaminase under these conditions. This table also showed that the degree of activation produced varies with the species, e.g. the effect of nucleoside triphosphate is greater in mouse and least in guinea-pig muscle.

Effect of ATP and GTP incubation on AMP deaminase in rabbit muscle homogenate

It can be seen from Fig. 1 that ATP produces an activation reaching a maximum after 60 min, while GTP produces a smaller but more rapid activation. Both ATP and GTP protect the enzyme, from inactivation at this temperature.

TABLE III

AMP DEAMINASE ACTIVITY IN SUPERNATANT EXTRACTS OF RABBIT MUSCLE, LIVER AND LUNG AFTER DIFFERENT CENTRIFUGATION TIMES

10% (w/v) homogenates of each organ in 0.25 M sucrose were prepared and centrifuged for the stated times in a MSE refrigerated centrifuge. Enzyme assays were performed by microdiffusion at 37 °C.

Centrifugation time (min)	Activity in supernatant (units/g)		
	Muscle	Liver	Lung
0	200	0.6	1.5
10	0.4	0.5	1.2
30	0.3	0.5	1.2

The AMP deaminase activity in sucrose homogenates of rabbit muscle was found to sediment rapidly on centrifugation in contrast to the enzyme in rabbit liver and lung.

In muscle the enzyme is bound in the form of myosin or actomyosin particles [14]. Such insoluble preparations give a hyperbolic substrate concentration versus enzyme velocity curve as shown in Fig. 2, Curve B. When this enzyme was purified by

the method of Smiley et al. [12] to the stage of elution from cellulose phosphate, preparations of spec.act. = 60 were obtained, (i.e. about 80% pure) which were now fully soluble in water and in which the myosin type complex had been disrupted by the purification. This enzyme was found to give a sigmoidal substrate concentration versus enzyme velocity curve as shown by Fig. 2, Curve A.

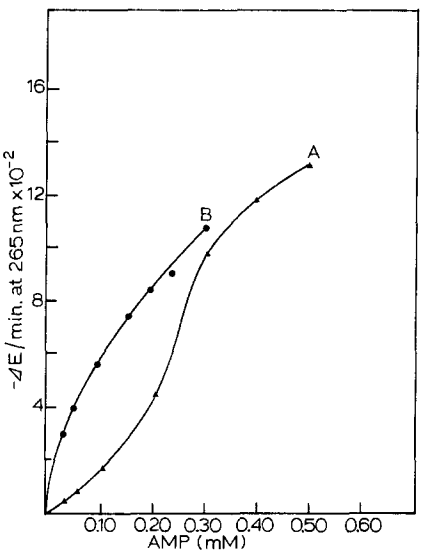

Fig. 2. Comparison of rabbit AMP deaminase activity versus substrate concentration in (A) a sample of purified enzyme and (B) in skeletal muscle homogenate. Enzyme purified as described in the text, and a 10% homogenate in 0.25 M sucrose was used. Enzyme assay was performed by measuring the fall in absorption at 265 nm at 37 °C as described in text. ▲—▲, A; ●—●, B.

It is clear that the enzyme present in muscle homogenate is almost entirely present in a water-insoluble form, while in rabbit liver and lung it is present largely in a water soluble form. These tissues show little activation by GTP [7].

There are a number of points of similarity between the enzyme present in dialysed extracts of rabbit or human erythrocytes as described by Razin and Mager [8] and that present in skeletal rabbit muscle homogenates described in this paper. Both enzymes are activated by GTP. In each case the degree of activation is about 50% of that produced by ATP. ATP affords complete protection against inactivation of the erythrocyte enzyme [8] at 50 °C and both ATP and GTP give considerable protection against loss of activity in muscle homogenates at 37 °C. Our results differ in one respect from those of Razin and Mager [8]. They obtained a sigmoidal curve for the substrate concentration versus enzyme velocity plot, while we obtained a hyperbolic curve with the particle-bound enzyme in muscle homogenate, but a sigmoidal curve with the purified enzyme. Our results are in better agreement with those of Rao et al. [14] who also reported a hyperbolic substrate concentration versus enzyme velocity curve for the membrane-bound erythrocyte enzyme which changed to a sigmoidal curve when the enzyme was solubilized (Fig. 1 (p. 653) in ref. 14). The effect of GTP on the erythrocyte enzyme activity was not tested by these authors.

A study of enzyme activity at "high" and "low" substrate concentration was undertaken. A final concentration of 5 mM nucleoside triphosphate (NTP) and 5 mM AMP was arbitrarily defined as "high" concentration and a final concentration of 0.2 mM NTP and 0.2 mM AMP was similarly defined as "low" concentration. From Table IV it can be seen that ATP and GTP behave differently at these two concentrations in rabbit muscle homogenate. At 5 mM, i.e high concentration, both ATP and

TABLE IV

EFFECT OF "HIGH" SUBSTRATE AND "HIGH" EFFECTOR CENTRATIONS (5 mM) AND "LOW" SUBSTRATE AND "LOW" EFFECTOR CONCENTRATIONS (0.2 mM) ON (A) AMP DEAMINASE ACTIVITY IN MUSCLE HOMOGENATE AND ON (B) PURIFIED DESALTED AMP DEAMINASE

Rabbit psoas muscle homogenate in 0.25 M sucrose (10% (w/v)) and enzyme purified as described in text were used. Assays in Column I were performed by microdiffusion. Assays in Column II were performed spectrophotometrically.

	Column I High substrate (5 mM)		Column II Low substrate (0.2 mM)	
	Units/g wet tissue	Activity %	Units/g wet tissue	Activity %
(A) Rabbit muscle homogenate				
AMP	35.7	100	2.1	100
+GTP	92.1	+258	0.8	−38
+ATP	141.5	+395	1.6	−76
(B) Purified AMP deaminase				
AMP	72.5	100	1.2	100
+GTP	46.4	−64	0.3	−25
+ATP	132	+182	43	+357

GTP cause activation of the enzyme. However, while 5 mM ATP causes activation of the purified enzyme, 5 mM GTP causes inhibition, and this provides the explanation for the inhibitory effect of GTP, generally reported in the literature [1–5]. Examination of these papers shows that, in each case, purified or partially purified enzyme, i.e. soluble enzyme was used, and this as shown in the present paper results in a inhibition by GTP. In the one case [8] where membrane-bound AMP deaminase was employed as enzyme, GTP, when tested, was found to cause activation of the enzyme as is also reported in this paper.

The behaviour of ATP and GTP at low concentration is again different. Using muscle homogenate both ATP and GTP cause inhibition while with purified enzyme ATP causes strong activation but GTP again causes inhibition.

Summarizing, we have shown that GTP incubation has different effects upon AMP deaminase depending upon the physical state of the enzyme. Water insoluble enzyme is stimulated by GTP and shows normal Michaelis kinetics while the purified and, hence soluble, enzyme is inhibited by GTP and shows sigmoidal kinetics. Thus the differences observed by Razin and Mager [8] and Rao et al. [14] and ourselves from those noted by Setlow et al. [2] and Smiley and Suelter [1], and the others [3–5] can be accounted for as due to differences in the physical states of the enzymes used.

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