BBA 66023

MUSCLE AMP AMINOHYDROLASE

III A COMPARATIVE STUDY ON THE REGULATORY PROPERTIES OF SKELETAL MUSCLE ENZYME FROM VARIOUS SPECIES

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(Received July 24th, 1969)

SUMMARY

1 The effect of K^+, nucleoside triphosphates, ADP and P_i on skeletal muscle AMP deaminase (AMP aminohydrolase, EC 3 5 4 6) from frog, pigeon, guinea pig, rabbit and rat, partially purified by the same method, was investigated

2 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

3 At pH 6 5 and at KCl concentrations higher than 100 mM, ATP, GTP, ITP and P_i inhibit the enzyme from all the species examined, however, at 50–100 mM KCl ATP activates the enzyme from guinea pig and rabbit At pH 7 1 and at 50–200 mM KCl ATP inhibits all the enzymes

4 AMP deaminase is inhibited to a limited extent by nucleoside triphosphates, the inhibition degree depends on the pH and on the sources, P_i completely inhibits the enzyme Creatine phosphate also is an inhibitor Kinetic evidences suggest two distinct sites of binding, one for the nucleoside triphosphate and the other for P_i

5 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 triphosphates and P_i

6 The data suggest that the enzyme in resting muscle is partially or completely inhibited, when ADP accumulates in muscle it removes the inhibition by nucleoside triphosphates, P_i and creatine phosphate and the enzyme results activated

INTRODUCTION

AMP deaminase (AMP aminohydrolase, EC 3 5 4 6) catalyzes the hydrolytic deamination of AMP to IMP and NH_3 The enzyme is widely distributed in animal tissues, however, in skeletal muscle AMP deaminase activity is much higher than in

other tissues including heart and smooth muscles[1,2] The distribution of the enzyme in skeletal muscle varies greatly, white muscles have higher enzyme concentration than red[3]

AMP deaminase was purified from rat and rabbit skeletal muscles and from calf brain[4-9] The kinetic properties of AMP deaminase from brain, erythrocytes and skeletal muscle have been extensively studied MENDICINO AND MUNTZ[10] first demonstrated that the brain enzyme is activated by ATP, the enzyme is also activated by monovalent cations and inhibited by GTP[9,11,12] AMP deaminase from various tissues of rat was found to be activated by ATP and inhibited by GTP[13] The enzyme from erythrocytes is activated by ATP, monovalent cations and inhibited by 2,3-diphosphoglyceric acid[14,15] LYUBIMOVA AND MATLINA[16] reported that AMP deaminase from rabbit skeletal muscle is activated by ADP and ATP An activation by ATP was also observed in carp muscle enzyme[17] However, in the presence of 0 6 M KCl crystalline AMP deaminase from rabbit skeletal muscle is inhibited by P_i but not activated by ATP and ADP[5-7] Recently SMILEY AND SUELTER[18] showed that the 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 P_i, ADP removes these inhibitions[19]

The different behavior of rabbit and rat enzyme towards the nucleoside di- and triphosphates can be due either to the different source of the two enzymes or to the different purification procedure or to the different assay conditions used (presence or absence of K^+)

In this paper we report the behavior of AMP deaminase from rabbit, guinea pig, pigeon and frog, purified by the same method, towards the nucleoside di- and triphosphates at various concentrations of K^+ The results show that these four enzymes behave similarly to the rat enzyme when assayed in the presence of K^+, the nucleoside triphosphates and P_i inhibit the enzymes and the inhibition is removed by ADP

EXPERIMENTAL PROCEDURE AND RESULTS

Nucleotides were obtained from Sigma Chemical Co or from Boehringer, other chemicals were of reagent grade The AMP deaminase activity was determined spectrophotometrically at 265 or 285 mμ (refs 18, 20), $\Delta\varepsilon_{mM}$ values of 8 86 and 0 23 were used at 265 and 285 mμ, respectively, for the calculation of the μmoles of substrate deaminated The spectrophotometric determinations were carried out in a Zeiss PM Q II spectrophotometer equipped with a Zeiss TE-converter and with a Varian G-2000 recorder The proteins were determined by the microbiuret method using bovine serum albumin as a standard

