ISOZYMES OF AMP DEAMINASE IN RED AND WHITE SKELETAL MUSCLES

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1. Introduction

A general differentiation in the metabolic pattern of white and red muscles is classically known [1]. Recently differences in these two types of muscles have been observed also at a molecular level with the demonstration of different types of isoenzymes of myosin ATPase [2], lactate dehydrogenase [3] and phosphorylase kinase [4].

The activity of adenylate deaminase of skeletal muscle is very high in comparison with that of all other tissues, including heart and smooth muscles [5]. Our previous distribution data showed that white muscles from different species deaminate AMP four to ten times more effectively than red muscles [6]. These differences can be due either to a different content of a single form of AMP deaminase or to the existence of isoenzymes with distinct kinetic properties, as observed for the above mentioned key regulatory enzymes.

The results of this paper give strong evidence that different types of adenylate deaminase exist in white and red muscles and we demonstrate that, besides quantitative, also qualitative dissimilarities exist.

2. Materials and methods

Muscle obtained from adult animals shortly after killing were freed of fat and tendons and stored at −30°C until the time of use; samples were finely minced and homogenized in 5 vol of ice cold 20 mM potassium phosphate buffer containing 0.1 M KCl and 1 mM 2-mercaptoethanol pH 7.0. After standing for 20 min the homogenate was centrifuged for 30 min at 20 000 *g*.

The supernatant was applied to a column of cellulose phosphate (0.9 × 10 cm) equilibrated with the same buffer; after washing with 50 ml of 20 mM phosphate buffer pH 7.0 containing 0.2 M KCl and 1 mM 2-mercaptoethanol, a linear gradient of KCl in the same buffer was applied: the reservoir and the mixing chambers each contained 50 ml of the appropriate solutions and fractions of about 1 ml were collected. AMP deaminase activity was routinely assayed spectrophotometrically adding samples of the eluate to the reaction mixture containing 50 mM imidazole HCl pH 6.5, 500 mM KCl and 0.1 mM AMP. The fractions used for kinetical studies were previously dialysed against two changes of 80 vol each of 1.0 M KCl containing 1 mM potassium phosphate pH 7.0. Effect of substrate concentration on the initial rate was determined at 20°C in 50 mM imidazole HCl pH 6.5 or 7.0, 100 mM KCl, following the absorbance decrease at 265 nm or the increase at 285 nm, depending on substrate concentration. All the spectrophotometric measurements were carried out with a Zeiss PM QII. One unit of enzyme activity, calculated according to [7], is defined as 1 μmol of AMP deaminated per minute. 5′ AMP was from Sigma; all other reagents were of analytical grade.

3. Results and discussion

Ogasawara et al. [8] reported recently that isoenzymes or rat AMP deaminase exist in several tissues, while kidney and muscle yielded only one form. Concerning muscle, previously Birnbaum et al. [9]

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reported the resolution of calf enzyme into two forms, and Zielke and Suelter [10] observed, during the purification of rabbit enzyme, the appearence of a small additional peak of activity which, in our experience, is also constantly present during the purification of rat muscle AMP deaminase (unpublished observation). By chromatography on cellulose phosphate carried out with the same procedure as described by Ogasawara, we confirmed the presence of only one form of adenylate deaminase in extracts of rat mixed skeletal muscle; however, with analysis of single muscles, multiple forms have been noted: when a linear gradient between 0.2 and 1.0 M KCl is applied (fig.1) the enzyme of white muscle (extensor digitorum longus) is eluted as a single asymmetrical peak at 0.6 M KCl (form B). This form of the enzyme is present in lower quantities also in red muscle (soleus), where an additional peak (form A) is released from the resin by a lower ionic strength (0.4 M KCl).

Because of the above reported evidence for the existence of clearcut differences between red and white muscle AMP deaminase, we have shifted our attention towards rabbit where muscles present a deep predominance of red or white fibers and the activity levels of adenylate deaminase are even more differentiated than in rat [6]. The data relative to rabbit enzyme are presented in fig.2; the enzyme from anconaeus muscle yields two peaks: the position of the former corresponds with that of the heart, form A, while the second possesses the same elution characteristics of the single form B of the white muscle (biceps femoris). Under the same experimental conditions of fig.2, we have studied the red muscle soleus and semitendinosus and the white muscle extensor digitorum longus and tibialis anterior: the chromatographic patterns are superimposable with those of anconaeus and biceps respectively.

