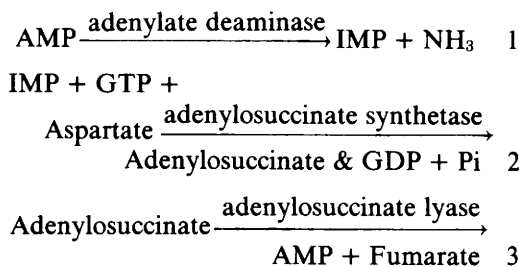


Enzymes of the Purine Nucleotide Cycle in Muscles from Normal and Dystrophic Chickens (40214)

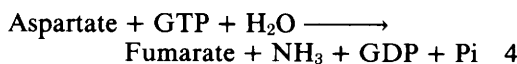
SANDRA W. CLARK, RICHARD W. PECKHAM, AND
FREDERICK B. RUDOLPH

Department of Biochemistry, Rice University, Houston, Texas 77001

It has been suggested that the three enzymes of the purine nucleotide cycle function as a cycle in muscle (1, 2) as follows:



with the following net reaction:



The ammonia production from AMP is proportional to the work done by the muscle (3) and the conversions involved have been demonstrated in rat muscle extracts (4, 5). The actual deamination does not appear to be directly involved in muscle contraction (6).

The function of the purine nucleotide cycle is not established but does seem to be involved in liberation of ammonia from amino acids, provision of citric acid cycle intermediates, regulation of the levels of the adenine pool and possibly as a modulator of glycolysis (2, 4). Modulation of the activities of enzymes that are involved with related reactions, such as adenylylase, may be a part of the overall metabolic consequences of the cycle.

Variations in the levels of a number of glycolytic and other enzymes occur with development of muscular dystrophy (7, 8). Adenylylase levels decrease markedly in dystrophic chickens (8), but no studies have been made on the other enzymes of the purine nucleotide cycle. As a part of a general study on purine metabolism, the levels of the three enzymes involved in the purine nucleotide cycle along with adenylylase were determined in red and white muscle of normal and

genetically dystrophic chickens.

Materials and Methods. Day-old New Hampshire Red chickens were obtained from the Department of Avian Sciences at the University of California, Davis. Control birds were Line 200 while the dystrophic birds were of the Line 308. This genetic strain was selected for the low muscle fat content and slower development of the myopathy (9). The chickens were maintained on commercial diet and sacrificed at three or 6 months of age by decapitation. The pectoral and sartorius muscles were immediately removed and 1 g portions were individually homogenized (10% w/v) in 0.03 M Hepes, pH 7.2 containing 0.1 M KCl, 5 mM EDTA and 0.1 mM dithiothreitol using a Tekmar Tissumizer. The homogenates were spun at 20,000g for 20 min and the supernatants used for the enzyme assays. For some of the assays the supernatants were dialyzed overnight as indicated against the homogenization buffer. Soluble protein concentrations were determined with biuret reagent with bovine serum albumin as a standard.

All nucleotides were provided by Sigma, and other chemicals were reagent grade. The PEI-cellulose sheets were a product of E. Merck. Adenylosuccinate was prepared from AMP and fumarate enzymatically as described by Carter and Cohen (10). 8-¹⁴C-IMP (10 mCi/mM) and 8-¹⁴C-AMP (22 mCi/mM) were obtained from New England Nuclear.

Adenylylase activity at 30° was determined from the decrease in absorbance at 265 nm as described by Smiley *et al.* (11). The reaction mixture contained 0.1 M potassium succinate, pH 6.5 with 50 μM AMP. Undialyzed supernatant was used for the assays and activity is expressed as nmoles IMP formed per minute.

Adenylosuccinate lyase activity was also determined at 30° spectrophotometrically from the decrease in absorbance at 280 nm

as described by Bridger and Cohen (12). The reaction mixture contained 20 mM Tris-chloride, pH 7.8 with 20 mM EDTA and 50 μ M adenylosuccinate. Undialyzed supernatants were used and activity is expressed as nmoles of AMP formed per min.