AMP deaminase from back and leg muscles of rabbit and guinea pig, from leg muscles of frog *(Rana esculenta)* and from wing muscles of pigeon was partially purified according to the method of CURRIE AND WEBSTER[4] up to the $(NH_4)_2SO_4$ fractionation step In Table I the total and the specific activities at the various steps of purification are reported For comparison the purification of the rat enzyme is also reported The incubation mixture for the determination of the enzyme activity contained 2 mM AMP, 50 mM imidazole HCl (pH 6 5) and 0 6 M KCl 1 unit of enzyme

TABLE I

PURIFICATION OF AMP DEAMINASE FROM FROG, PIGEON, GUINEA PIG, RABBIT AND RAT SKELETAL MUSCLE

The enzyme from the various sources was purified according to the method used by CURRIE AND WEBSTER[4] for AMP deaminase from rat skeletal muscle. The dissociation of the enzyme from actomyosin was carried out with phosphate buffer (pH 7.2) at an ionic strength of 0.05 for pigeon, rabbit and rat preparations and at an ionic strength of 0.1 for frog and guinea pig preparations. 1 enzyme unit is the amount of enzyme which catalyzes the deamination of 1 μmole of AMP per min at 25°

Fraction	*Frog*		*Pigeon*		*Guinea pig*		*Rabbit*		*Rat*	
	Total activity (units)	*Specific activity (units/mg)*	*Total activity (units)*	*Specific activity (units/mg)*	*Total activity (units)*	*Specific activity (units/mg)*	*Total activity (units)*	*Specific activity (units/mg)*	*Total activity (units)*	*Specific activity (units/mg)*
Muscle extract*	3850	0.20	10 400	0.40	7800	0.18	15 200	0.80	8100	0.36
Actomyosin preparation	2720	0.35	15 500	0.44	4100	0.25	11 700	1.50	6400	0.42
Phosphate supernatant	390	1.56	2 800	3.90	1900	1.35	5 300	3.80	4900	7.20
$(NH_4)_2SO_4$ fraction	210	3.60	900	6.70	1100	3.00	2 600	12.20	3400	21.20

* From 100 g of skeletal muscle

activity is defined as the amount which catalyzes the deamination of 1 μmole of AMP per min at 25° and 2 mM AMP The purification ratio and the recovery (Table I) of frog, pigeon, guinea pig and rabbit AMP deaminase are lower than those of the rat enzyme Purification procedures were described for AMP deaminase from pigeon and rabbit skeletal muscle[5–8,21], however, we preferred to purify all the enzymes by the same method The most evident differences in the purification procedure of AMP deaminase from the five species have been observed in the phosphate dissociation step Phosphate dissociates the enzyme from actomyosin preparations of all the species examined, but the purification ratio is low as compared to that of the rat The same result was obtained by CURRIE AND WEBSTER[4] with rabbit actomyosin The dissociation with phosphate was also used by SMILEY *et al* [8] in the purification of rabbit AMP deaminase For the dissociation of AMP deaminase from guinea pig and frog actomyosin the ionic strength of the phosphate buffer (pH 7 2) was 0 1 instead of 0 05 as used for the purification of the other enzymes The rise in the ionic strength was necessary to increase the yield of the dissociated enzyme Although the purification and the recovery were lower than those observed for the rat enzyme, the enzymes from frog, guinea pig, pigeon and rabbit skeletal muscle after $(NH_4)_2SO_4$ fractionation were enough purified for the kind of study reported in this paper In fact the enzyme preparations were free of 5′-nucleotidase, myokinase, nucleoside diphosphokinase and ATPase activities and only the guinea pig preparation showed a small adenosine deaminase activity

Like AMP deaminase from rabbit and rat skeletal muscle, the frog, pigeon and guinea pig enzymes are activated by K^+ In Fig 1 the effect of increasing KCl concentrations is reported The activity was assayed in 50 mM imidazole HCl (pH 6 5) and 150 mM tetramethylammonium chloride AMP deaminase from pigeon skeletal