Although in both rat and rabbit the chromatograpic pattern is essentially similar, differences are evident

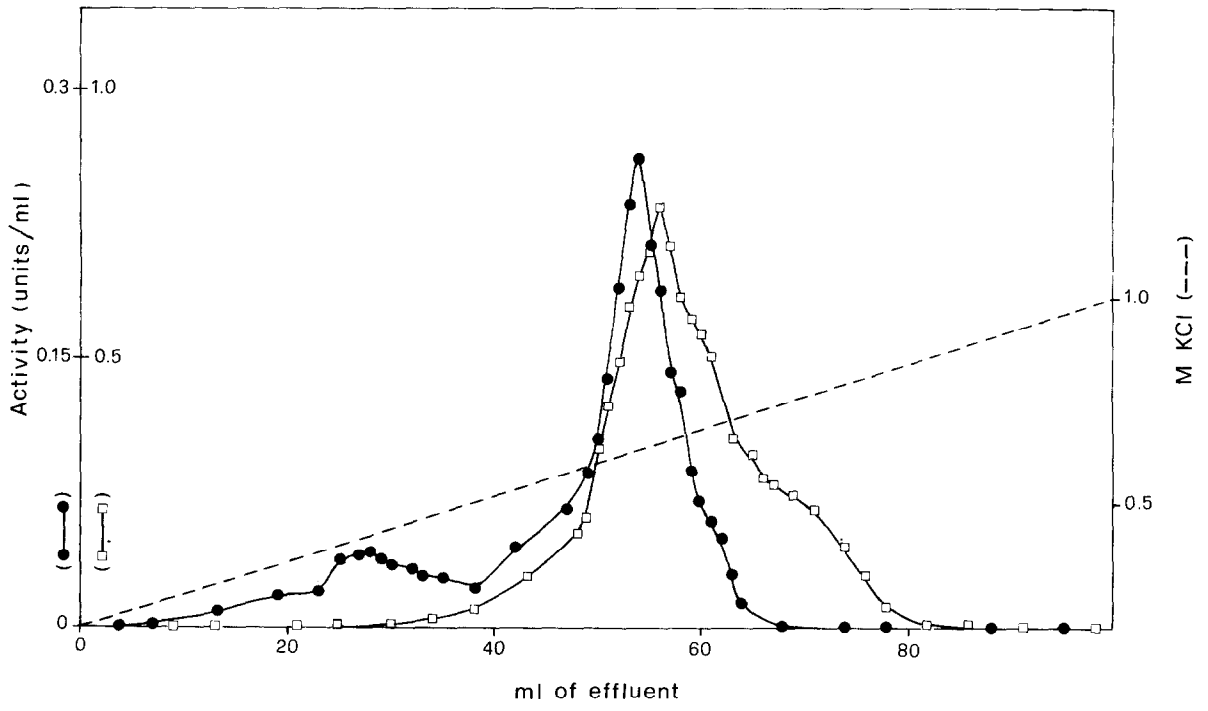

Fig.1. Phosphocellulose column chromatography of AMP deaminase from extracts of rat skeletal muscles: 3.4 g of soleus muscle (•) and 4.2 g of extensor digitorum longus (□) were used. The experiments were performed as described under Materials and methods.