Adenylosuccinate synthetase was assayed at 30° either spectrophotometrically by the absorbance increase at 280 nm as previously described (13) or from measurement of the conversion of 8-¹⁴C-IMP into adenylosuccinate. Dialyzed supernatant was used for either assay. It was found in using the isotope assay that the synthetase level did not change upon dialysis but most of the adenylosuccinate lyase activity was absent in the dialyzed supernatant. The assay mixture for both assays contained 0.02 M HEPES, pH 7.0 with 1 mM magnesium acetate, 5 mM aspartate, 60 μ M GTP, and 150 μ M IMP. The separation of the [¹⁴C]labeled adenylosuccinate was made on PEI, cellulose sheets as described by Crabtree and Henderson (14). The reactions were stopped by addition of cold 1 M potassium hydroxide in methanol. After centrifugation to remove protein the solutions were neutralized with perchloric acid and the resulting potassium perchlorate removed by centrifugation. Methanolic KOH was used to inactivate the adenylate kinase activity present in the homogenate. The solutions were dried and dissolved in a minimal volume of H₂O for spotting on the thin-layer sheets. Standards were run on each sheet and the amount of isotope in each compound determined by liquid scintillation counting. The amount of conversion was determined from the number of counts in the adenylosuccinate spot divided by the total counts applied to the sheet. The synthetase activity was expressed as nmoles of adenylosuccinate formed per min with either assay. Nearly identical values for the specific activity in the tissues were obtained with the two assays.

Adenylate kinase was assayed at 30° in a reaction mixture containing 0.02 M HEPES, pH 7.0 with 0.4 mM magnesium sulfate, 0.2 mM ATP and 0.05 mM AMP with 8-¹⁴C-AMP. Dialyzed supernatants were used and the labeled ADP was separated and determined on PEI-cellulose sheets as described above for the synthetase.

The assays were dependent on the presence of all reactants and no significant degradation

of reactants or products was observed under the assay conditions. Multiple time points were taken with the radioactive assays to insure measurement of true initial rate of activity.

Results and discussion. The levels of adenylosuccinate synthetase and lyase and adenylate deaminase and kinase in the pectoralis and sartorius muscles of normal and dystrophic chickens are listed in Table I. The pectoralis is a main site of the dystrophic process in chickens while the sartorius does not appear to undergo gross wasting (15) allowing the enzyme levels in the sartorius to serve as an internal control to some extent.

Adenylate deaminase levels do decrease in dystrophic pectoralis relative to controls as described previously (8). The levels of the dystrophic pectoral enzyme are more similar to the sartorius than normal pectoral consistent with previous studies suggesting that the dystrophic white muscle fibers take on the characteristics of red fibers (16). The enzyme level is elevated in the younger dystrophic sartorius relative to the control. A number of enzymes show early elevation followed by a decrease as the disease develops (7). This is likely another indication of the slower development of the disease in the sartorius.

Similar to adenylate deaminase, the levels of adenylosuccinate synthetase and adenylosuccinate lyase do change significantly in dystrophic tissues. The synthetase activity is slightly lower in the dystrophic tissues ($P > 0.02$) except in the 3-month breast sample and rises in all tissues with age. The lyase is significantly elevated in the dystrophic pectoralis only, particularly at three months. Levels of the two adenylosuccinate enzymes had not been compared previously in normal and dystrophic tissue. Adenylate kinase activity was not altered in either tissue at either age. This result is in contrast to hamster muscle where the level of the enzyme decreased in dystrophic tissue (17).

Thus, it appears that the levels of adenylate deaminase in dystrophic tissue do not change in a coordinated fashion with the enzymes of the purine nucleotide cycle. The synthetase activity is generally lower while the lyase is elevated. The proposed functions of the purine nucleotide cycle include liberation of ammonia from amino acids thereby providing citric acid intermediates, regulating rela-

TABLE I. Activities of Purine Metabolizing Enzymes in Normal and Dystrophic Chicken Muscle. ^a