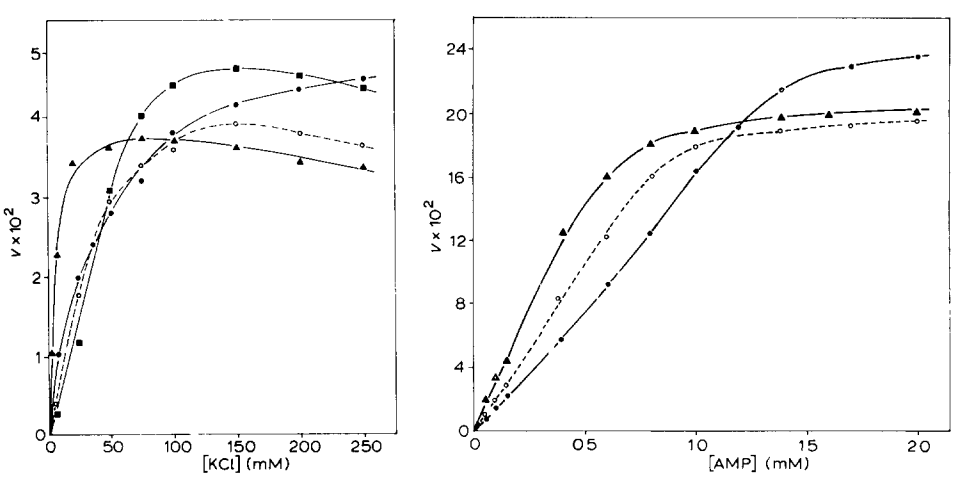


Fig 1 Effect of KCl on skeletal muscle AMP deaminase from frog (●), pigeon (▲), guinea pig (○) and rabbit (■) The reaction mixture contained 100 μM AMP, 50 mM imidazole HCl (pH 6 5) and 150 mM tetramethylammonium chloride Temperature, 20

Fig 2 Effect of AMP concentration on initial velocity of skeletal muscle AMP deaminase from frog (●), pigeon (▲) and guinea pig (○) The reaction mixture contained 50 mM imidazole HCl (pH 6 5) and 150 mM KCl Temperature, 20°

TABLE II

EFFECT OF ADP ON AMP DEAMINASE INHIBITION BY SOME NUCLEOSIDE TRIPHOSPHATES AND P_i AT pH 6 5

Each assay system contained 100 μM AMP, 50 mM imidazole HCl (pH 6 5) and 150 mM KCl Temperature 20° An arbitrary value of 100 is assigned to the activity obtained in the absence of effectors

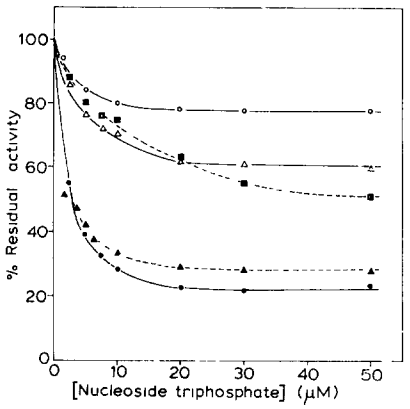
Additions	*Frog*		*Pigeon*		*Guinea pig*		*Rabbit*	
	Without ADP	*With ADP (50 μM)*	*Without ADP*	*With ADP (50 μM)*	*Without ADP*	*With ADP (50 μM)*	*Without ADP*	*With ADP (50 μM)*
None	100	160	100	108	100	137	100	134
ATP (50 μM)	80	143	82	100	91	131	72	130
GTP (10 μM)	72	140	75	105	74	137	31	134
ITP (10 μM)	75	145	89	109	83	124	45	133
P_i (5 mM)	70	125	63	107	62	129	31	133

muscle is almost completely activated at 20 mM KCl while for the rabbit and guinea pig enzymes the highest activity is obtained with 100–150 mM KCl, higher KCl concentrations are necessary for a complete activation of frog enzyme The rat enzyme behaves similarly to pigeon AMP deaminase[19] In the absence of tetramethylammonium chloride higher KCl concentrations are required for the complete activation of the enzyme (Table IV), for the rat enzyme see ref 19

