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DIFFERENTIAL RESPONSE OF AMP DEAMINASE ISOZYMES TO CHANGES IN THE ADENYLATE ENERGY CHARGE

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Summary

AMP deaminase has been prepared from white skeletal muscle fibers, red skeletal muscle fibers, cardiac muscle and liver. The isozymes from skeletal muscle, cardiac muscle and liver can be readily distinguished from one another by the shape of the adenylate energy charge response curve. However, the enzyme prepared from different skeletal muscles which consists of predominately red or white fibers are indistinguishable from one another by this criterion.

AMP deaminase is believed to be important in the regulation of several intracellular activities including the stabilization of the adenylate energy charge (1) and the interconversion of adenine, guanine and inosine nucleotides through its participation in the purine nucleotide cycle (2-4). Previous studies in the rat (5) have demonstrated the existence of tissue-specific isozymes which differ from one another with respect to their chromatographic, kinetic and immunological properties. The forms of this enzyme found in skeletal muscle, liver and cardiac muscle have been designated as isozymes A, B and C respectively. In addition to the identification of these three distinct and tissue-specific isozymes, Raggi et al. (6) have further fractionated skeletal muscle AMP deaminase into two forms which differ in both their chromatographic and kinetic properties. Moreover, a difference in the distribution of these two forms of the enzyme in various types of muscle was observed. These observations have led Raggi et al. (6) to suggest that different isozymes of AMP deaminase exist

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in white and red muscle fibers and that the type found in red muscle is identical to the isozyme found in cardiac muscle.

In this communication, we have prepared the enzyme from red and white muscle, as well as from cardiac muscle and liver, and have examined these preparations for differences in their response to changes to the adenylate energy charge. The results of these experiments indicate that AMP deaminase from both red and white skeletal muscle fibers can be readily distinguished from the enzyme found in either cardiac muscle or liver on the basis of the profile generated by measuring the activity as a function of energy charge. However, the two chromatographically distinguishable forms of AMP deaminase found in red and white skeletal muscle fibers are identical to one another by this criterion.

MATERIALS AND METHODS

Materials. Tissues were taken from an adult rabbit shortly after killing and were freed of fat and connective tissue. The skeletal muscles taken for these experiments were the anconeus (predominantly red) and the biceps femoris (predominantly white).

Purification of the Enzyme. The method used for the purification of rabbit liver AMP deaminase was identical to that reported by Ogasawaea et al. (7). The purification of the enzyme from skeletal and cardiac muscle followed the procedure described by Raggi et al. (6) with a few minor modifications. Samples of muscle were minced and homogenized in 2.5 volumes of cold buffer containing 20 mM potassium phosphate, 0.1 M KCl and 1 mM 2-mercaptoethanol (pH 7.0). After standing on ice for 50 min, the homogenate was centrifuged at 20,000 x g for 30 min. The supernatant was absorbed onto a column (1 x 15 cm) of cellulose phosphate which had been previously equilibrated with 40 volumes of 20 mM potassium phosphate buffer (pH 7.0) containing 0.2 M KCl and 1 mM 2-mercaptoethanol. After loading the sample, the column was washed with 100 ml of the equilibration buffer prior to elution. The enzyme was eluted with a linear gradient consisting of 50 ml each of 0.2 M KCl and 2.0 M KCl both in buffer containing 20 mM potassium phosphate and 1 mM 2-mercaptoethanol (pH 7.0).

Enzyme Assay. Unless otherwise indicated, enzyme activity was determined spectrophotometrically using a Gilford 240 spectrophotometer equipped with an expanded scale recorder. The amount of AMP converted to IMP was monitored by following the increase in absorbance at 285 nm as a function of time. The absorbance changes were converted to units of

concentration using a Δ_{mM} of 0.23 (8). The standard assay used in monitoring the enzyme from cardiac and skeletal muscle was performed in cuvettes of 1 cm light path and with a total volume of 1.0 ml containing 50 mM imidazole-HCl, 100 mM KCl and 2 mM AMP (pH 6.5). For monitoring the enzyme from liver throughout purification, the generation of ammonia was measured by coupling it to the glutamate dehydrogenase reaction and following NADH oxidation at 340 nm as previously described (1).

