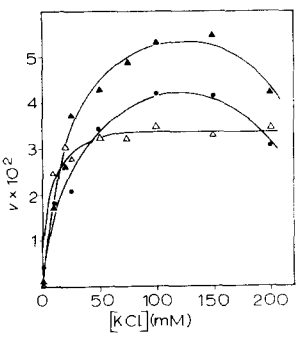
BBA 63346

Muscle AMP aminohydrolase. I. Some regulatory properties of rat skeletal muscle enzyme

AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) is widely distributed in animal tissues; skeletal muscle is the richest source[1]. The enzyme has been purified from rabbit and rat muscle[2-4] and from calf brain[5]. The kinetic properties vary depending on the source of the enzyme. Calf brain AMP deaminase is activated by Li^+, Na^+, K^+ and ATP[5]; the enzyme from various tissues of the rat was found to be activated by ATP and inhibited by GTP[6]. No activation by ATP was observed by LEE[2] in rabbit muscle AMP deaminase; recently, however, SMILEY AND SUELTER[7] demonstrated an activation by ATP and ADP in the absence of K^+ or Na^+.

In this paper some regulatory properties of AMP deaminase from rat muscle are reported. The enzyme was purified according to CURRIE AND WEBSTER[4] with some minor modifications. The modified method yielded a homogeneous enzyme having a specific activity of 510 μmoles of AMP deaminated per min per mg of protein at 20°



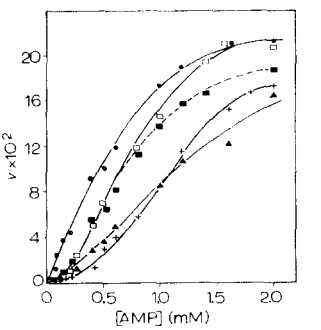


Fig. 1. Effect of KCl on AMP deaminase activity. The reaction mixture contained 100 μM AMP, 0.05 M imidazole·HCl (pH 6.5) (●) or imidazole buffer containing 0.1% bovine serum albumin (▲) or 0.2 M tetramethylammonium chloride (△).

Fig. 2. Effect of some anions on AMP deaminase activity. The reaction mixture contained 100 mM K^+, 0.1% bovine serum albumin, 0.05 M imidazole·HCl buffer (pH 6.5) and 40 mM citrate (▲) or 100 mM Cl^- (●), acetate (□), lactate (■) or lactate ions *plus* 5 mM potassium phosphate (pH 6.5) (+).

and with 2 mM AMP. The final enzyme preparation was free from any detectable myokinase, 5'-nucleotidase and ATPase activity. The enzyme activity was followed spectrophotometrically at 265 or 285 mμ according to KALCKAR[8]. For the calculation, $\Delta\varepsilon_{mM}$ values of 8.86 and 0.23 were used at 265 and 285 mμ, respectively.

The enzyme activity was tested in many different buffers. The highest activity was obtained using the following buffer: 0.05 M imidazole·HCl (pH 6.5), 0.1% bovine serum albumin and 100 mM KCl. Crystalline serum albumin was dialysed for 2 days against imidazole·HCl buffer (pH 6.5). In Fig. 1 the effect of increasing concentrations of KCl on the enzyme activity is reported. The highest activity was obtained with 100–150 mM KCl when imidazole or albumin–imidazole buffer was used. With imida-

zole buffer and 200 mM tetramethylammonium chloride, an almost complete activation was obtained with 20 mM KCl. With citrate, succinate or cacodylate buffer under the same experimental conditions, lower values of the enzymatic activity were obtained.

The effect of the substrate concentration on the initial velocity was studied. With albumin–imidazole buffer in the presence of 100 mM KCl, the enzyme gives normal hyperbolic kinetics; the K_m for AMP is 0.5 mM (Fig. 2). However when Cl^- is substituted by organic anions like lactate, acetate or citrate, the enzyme follows sigmoid kinetics (Fig. 2). With acetate the v_{max} is the same as that observed with Cl^-, while with lactate and citrate the v_{max} value slightly decreases. The apparent K_m for AMP is 0.7 mM with acetate and lactate and 1.2 mM with citrate. The Hill plots from the data of Fig. 2 give slopes of 1.1, 1.8, 1.7, 1.3 for Cl^-, acetate, lactate and citrate, respectively.

The effect of ATP, ADP, GTP and P_i on the enzyme activity is reported in Table I. The incubation mixtures contained albumin–imidazole buffer (pH 6.5), 100 mM K^+ and 100 mM Cl^-, lactate or acetate or 40 mM citrate anions. When Cl^-,

TABLE I

EFFECT OF ANIONS, P_i AND NUCLEOTIDES ON AMP DEAMINASE

Each assay system contained 100 μM AMP, 0.05 M imidazole·HCl (pH 6.5), 0.1% bovine serum albumin, 100 mM K^+, 100 mM Cl^-, acetate or lactate, or 40 mM citrate ions. The arbitrary value of 100 is assigned to the activity obtained with Cl^- and without additions.

Additions	*Relative velocity in the presence of*			
	Cl⁻	*Acetate*	*Lactate*	*Citrate*
None	100	39	38	27
ADP (25 μM)	100	62	65	27
ATP (50 μM)	27	21	20	20
GTP (1.5 μM)	15	3	3	25
P_i (1.5 mM)	60	11	13	16

acetate or lactate were present, ATP and GTP inhibited the enzyme activity, while with citrate they were without effect. GTP is a much more powerful inhibitor than ATP. P_i inhibited with all the anions used. ADP activated the enzyme inhibited by acetate and lactate.