In Fig 2 the substrate–velocity curves of the enzymes from frog, pigeon and guinea pig are reported The curves were obtained in 50 mM imidazole HCl (pH 6 5) and 150 mM KCl The apparent K_m for AMP, calculated on the curves of Fig 2, is 0 35, 0 5 and 0 75 mM for pigeon, guinea pig and frog, respectively The substrate–velocity curve for the rabbit enzyme is similar to that reported by SMILEY AND SUELTER[18]

The effect of some nucleoside di- end triphosphates and P_i on AMP deaminase from frog, pigeon, guinea pig and rabbit is shown in Table II The table also reports the activity in the simultaneous presence of ADP and ATP, GTP, ITP or P_i Assay conditions were 50 mM imidazole HCl (pH 6 5), 150 mM KCl and 100 μM AMP The nucleoside triphosphates and P_i inhibit the enzyme from all the sources while ADP slightly activates AMP deaminase when tested in the absence of inhibitors and removes the inhibition by nucleoside triphosphates and P_i In the absence of K^+, ADP strongly activates the enzyme from all the sources considered

The effect of varying inhibitor concentrations on AMP deaminase activity is reported in Figs 3 and 4 A finite value of inhibition is obtained when the enzyme activity is assayed in the presence of increasing concentrations of nucleoside triphosphates, while an almost complete inhibition is observed in the presence of increasing concentrations of P_i The effect of mixing two inhibitors on the enzyme activity was also tested In Table III the residual activity in the presence of each inhibitor alone or of a mixture of two inhibitors is shown In the same table the product of the residual activities obtained in the presence of each inhibitor alone is reported in parentheses It appears that the simultaneous presence of a nucleoside triphosphate and P_i leads to a residual activity lower than the product of the separate residual activities, while when both ATP and GTP are present in the assay the residual activity is higher



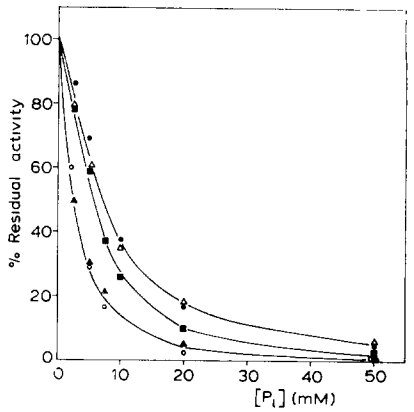


Fig. 3. Effect of GTP on skeletal muscle AMP deaminase from pigeon (■), guinea pig (□) and rabbit (●) and of ATP on skeletal muscle enzyme from frog (○) and rat (▲) at pH 6.5. The reaction mixture contained 100 μM AMP, 50 mM imidazole HCl (pH 6.5) and 150 mM KCl. Temperature, 20°.

Fig. 4. Effect of P_i on skeletal muscle AMP deaminase from frog (●), pigeon (△), guinea pig (■), rabbit (▲) and rat (○). The reaction mixture contained 100 μM AMP, 50 mM imidazole HCl (pH 6.5) and 150 mM KCl. Temperature, 20°. The P_i was added as sodium phosphate buffer (pH 6.5).

The effect of nucleotides was also tested at different KCl concentrations. In fact it was reported that in the absence of monovalent cations ATP activated the rabbit AMP deaminase[18]. Table IV shows the effect of ATP, GTP and ADP on AMP deaminase from guinea pig, rabbit and rat at four concentrations of KCl. The assays were carried out in 100 μM AMP, 50 mM imidazole HCl (pH 6.5) and 50, 100, 150 or 200 mM KCl. An arbitrary value of 100 is assigned to the activity obtained in the

TABLE III

INHIBITION OF AMP DEAMINASE BY PAIRS OF INHIBITORS (NUCLEOSIDE TRIPHOSPHATES OR P_i)

Each assay system contained 100 μM AMP, 50 mM imidazole HCl (pH 6.5) and 150 mM KCl. Temperature, 20°. An arbitrary value of 100 is assigned to the activity obtained in the absence of inhibitors. The products of the residual activities obtained in the presence of each inhibitor alone are reported enclosed in brackets. For example there was 75% residual activity in the presence of 10 μM GTP and 80% in the presence of 2.5 mM P_i for AMP deaminase from pigeon skeletal muscle: the product of the residual activities reported in parentheses is (0.75 × 0.80) × 100 or 60%.