Generation of Energy Charge Ratios. Mixtures of ATP, ADP and AMP corresponding to varying energy charge ratios from 0.9 to 0.1 were generated as described below. Mixtures of AMP and ATP were made in proportions ranging from 1 part AMP/9 parts ATP to 9 parts AMP/1 part ATP respectively. The total concentration of adenine nucleotides was kept constant at 10 mM, and $MgCl_2$ was added to give a final concentration of 25 mM. Adenylate kinase (1.0 μg, Sigma Grade III, 1400 units/mg) was added to 10 ml of each of the AMP/ATP mixtures, and the reaction was allowed to proceed for 2 hr at 30°. This amount of time was more than sufficient to achieve an AMP/ADP/ATP equilibrium mixture. The concentrations of AMP, ADP and ATP at equilibrium were determined directly, and the individual nucleotide concentrations were found to be within 5% of those predicted from the initial AMP/ATP ratio and the equilibrium constant for the adenylate kinase reaction.

RESULTS AND DISCUSSION

The elution profiles of AMP deaminase preparations from white and red skeletal muscle and cardiac muscle obtained by chromatography on cellulose phosphate are shown in Fig. 1. The enzyme from biceps femoris which consists predominantly of white fibers elutes as a symmetrical peak of activity (Fig. 1A) which is released from the resin by 0.64 M KCl. In contrast, the elution profile for AMP deaminase from anconeus muscle (Fig. 1B) which is rich in red fibers, shows shoulders which both precede and follow the main peak of activity. The major peak of activity from the red anconeus muscle is eluted with 0.56 M KCl, a salt concentration considerably lower than that required to elute the enzyme from white muscle. Not only is the enzyme in red skeletal muscle chromatographically distinct from that in white muscle, but its properties are also different from the cardiac muscle isozyme. These data are different from the results reported by Raggi et al. (6) in which they concluded that the major

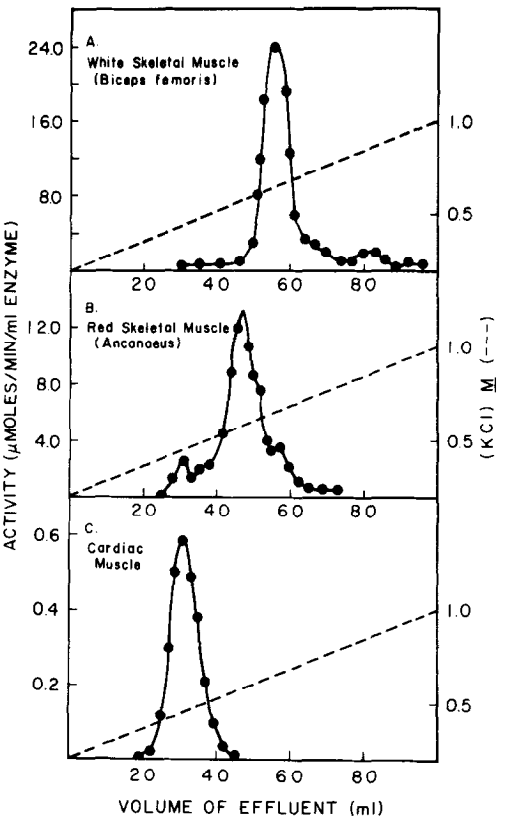

Fig. 1. Comparison of chromatographic properties on cellulose phosphate of AMP deaminase from rabbit biceps femoris (A), anconeus (B) and cardiac muscle (C). Details of the experiment are described under Materials and Methods.

form of enzyme in red muscle is identical to the heart muscle isozyme. As can be seen by comparing Fig. 1B and Fig. 1C, most of the enzyme from red muscle is adsorbed to cellulose phosphate much more tightly than the enzyme found in cardiac muscle. We do, however, observe that a small portion (10%) of the total AMP deaminase activity in red muscle elutes with a salt concentration identical to that required to elute the enzyme from cardiac muscle.

