SHORT COMMUNICATIONS

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Muscle AMP aminohydrolase. II. Distribution of AMP aminohydrolase, myokinase and creatine kinase activities in skeletal muscle

AMP deaminase (AMP aminohydrolase, EC 3.5.4.6), first systematically studied by SCHMIDT[1], is widely distributed in animal tissues; however, the enzyme activity in skeletal muscle is very high as compared with all other tissues including smooth and heart muscles[2,3]. Although the high content of AMP deaminase activity in skeletal muscle indicates that this enzyme is associated with the specialized chemistry of this tissue, its biochemical role and its functions in muscle operation have been unclear up to now. It does not appear, however, that AMP deaminase is essential for the contractile process, since the muscles of some invertebrates are lacking in AMP deaminase[4,5]. In this paper the distribution of AMP deaminase activity in the skeletal muscles of rabbit, pigeon and rat is reported. The activity of two other enzymes, myokinase (EC 2.7.4.3) and creatine kinase (EC 2.7.3.2), which affect the metabolism of adenine nucleotides, was also examined. It is known that the skeletal muscle consists of two types of striated muscle cells: the red and white fibers. Both types are found in all muscles of the majority of animals; however, the distribution of white and red fibers in muscle varies to a great extent. The rabbit is peculiar in that red or white fibers generally predominate in its muscles[6]. Some metabolic differences exist between white and red muscles. White muscles, in contrast to red, have larger high-energy phosphate reserves and a higher capacity to derive energy from glycolytic reactions[6,7], react quickly to a stimulus, are capable of short periods of intense activity, and fatigue more rapidly than do red muscles[6]. Myosin from rabbit red muscle has 20–30% of the ATPase activity of white preparations[8]. The content of several other enzyme activities is different in white and red muscle[7,9,10]. KLEINE AND CHLOND[11] found that both myokinase and creatine kinase activities are higher in human white skeletal muscle than in red muscles.

The chilled muscles of three adult animals were pooled, cut in small pieces and homogenized in 0.6 M KCl. The 5% homogenates were centrifuged to remove the debris. A fraction of these homogenates was diluted with the extraction medium and used for the determination of the protein concentration and AMP deaminase activity. The proteins were determined by the biuret method using bovine serum albumin as a standard; the AMP deaminase activity was determined both spectrophotometrically and by measuring the NH_3 formation. The incubation medium in a final volume of 2 ml containing 0.1 M potassium succinate buffer (pH 6.5), 4 mM AMP and 100 mM KCl was incubated at 25°. The reaction was stopped by the addition of 1 ml of 15% (w/v) trichloroacetic acid, and portions of protein-free filtrates were taken for NH_3 determinations. The same incubation medium containing 2 mM AMP was used for the spectrophotometric measurement[12]. The two methods give similar results. Fractions of the 5% homogenates were conveniently diluted with 0.1 M Tris–HCl buffer (pH 7.5) or with 0.05 M phosphate buffer (pH 7.5) and used for the determination of myokinase and creatine kinase activity. The myokinase activity was assayed at 25° by the

TABLE I

DISTRIBUTION OF AMP DEAMINASE, MYOKINASE AND CREATINE KINASE ACTIVITY IN RABBIT MUSCLES

Muscle	*Protein concn. of 5% homogenates (mg/ml)*	*AMP deaminase (μmoles/g fresh wt. per min at 25°)*	*Myokinase (μmoles/g fresh wt. per min at 25°)*	*Creatine kinase (μmoles/g fresh wt. per min at 30°)*
Red muscles				
Soleus	9.8	15.4	30	340
Vastus internus	11.0	16.4	35	418
Anconaeus internus	10.1	16.9	45	403
Semitendinosus	11.1	23.1	37	337
Mm. intertransversales	11.8	34.6	40	345
White muscles				
Adductor femoris magnus	9.3	205	250	965
Biceps femoris caput longum	8.0	215	225	988
Biceps femoris caput brevis	9.3	210	245	1050
Vastus lateralis	10.1	93	190	886
Longissimus dorsi	10.0	98	215	811
Gleno ulnaris	8.2	90	220	737

procedure described by COLOWICK[13]; the creatine kinase was determined at pH 7.5 and 30° by measuring the liberation of creatine[14].