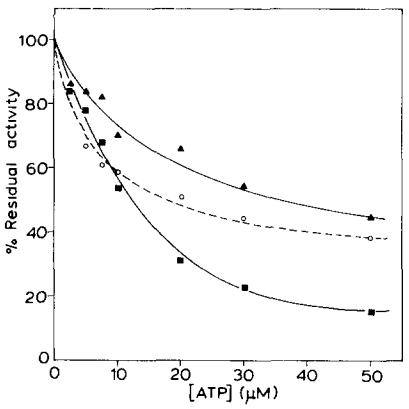
Additions	*Pigeon*	*Guinea pig*	*Rabbit*
None	100	100	100
ATP (50 μM)	82	91	72
GTP (10 μM)	75	74	31
ITP (10 μM)	89	83	45
P_i (2.5 mM)	80	79	50
P_i (5.0 mM)	63	62	31
ATP (50 μM) + P_i (5 mM)	44 (52)	38 (56)	14 (22)
GTP (10 μM) + P_i (2.5 mM)	43 (60)	46 (58)	8 (15)
ITP (10 μM) + P_i (2.5 mM)	64 (71)	46 (65)	12 (22)
ATP (50 μM) + GTP (10 μM)	81 (62)	89 (67)	48 (22)

TABLE IV

EFFECT OF ATP, GTP AND ADP ON SKELETAL MUSCLE AMP DEAMINASE FROM VARIOUS SPECIES AT DIFFERENT KCl CONCENTRATIONS AND AT pH 6.5

Each assay system contained 100 μM AMP, 50 mM imidazole HCl (pH 6.5) and the indicated KCl concentrations. Temperature, 20°. An arbitrary value of 100 is assigned to the activity obtained in the presence of 50 mM KCl and in the absence of effectors.

KCl concn. mM	Additions	Guinea pig				Rabbit				Rat			
		None	ATP (50 μM)	GTP (50 μM)	ADP (50 μM)	None	ATP (50 μM)	GTP (50 μM)	ADP (50 μM)	None	ATP (50 μM)	GTP (50 μM)	ADP (50 μM)
50		100	100	68	455	100	237	33	—	100	42	4	280
100		180	240	140	500	206	282	53	620	235	68	8	240
150		350	315	240	485	450	325	98	605	250	75	11	230
200		460	420	320	435	570	430	195	560	230	96	12	220



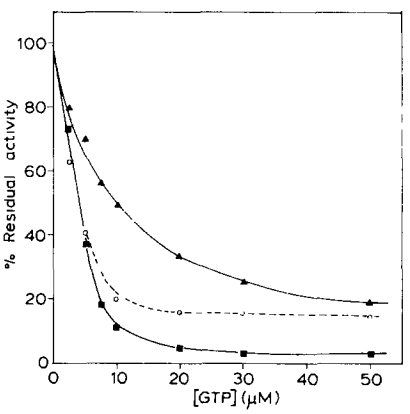


Fig 5 Effect of ATP on skeletal muscle AMP deaminase from guinea pig (▲), rabbit (○) and rat (■) at pH 7 1 The reaction mixture contained 100 μM AMP, 50 mM imidazole HCl (pH 7 1) and 150 mM KCl Temperature, 20°

Fig 6 Effect of GTP on skeletal muscle AMP deaminase from guinea pig (▲), rabbit (○) and rat (■) at pH 7 1 The reaction mixture contained 100 μM AMP, 50 mM imidazole HCl (pH 7 1) and 150 mM KCl Temperature, 20°

presence of 50 mM KCl without additions The enzymes from the three sources examined show a different behavior towards ATP Guinea pig and rabbit enzymes are activated by ATP when assayed at 50 and 100 mM KCl while ATP inhibits at 150–200 mM KCl The enzyme from rat is inhibited by ATP at each KCl concentration tested GTP inhibits the enzymes in every case The effect of ITP (not reported in the table) is similar to that of GTP At 50–100 mM KCl concentration ADP is a better activator than ATP while at 200 mM KCl ADP is without effect on the enzyme activity