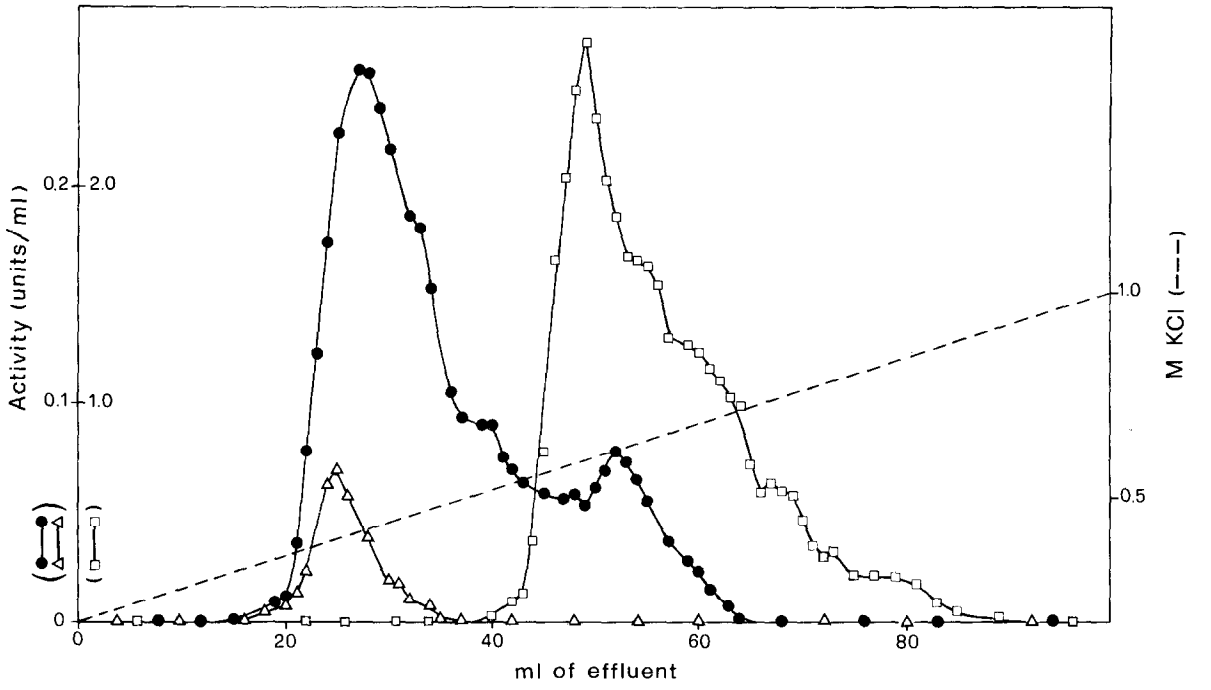

Fig.2. Phosphocellulose chromatogram of AMP deaminase from 2 g of rabbit cardiac muscle (△), 2.3 g of anconaeus (●) and 5 g of biceps femoris (□). The experiments were performed as described under Materials and methods.