With ATP, GTP or P_i in the presence of Cl^-, the substrate–velocity curves are sigmoid; in the presence of lactate or acetate, the sigmoidicity is increased. In Fig. 2 a curve obtained with 5 mM P_i in the presence of 100 mM lactate is reported; the Hill plot gives a slope of 2.2.

The effect of Ca^{2+} and Mg^{2+} on the enzyme activity has also been tested. Ca^{2+} and Mg^{2+} added as chlorides, slightly activate the enzyme inhibited by lactate. They also remove the ADP activation and the GTP inhibition. The inhibition by ATP is increased at 5 mM Mg^{2+} and Ca^{2+} but is removed at higher concentrations. In Table II the effect of Mg^{2+} is reported. Similar results have been obtained with Ca^{2+}.

UTP and CTP inhibit the enzyme and the inhibition is removed by 5 mM Ca^{2+} and Mg^{2+}. The effect of GDP is similar to that of ADP. ADP and GDP remove the inhibition by nucleoside triphosphates. The inhibition by organic anions and by

nucleoside triphosphates and the activation by nucleoside diphosphates were also observed in the absence of albumin in the incubation mixtures.

From these data it appears that rat muscle AMP deaminase in the presence of 100 mM K^+ is affected by many compounds. Nucleoside triphosphates, P_i and concentrations of lactate in the same range as that found during the anaerobic contraction of muscle[9], inhibit the enzyme. ADP and GDP counteract this inhibition. Ca^{2+} and Mg^{2+} remove the activation and inhibition by nucleotides probably through the formation of inactive nucleotide–divalent cation complexes.

TABLE II

EFFECT OF Mg^{2+} AND OF SOME NUCLEOTIDES ON AMP DEAMINASE INHIBITED BY LACTATE

Each assay system contained 100 μM AMP, 0.05 M imidazole·HCl (pH 6.5), 0.1% bovine serum albumin and 100 mM potassium lactate. An arbitrary value of 100 is assigned to the activity obtained in the absence of nucleotides and Mg^{2+}. In the presence of 100 mM KCl instead of potassium lactate the relative initial velocity was 270.

Additions	*Relative velocity in the presence of* Mg^{2+}			
	0 mM	*5 mM*	*12.5 mM*	*25 mM*
None	100	140	170	175
ADP (50 μM)	250	190	185	175
ATP (50 μM)	65	30	60	125
GTP (1.5 μM)	15	100	165	175

All these compounds may be significant in the regulation of the *in vivo* enzyme activity. In fact when the ratios of nucleoside triphosphates to nucleoside diphosphates are high, the enzyme should be at least partially inhibited; at lower ratios, the enzyme should be activated. However, the concentrations of nucleotides bound to divalent cations should be taken into account and excluded from the ratios (the only exception might be ATP which is a more powerful inhibitor in the presence of 5 mM Mg^{2+} or Ca^{2+}). The nucleotides bound to the muscle proteins should also be excluded[10]. On the basis of the results reported above, changes of the enzyme activity in the muscle are conceivable without changes in the ratio between the total (bound and not bound) nucleoside tri- and diphosphates.

More detailed studies on the regulatory properties of the enzyme are in progress.

This work was supported by Italian CNR.

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Received July 15th, 1968

Biochim. Biophys. Acta, 167 (1968) 626–629

BBA 63351

The identity of vitamin A esterase activity of rat pancreatic juice

It is well documented that homogenates from the pancreas and other tissues catalyze the hydrolysis of vitamin A esters (see ref. 1). The identity of the enzyme(s) involved has, however, not been established and it has in fact been suggested that there is more than one enzyme involved in this reaction in the different organs. The basis for this suggestion was the finding that sodium taurocholate had different effects on this reaction depending on the enzymatic source[1]. The vitamin A esterase activity of extract from the pancreatic gland was low in the absence of bile salt but strongly stimulated by its presence. As vitamin A esters are insoluble in water and disperse as an emulsion, it was conceivable that they could be substrate for pancreatic lipase (glycerol-ester hydrolase, EC 3.1.1.3). It also seemed possible, however, that vitamin A esters in the presence of bile salt could be substrate for another enzyme (carboxylic ester hydrolase) recently found in this laboratory to split a variety of water-soluble or bile salt-dispersed esters including monoglycerides with long-chain fatty acids[2–4].

The experiments of the present investigation were undertaken to identify the vitamin A esterase activity of rat pancreatic juice. Vitamin A palmitate was a gift of Hofmann-La Roche, Basel, Switzerland, and was freed from vitamin A by thin-layer chromatography.

Emulsified vitamin A palmitate was produced as follows. 10 μmoles vitamin A palmitate dissolved in 2 mg hexadecane were dispersed per ml 0.05 M Tris–HCl buffer (pH 8.6) by sonication using a Branson sonifier. Bile salt dispersed vitamin A palmitate was obtained by insonating 10 μmoles vitamin A palmitate per ml of a solution 6 mM in sodium taurodeoxycholate in 0.15 M phosphate buffer (pH 6.3). The taurodeoxycholate was synthesized according to HOFMANN[5] and at least 97% pure by thin-layer chromatography.

1-ml aliquots of the substrates were mixed with 200 μl of enzyme source and incubated for 1 h at 37°. After the end of the incubation period the emulsified substrate incubation was mixed with 1 ml 1 M acid phosphate (to decrease the pH to enable extraction of the fatty acids). Both incubations were then mixed with 3 times their volume of a mixture of equal parts heptane–diethyl ether and ethanol and shaken. After separation of the phases, aliquots of the upper phases were taken, evaporated to dryness, dissolved in ethanol and titrated with 0.02 M NaOH using Nile blue as indicator.

Lyophilized rat pancreatic juice was separated by gel filtration or by ion-exchange chromatography as earlier described[2,3] and the fractions tested for activity