The extent of inhibition by nucleoside triphosphates depends on the pH of the incubation mixture In fact in 50 mM imidazole HCl (pH 7 1) containing 150 mM KCl (Table V) the inhibition by ATP and GTP of skeletal muscle AMP deaminase

TABLE V

EFFECT OF ADP ON AMP DEAMINASE INHIBITION BY ATP, GTP, P_i AND CREATINE PHOSPHATE AT pH 7 1

Each assay system contained 100 μM AMP, 50 mM imidazole HCl (pH 7 1) and 150 mM KCl Temperature, 20° An arbitrary value of 100 is assigned to the activity obtained in the absence of effectors

Additions	*Guinea pig*		*Rabbit*		*Rat*	
	Without ADP	*With ADP (50 μM)*	*Without ADP*	*With ADP (50 μM)*	*Without ADP*	*With ADP (50 μM)*
None	100	180	100	195	100	120
ATP (50 μM)	44	157	37	180	16	68
GTP (10 μM)	52	150	18	167	12	65
P_i (5 mM)	58	147	43	158	38	87
Creatine phosphate (5 mM)	63	135	34	145	30	67

TABLE VI

EFFECT OF ATP, GTP AND ADP ON SKELETAL MUSCLE AMP DEAMINASE FROM VARIOUS SPECIES AT DIFFERENT KCl CONCENTRATIONS AND AT pH 7.1

Each assay system contained 100 μM AMP, 50 mM imidazole HCl (pH 7.1) and the indicated KCl concentrations. Temperature, 20°. An arbitrary value of 100 is assigned to the activity obtained in the presence of 50 mM KCl and in the absence of effectors.

KCl concn (mM)	*Additions*	*Guinea pig*				*Rabbit*				*Rat*			
		None	*ATP (50 μM)*	*GTP (50 μM)*	*ADP (50 μM)*	*None*	*ATP (50 μM)*	*GTP (50 μM)*	*ADP (50 μM)*	*None*	*ATP (50 μM)*	*GTP (50 μM)*	*ADP (50 μM)*
50		100	59	25	497	100	81	40	733	100	22	7	356
100		296	194	66	1240	170	96	47	768	243	31	8	325
150		750	326	128	1300	538	192	67	1030	257	38	8	291
200		943	314	113	1220	731	272	76	940	261	68	12	253

from guinea pig, rabbit and rat is higher than at pH 6 5, while the inhibition by P_i is about the same (see Table II for guinea pig and rabbit and ref 19 for rat), creatine phosphate is effective as P_i in the inhibition of the enzyme A finite value of inhibition is obtained when the enzyme is assayed in the presence of increasing concentrations of ATP and GTP but the extent of inhibition is higher at pH 7 1 than at pH 6 5 (Figs 3, 5 and 6) However, at pH 7 1 and at 50–100 mM KCl, in contrast to pH 6 5, no activation by ATP of rabbit and guinea pig enzyme is observed (Table VI) ADP removes the inhibition by ATP, GTP and P_i also at pH 7 1 (Table V)

DISCUSSION

The comparative study on the behavior of skeletal muscle AMP deaminase from five different species towards K^+, nucleotides and P_i shows that the enzymes, purified by the same method, have some fundamental common characteristics which suggest a common regulatory mechanism, however, some minor differences were also observed K^+ activates the enzyme from all the sources examined but lower KCl concentrations are necessary for the complete activation of rat[19] and pigeon enzyme KCl concentration and pH influence the behavior of AMP deaminase towards ATP and this phenomenon may explain the different behavior of AMP deaminase towards this nucleotide observed by various authors[5–7,16–19]