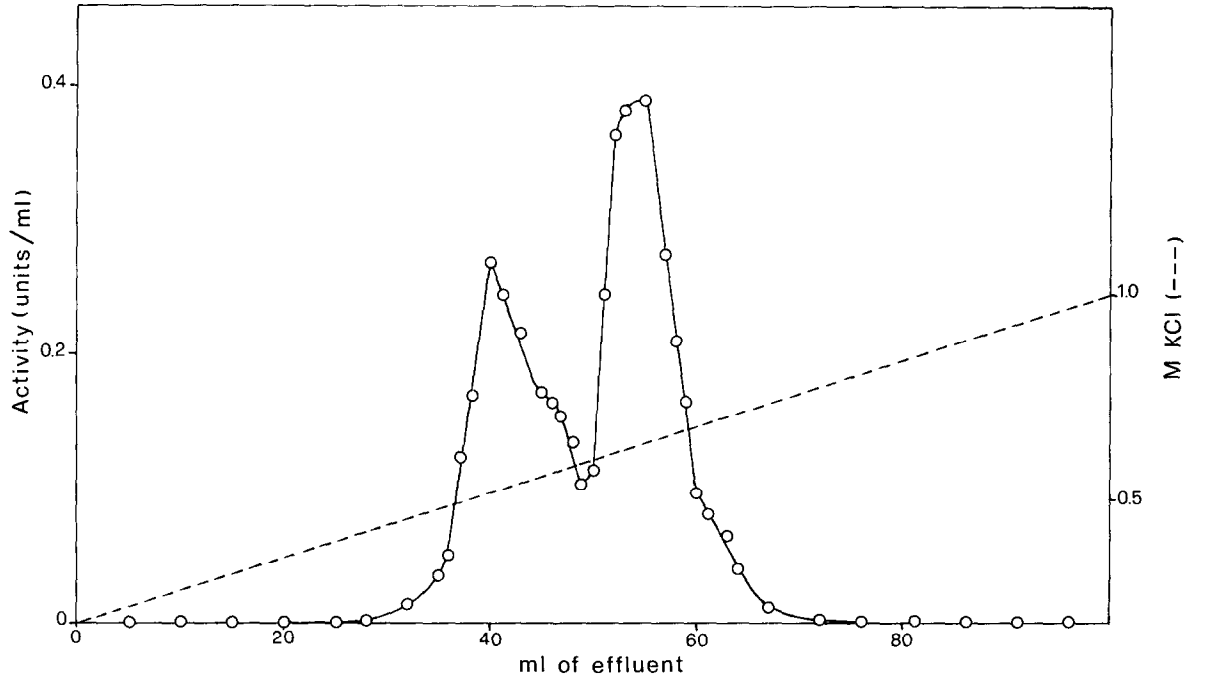

Fig.3. Phosphocellulose chromatogram of AMP deaminase from 4 g of human pectoralis maior. The experiments were performed as described under Materials and methods.

between them: in the red muscle, form A is quantitatively a minor component (about 20% of the total activity) in the rat, while in rabbit it accounts for about 70%. In addition, the asymmetry of the elution peaks, which may indicate the presence of multiple subforms, is much more accentuated in the rabbit muscles. The chromatograms of rabbit white muscles reveal the constant appearence of three shoulders of form B: they do not represent artifacts due to limited proteolysis since experiments performed with fresh tissues, without freezing, have given similar patterns. The existence of subforms is further supported by the results obtained with the human muscle pectoralis maior (fig.3), which presents two sharp peaks in the elution region of isoenzymes B (0.52 and 0.62 M KCl, respectively). Form A and B of rabbit muscle adenylate deaminase have been used for kinetic studies and the results are summarized in table 1: under the experimental conditions adopted, both isoenzymes present a Hill coefficient greater than 1, which indicates positive cooperativity towards substrate. A shift of the pH values from 7.0 to 6.5 affects the enzymes differently, since form A is quite unsensitive, while n_H for form B decreases from 1.9 to 1.4; independently of the pH, the $S_{0.5}$ values are higher for form A than for form B. The data of this paper demonstrate the existence of at least two AMP deaminase isozymes in striated muscle. We must point out that their distribution is quantitatively different in the various types of muscles examined. In the heart, the whole activity can be accounted for by form A, which is still present in the red muscles characterised by a prevalently aerobic metabolism. White muscles, which are mainly composed of glycolytic fibers, present exclusively isozyme B which accounts for the greatly increased activity. The interest of these observations is connected with the statement that AMP deaminase increases during development from the foetal to the adult type of muscle [11], which is characterised by a more intense anaerobic metabolism as demonstrated by the distribution data of activity [12] and isoenzymatic pattern [3] of lactate dehydrogenase. Previous in vitro studies had shown the existence of a purine nucleotide cycle [13] in muscle, which is correlated with oscillations of the glycolytic pathway [14]. A narrow link between phosphofructokinase and AMP deaminase in white muscle of rat, but not in red muscles, has been demonstrated in a very recent report [15]. The results of our work establish an enzymological basis for this correlation between anaerobic metabolism and AMP deaminase activity in white muscle.

The isoenzymatic pattern of AMP deaminase is also consistent with the possible biological function of this enzyme in protecting cells against sudden changes of energy charge [16], which occur to different extent during the activity of the various types of muscles. The hypothesis of a different role of AMP deaminase isoenzymes is strengthened by the recent evidence of different sensitivity of white and red muscle enzyme to a restricted protein diet [17]. We are presently working on the molecular basis of the differences between isozymes A and B.

Table 1
Kinetic parameters for AMP deaminase isozymes

	Form A		Form B	
pH	6.5	7.0	6.5	7.0
n_H	1.3	1.3	1.4	1.9
$S_{0.5}$	0.9	1.3	0.5	0.4

The effect of substrate on the initial was determined as described under Materials and methods, using as enzyme source the pooled and dialysed fractions corresponding to the isozymes A and B. The $S_{0.5}$ values are reported as mM AMP.

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