ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 83, 350–352 (1959)

LETTER TO THE EDITORS

The Amination of Inosinic Acid to Adenylic Acid in Muscle Extracts.[1]

The primary synthesis of AMP and its regeneration from IMP has been shown, with the aid of isotopically labeled substrates, to occur in bone marrow (1), in *E. coli* (2), and in muscle (3). The formation of AMP proceeds by transfer of NH_2 groups from L-aspartic acid to IMP via adenylosuccinate (4) as the intermediate. With enzyme preparations from *E. coli*, GTP acts as the specific high-energy cofactor (2); with bone marrow (1) and muscle (3) ATP is adequate.

With a view to the possible role of indirect deamination via AMP as a subsidiary path of amino acid catabolism in animals (cf. 5), we studied the amination of IMP in enzyme extracts from muscle. This process can be observed in a relatively simple assay (modified from that of Newton and Perry) (3) by means of conventional enzymic and chromatographic methods, without the use of isotopes. The enzyme system is remarkably stable, and under suitable conditions all of the added aspartic acid is converted to AMP.

The enzyme preparation is obtained as follows. Pigeon breast muscle is homogenized in a 0.003 *M* aqueous solution of EDTA (1:3), centrifuged at 9000 × *g*, and the supernatant is precipitated in the cold with 6 volumes of chilled acetone. Dialyzed aqueous extracts of the acetone powder are used for the tests.

The complete test system consists of 0.2 ml. enzyme solution, 10 μmoles 3-phosphoglycerate, 3 μmoles $MgCl_2$, 3 μmoles ATP, from 7 to 10 μmoles IMP, from 3 to 10 μmoles L-aspartic acid (or twice these amounts of DL-aspartic acid), 10^{-2} *M* phosphate buffer (pH 7.4); total volume, 1.5 ml. The test samples and all necessary controls are incubated from 1 to 4 hr. at room temperature (22–24°C.), fixed by heating to boiling, and analyzed.

Two procedures, checked by calibration experiments, were used for quantitative determination of the total content of adenosine phosphates (practically only ATP) in the fixed incubation mixtures:

(A) The samples are incubated 60 min. at 37° with purified potato Apyrase (6) and adenylate deaminase from rabbit myosin (7). The ΔNH_3 values thus obtained are a measure of the total adenosine-5-phosphates.

(B) After incubation with Apyrase alone, aliquots of the samples are chromatographed on paper for 72 hr. in *n*-propanol–conc. aqueous ammonia–2.10^{-3} *M* EDTA (6:3:1) as the solvent (8). The main UV-absorbing spots (IMP and AMP) are eluted and estimated in a Beckman-type spectrophotometer at 250 resp. 260 mμ.

The results of AMP determinations in the samples by methods A and B usually agree within ±3% or better, as shown in Table I, which represents the values of AMP formation after 1 hr. incubation.

If the incubation lasts 3 or 4 hr., the ΔAMP may correspond to practically 100% of the added L-aspartic acid (Fig. 1).

[1] Abbreviations: IMP, inosinic acid; AMP, adenylic acid; ATP, adenosine-5-triphosphate; GTP, guanosine-5-triphosphate; α-MeAsp, α-methyl-DL-aspartate; Apyrase, adenylic pyrophosphatase; EDTA, ethylenediaminetetraacetic acid.

TABLE I

The Synthesis of AMP from IMP (7.5 μmoles) and L-Aspartic Acid (3 μmoles) in Pigeon Breast Muscle Extracts[a]

Expt. No.	Δ AMP (μmoles)	
	Procedure A	Procedure B
1	0.75	0.73
2	0.77	0.81
3	0.61	0.69
4	1.53	1.40
5	1.52	1.42

[a] Incubation time, 1 hr.

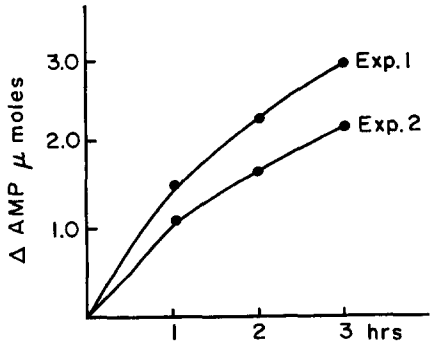

FIG. 1. Formation of AMP from IMP (8 μmoles) and aspartic acid (3 μmoles)

With extracts from pigeon heart muscle the rates of AMP formation were considerably lower than with skeletal muscle.

In the standard assay, asparagine, β-alanine, γ-aminobutyrate (cf. 9), L-cysteic acid, glutamine, and L-glutamic acid fail to act either as substitutes for aspartic acid or as inhibitors in its presence. Glutamic acid can act as the N-donor in the presence of oxaloacetate, owing to transamination (ΔAMP is about half of that obtained with aspartic acid as the donor). α-MeAsp, an effective inhibitor of argininosuccinate synthesis in the ornithine cycle of ureogenesis (10), produces no inhibition of the closely analogous condensation step in the amination of IMP, even at a ratio of α-MeAsp to aspartic acid of 100:1.

It was also found that GTP[2] was inadequate as a substitute for ATP in the enzyme system from muscle (in contrast to the *E. coli* system) (2).

A detailed account of this work will be published elsewhere.

After submitting this note for publication, a paper by C. L. Davey (*Nature*, **183**, 995 (1959)) came to our notice, reporting the presence in enzyme extracts of rabbit muscle of tightly bound GTP which is essential for the condensation of aspartate with inosinic acid.

Davey claims to have demonstrated accumulation of adenylisuccinate in test

[2] We are indebted to Dr. J. M. Buchanan for the generous gift of GTP.

systems similar to those of Neuton and Perry, but this intermediate was apparently identified only by differential spectrophotometry which lacks specificity.

References

1. Abrams, R., and Bentley, M., *J. Am. Chem. Soc.*, **77,** 4179 (1955); *Arch. Biochem. Biophys.* **56,** 184 (1955).
2. Lieberman, I., *J. Am. Chem. Soc.* **78,** 251 (1956).
3. Newton, A. A., and Perry, S. V., *Nature* **179,** 49 (1957).
4. Carter, C. E., and Cohen, L. H., *J. Biol. Chem.* **222,** 17 (1956).
5. Braunstein, A. E., *Advances in Enzymol.* **19,** 335 (1957).
6. Lee, K. H., and Eiler, J. J., *Science* **114,** 393 (1951).
7. Lyubimova, M. N., and Engelhardt, V. A., *Biokhimiya* **4,** 716 (1939); cf. *Doklady Akad. Nauk SSSR* **94,** 927 (1954).
8. Hanes, C. S., and Isherwood, F. A., *Nature* **164,** 1107 (1948).
9. Kometiani, P. A., and Klein, E. E., *Biokhimiya* **21,** 389 (1956).
10. Braunstein, A. E., Severina, I. S., and Babskaya, J. E., *Biokhimiya* **21,** 738 (1956).

Institute of Biological and Medical Chemistry,
The USSR Academy of Medical Sciences,
Moscow, U.S.S.R.

E. F. Yefimochkina
A. E. Braunstein

Received May 25, 1959