The enzyme is inhibited to a limited extent by nucleoside triphosphates These compounds probably have an identical site of binding on the enzyme molecule In fact the residual activity obtained in the simultaneous presence of two nucleoside triphosphates is higher than the product of the residual activities obtained with each inhibitor alone ATP which inhibits the enzyme to a minor extent than GTP, partially removes the inhibition by GTP In the presence of P_i and nucleoside triphosphates the residual activities are lower than the product of the residual activities obtained in the presence of one inhibitor alone This phenomenon was observed with other enzymes and was explained assuming distinct sites of binding for the inhibitors[22,23] This is probably true also for AMP deaminase in which two different sites, one for P_i and the other for the nucleoside triphosphate, can be hypothesized However, a more detailed kinetic study, which is not the aim of the present work, will be necessary to elucidate this point

Nucleoside triphosphates, ADP, creatine phosphate and P_i at concentrations near or lower than those found in muscle affect the AMP deaminase activity from five different species, when assayed at pH 7 1 and at physiological concentrations of K^+ (100–150 mM) and AMP (100 μM) All these compounds appear to be significant in the regulation of the activity *in vivo* The ATP, creatine phosphate and P_i concentrations in muscle and the fact that a great part of ADP is bound to muscle proteins[25] suggests that in resting muscle AMP deaminase is at least partially, if not completely, inhibited In the conditions in which free ADP accumulates in muscle, it removes the inhibition by P_i and ATP and the enzyme results activated On this aspect muscle AMP deaminase seems to help the myokinase reaction in utilizing the high-energy phosphate of ADP for muscular contraction and in maintaining a high ATP/ADP ratio[26] (myosin ATPase is inhibited by ADP[27]) In fact muscle myokinase, specific for adenine nucleotides, is inhibited by AMP which has a K_i value lower than K_m for ADP[28] Such a kind of regulation of AMP deaminase could explain the ob-

servations that IMP accumulates in muscle subjected to strong tetanic contractions and is reaminated to AMP during the aerobic recovery period, while it is impossible to demonstrate a deamination of AMP in resting muscle or during a moderate muscular work[29–35] Although it cannot be excluded that in these conditions a deamination of AMP occurs, in fact if the rate of IMP reamination is higher than or equal to the rate of AMP deamination no accumulation of IMP is observed, however, because the capacity of the muscle to reaminate IMP is much less than its potential AMP deaminating capacity[36–38], it must be concluded that AMP deaminase is inhibited to a great extent in resting muscle or during moderate muscular work

The studies carried out with poisoned muscles in which ATP synthesis is completely or partially inhibited are useful to confirm that IMP is produced when ADP tends to accumulate in muscle LANGE[39] and HERMANS[40] with muscles poisoned with iodoacetate and MARÉCHAL AND BECKERS-BLEUKX[41] with frog muscles poisoned with 1-fluoro-2,4-dinitrobenzene observed that the ATP used for muscular contraction is almost quantitatively replaced by IMP, however, CAIN AND DAVIES[26] reported that during the contraction of frog muscles poisoned by 1-fluoro-2,4-dinitrobenzene ATP is stoichiometrically converted to ADP and AMP These contradictory findings can be explained assuming that in the poisoned muscles studied by the different authors AMP deaminase is inactivated by this drug to a different extent AMP deaminase, in fact, from frog, guinea pig and rat is inactivated by 1-fluoro-2,4-dinitrobenzene (G RONCA, unpublished observation)

Besides nucleoside triphosphates, ADP, creatine phosphate and P_i other factors may be significant in the regulation of AMP deaminase *in vivo* As it appears from the pH dependence of enzyme activity[5–7,17] and of nucleoside triphosphate inhibition, a decrease of pH in muscle results in increasing enzyme activity Variations of pH during muscle activity were described[42] It was reported that lactate is an inhibitor of rat AMP deaminase and Mg^{2+} and Ca^{2+} influence both ADP activation and P_i and nucleoside triphosphate inhibition[19] The skeletal muscle enzyme from all the five species examined is inhibited by 2,3-diphosphoglyceric acid (G RONCA, unpublished observation) as AMP deaminase from erythrocytes[14] These data suggest a more complex regulation of muscle AMP deaminase than that discussed above

ACKNOWLEDGMENT

The authors thank Miss P Galbani for her skilled technical assistance This work was supported by the Consiglio Nazionale delle Ricerche, Italy

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