Enzyme	Specific Activity (nmol product/min/mg protein)			
	Control pectoralis	Dystrophic pectoralis	Control sartorius	Dystrophic sartorius
Adenylate deaminase				
3-month-old chicks	17.4 ± 2.9 (12)	11.5 ± 2.1 (9) (<i>P</i> > 0.01)	9.55 ± 1.4 (12)	18.1 ± 1.4 (9) (<i>P</i> > 0.01)
6-month-old chicks	32.4 ± 1.9 (11)	17.3 ± 3.8 (9) (<i>P</i> > 0.01)	21.7 ± 2.3 (11)	25.7 ± 4.3 (9) (N.S.D.) ^c
Adenylosuccinate lyase				
3-month-old chicks	6.3 ± 0.4 (12)	11.2 ± 0.9 (9) (<i>P</i> > 0.01)	8.9 ± 1.2 (12)	8.8 ± 0.7 (9) (N.S.D.)
6-month-old chicks	5.3 ± 0.5 (11)	6.6 ± 0.6 (9) (<i>P</i> > 0.01)	5.1 ± 0.4 (11)	5.8 ± 0.6 (9) (N.S.D.)
Adenylosuccinate synthetase ^b				
3-month-old chicks	1.6 ± 0.1 (12)	1.4 ± 0.2 (9) (N.S.D.)	2.2 ± 0.3 (12)	1.4 ± 0.5 (9) (<i>P</i> > 0.01)
6-month-old chicks	6.4 ± 1.3 (11)	4.9 ± 0.8 (9) (<i>P</i> > 0.02)	5.1 ± 0.8 (11)	3.8 ± 0.6 (9) (<i>P</i> > 0.02)
Adenylate kinase				
3-month-old chicks	8.4 ± 0.9 (12)	8.9 ± 1.3 (9) (N.S.D.)	7.5 ± 1.1 (12)	7.9 ± 1.3 (9) (N.S.D.)

^a Assays were done as described in "Methods and Materials." Control birds were New Hampshire Line 200 and dystrophic birds were Line 308. Values are means ± SE for the number of birds in parentheses.

^b Data given is derived from the spectrophotometric assays; similar results were obtained from the radioactive assays.

^c Not statistically different (*P* > 0.05).

tive adenine nucleotide pool levels and possibly regulating glycolysis (2). These roles will vary depending on the needs of the tissue and will likely be different in different muscles as indicated by the control data. A related study has shown that the effects of exercise on adenylate deaminase and adenylosuccinate lyase tissue levels are also not coordinated (18). It was suggested in that study that the deaminase levels decrease along with the levels of glycolytic enzymes consistent with some studies on dystrophic tissue (7). Farrell and Olson (19) have shown that the ATP/AMP ratio is decreased in dystrophic chickens which is consistent with a lowered activity of adenylate deaminase.

The lack of coupling between levels of the enzymes of the purine nucleotide cycle is not necessarily an argument against the functioning of such a sequence *in vivo*. The deamination occurs in a different physiological state than reamination so controls may well be expected to be different. The consequences of the deamination will vary depending on the metabolic state of the muscle, whether red or white, or possibly on the pathogenesis of the disease in cases such as dystrophy.

The reason for elevation of the adenylosuccinate lyase in the dystrophic pectoralis is not clear. It has generally been presumed that the synthetase is the rate-limiting enzyme in conversion of IMP to AMP and that muscle

does not synthesize *de novo* a significant amount of purines. It has been shown, however, that heart muscle synthesizes adenine nucleotides at a significant rate (20) and the synthesis varies depending on the metabolic state. The rise in adenylosuccinate lyase may be a result of the muscle trying to synthesize purine nucleotides to restore metabolic balance to the cells. Adenylosuccinate lyase is a dual function enzyme catalyzing the conversion of aminoimidazole succinocarboxamide ribonucleotide (SICAR) to aminoimidazole-carboxamide ribonucleotide (AICAR), a step in *de novo* purine synthesis. It may well be that its levels are regulated relative to *de novo* pathway and not the purine nucleotide cycle. If this is true, then the synthetase and deaminase levels may be related since they both show similar trends in the dystrophic tissue.

Summary. The tissue levels of the enzymes involved in the proposed purine nucleotide cycle in muscle, adenylosuccinate synthetase and lyase and adenylate deaminase, have been determined in the sartorius and breast muscles of normal and genetically dystrophic chickens. The deaminase and synthetase levels in the breast decrease while the lyase levels rise.

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