In Table I the protein concentrations of the 5% homogenates and the AMP deaminase, myokinase and creatine kinase activities of rabbit muscles are reported. The white muscle extracts deaminate AMP about 8 times more than do red muscles under the same experimental conditions. The lower AMP deaminase activity of red muscles could be due to the presence of an inhibitor. However the same differences between red and white muscles have been observed with dialyzed extracts; furthermore there was a linear relationship between the initial rate of AMP deamination by red muscle homogenates and protein concentration. The AMP deaminase activity of a mixture of red and white muscle extracts was equal to the sum of activities of the extracts measured separately.

The red muscles have about 15% of the myokinase and 40% of the creatine kinase activity of white muscles. Probably these differences are higher in a single red and white fiber. In fact with the exception of soleus muscle, the other muscles are prevalently but not completely composed of red or white fibers.

The AMP deaminase, myokinase and creatine kinase activities of white muscle extracts are similar to those observed in leg and dorsal rabbit muscles[12–14]. This is not surprising because the rabbit skeletal muscle apparatus is generally composed of white muscle.

In Table II the distribution of the three activities in pigeon muscles is reported. The muscles of the leg (the first 4 muscles of the table) have about 25% of AMP deaminase and 50% of myokinase activity of wing muscles, while creatine kinase activity is uniformly distributed. The AMP deaminase activity of three rat muscles was also examined (Table III). The soleus muscle which is a red muscle has 30% of the AMP deaminase activity of both white levator ani muscle and mixed tibialis anterior muscle.

TABLE II

DISTRIBUTION OF AMP DEAMINASE, MYOKINASE AND CREATINE KINASE ACTIVITY IN PIGEON MUSCLES

Muscle	*Protein concn. of 5% homogenates (mg/ml)*	*AMP deaminase (μmoles/g fresh wt. per min at 25°)*	*Myokinase (μmoles/g fresh wt. per min at 25°)*	*Creatine kinase (μmoles/g fresh wt. per min at 30°)*
Gastrocnemius	13.6	22	114	785
Ileotrochantericus	12.6	32	103	762
Semitendinosus	14.0	25	125	709
Ileotibialis	14.0	34	97	810
Biceps brachii	10.6	126	217	579
Deltoideus anterior	13.0	95	217	693
Deltoideus posterior	11.2	105	211	625
Costoscapularis	10.1	95	195	588

KALCKAR AND RITTENBERG[15] have shown that ^{15}N of labeled ammonium citrate fed to rats is incorporated into the 6-NH_2 group of the skeletal muscle adenine nucleotides 15 times more rapidly than into the purine ring nitrogen, while in liver the ^{15}N distributes uniformly in the nitrogen atoms of the purine ring and of the 6-NH_2 group. During muscle contraction IMP is produced[16–19]. Skeletal muscle extracts catalyze the amination of IMP by aspartic acid[20,21]. These data indicate that the muscle adenine nucleotides undergo a cycle of 6-NH_2 deamination and reamination, but neither the extent of this process nor the physiological role of this cycle are known. It is evident however that through this cycle it is possible to control the AMP levels in skeletal muscles. In contrast to smooth and cardiac muscles, no dephosphorylation of AMP has been observed[3,14] in this tissue. Because energy is consumed in this cycle, it is necessary to regulate some of the enzymes. AMP deaminase of rat and rabbit skeletal

TABLE III

DISTRIBUTION OF AMP DEAMINASE ACTIVITY IN RAT MUSCLES

Muscle	*Protein concn. of 5% homogenates (mg/ml)*	*AMP deaminase (μmoles/g fresh wt. per min at 25°)*
Soleus	9.5	23
Tibialis anterior	11.0	88
Levator ani	10.2	82

muscles are regulated enzymes[12,22]. Nothing is known of the regulation of the other enzymes of the cycle. However, adenylosuccinate synthetase from *Escherichia coli* is inhibited by AMP[23].

From these data and from those of DAVEY[21] on the distribution of adenylo-

succinate synthetase in rabbit muscles, it appears that the muscles with larger glycogen and high-energy phosphate reserves and with a higher capacity to derive energy from glycolytic reactions have the highest content of both AMP deaminase and adenylosuccinate synthetase; in the muscles (cardiac and red muscles) which produce energy generally through oxidative phosphorylation reactions, the content of the two enzymes is low. This observation corroborates that of KENDRICK-JONES AND PERRY[14] who observed that AMP deaminase, myokinase, creatine kinase and aldolase activities increase in the skeletal muscles during development from fetal to adult type; the adult skeletal muscle, in contrast to other tissues, has a more highly developed anaerobic metabolism than its fetal counterpart[14].

Further studies are in progress concerning a correlation between the AMP deamination–reamination cycle and the carbohydrate metabolism in skeletal muscle.

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