In order to insure that the elution profiles shown in Fig. 1 were representative of the total AMP deaminase activity in the crude extracts,

TABLE 1

RECOVERY OF AMP DEAMINASE FROM CELLULOSE PHOSPHATE

Type of Muscle	Total Units in Crude Extract from 10 g Tissue	Units Applied to Column	Units Recovered from Column	Percent Recovery
White Skeletal (Biceps Femoris)	80	60	50	83
Red Skeletal (Anconeus)	42	40	35	87
Cardiac Muscle	1.5	1.1	1.0	91

the recovery from each of the cellulose phosphate columns was carefully monitored. As can be seen in Table 1, between 80 and 90 percent of the activity in each of the crude muscle extracts could be accounted for in the fractions recovered from cellulose phosphate chromatography. Furthermore, when the fractions containing enzyme activity were pooled separately for each column, diluted to the proper ionic strength, and rechromatographed on the same column and under the same conditions, the elution profiles generated for all three samples were identical to those shown in Fig. 1. Therefore, it can be concluded that the AMP deaminase activity from white muscle, red muscle and cardiac muscle are all distinguishable from one another with respect to their chromatographic properties.

In view of the presence of tissue-specific isozymes of AMP deaminase, it seems likely that the physiological role of this enzyme may vary from tissue to tissue and may be dictated by unique metabolic patterns within a particular tissue. One of the several physiological functions which has been suggested for this enzyme is to regulate or stabilize the adenylate energy charge within the cell. Therefore, we decided to examine the

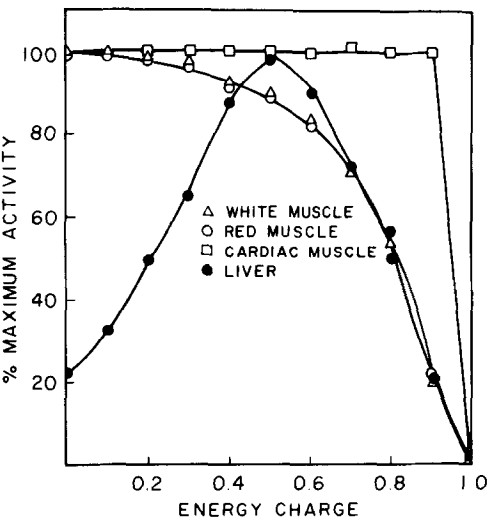

Fig. 2. Response of AMP deaminase isozymes to variations in the adenylate energy charge. The assay mixture contained 50 mM imidazole-HCl (pH 6.5), 100 mM KCl and adenine nucleotides at a total concentration of 4.0 mM and at the energy charge values indicated on the abscissa. The enzyme used was prepared from rabbit white skeletal muscle (Δ), red skeletal muscle (o), cardiac muscle (□) and liver (●).

activity of the enzyme from various tissues as a function of alterations in the adenylate energy charge. The results of these experiments are summarized in Fig. 2. It is clear from these data that the enzyme from skeletal muscle, cardiac muscle and liver can be distinguished from one another by the shape of the curve generated by measuring the activity in the presence of mixtures of ATP, ADP and AMP which correspond to energy charge values ranging from zero to one. In all of these experiments, the total adenylate pool size was kept constant at a concentration of 4.0 mM. Under these conditions, the cardiac muscle enzyme shows virtually no variation in activity with the energy charge between zero and 0.9. On the other hand, the enzyme activity from both red and white muscle fibers increases linearly as the energy charge decreases from approximately 0.9 to 0.7, and then continues to increase more slowly until maximum activity

is reached at an energy charge value of about 0.2. Quite clearly, the preparations of enzyme from red and white muscle fibers behave identically throughout the entire range of energy charge values. The isozyme from liver is indistinguishable from the skeletal muscle isozyme at energy charges greater than approximately 0.65. However, the shape of the response curve for the liver isozyme, is very different from that of the skeletal muscle enzyme at energy charge values below 0.6, with the liver isozyme showing maximum activity at 0.5 and decreasing rapidly at lower energy charge values.

These data provide an additional criterion by which the immunologically distinct isozymes of AMP deaminase can be quickly and easily distinguished from one another. The shape of the adenylate energy charge response curve appears to be very different for the enzyme from skeletal muscle, cardiac muscle and liver. On the other hand, the enzyme preparations from skeletal muscle containing either predominately red fibers or predominantly white fibers behave identically with respect to alterations in the energy charge. These data suggest that although the red and white muscle AMP deaminase activities can be separated from one another on columns of cellulose phosphate, they are functionally either very similar or identical to one another.

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