ENZYMIC BREAKDOWN OF ADENOSINE TRIPHOSPHATE IN THE LIVER

by

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In order to ascertain the mechanism underlying the enzymic breakdown of ATP in the liver, a study was carried out on the formation of the end-products of the breakdown of ATP by rat liver homogenates, *i.e.* orthophosphate (P), pyrophosphate (PP), muscle adenylic acid (AMP) and adenosine diphosphate (ADP).

We have found that besides enzymic P liberation (the liver phosphatase system has already been studied by many authors[1,2,3,4]), PP is produced by the action of rat liver homogenates on ATP.

On this basis a study of the adenosine phosphates (ADP, AMP) formed in the breakdown of ATP appeared necessary. These compounds were therefore determined under the same experimental conditions as P and PP.

The results indicating P and PP liberation, plotted in Fig. 1, most likely represent the true amounts of P and PP enzymatically formed from ATP. They were calculated taking into account inorganic PPase activity of rat liver, which was severally tested under the same conditions as for P and PP formation from ATP. P liberated from PP by this enzyme (PP $\rightarrow$ 2 P) was subtracted from P or alternatively added to PP, values for these compounds being determined on samples in which ATP was used as substrate.

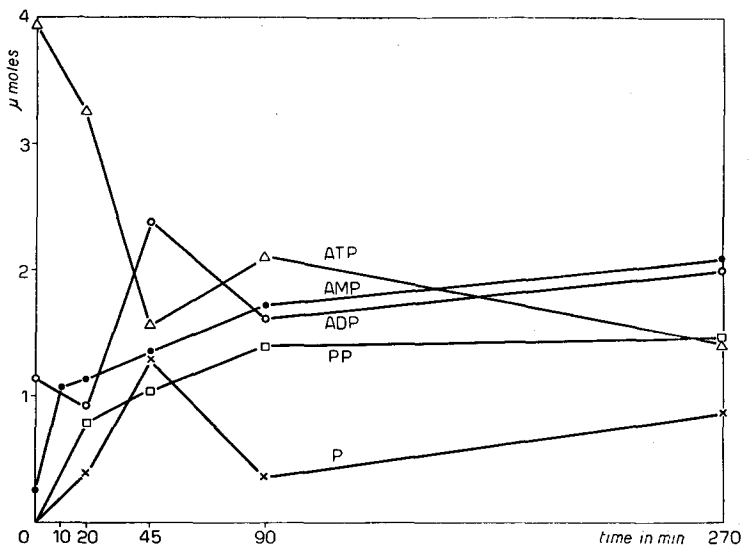

Fig. 1. End products of ATP breakdown by rat liver enzymes. 0.15 ml diethylbarbiturate buffer pH 7.4, 0.05 ml $5.2 \cdot 10^{-3}$ *M* $MgCl_2$, 3.94 µmoles ATP Na salt, 0.20 ml liver homogenate (1 g tissue in 100 volumes H_2O) brought to 0.65 ml with H_2O. Incubation temperature 37°. P determined according to LOWRY AND LOPEZ[5], PP according to KORNBERT,[6] ATP, ADP, AMP according to KALCKAR[7]

The formation of both P and PP shows that, when incubated with liver homogenates, ATP undergoes a twofold splitting: namely, one reaction transforms it into ADP, another reaction transforms it directly into AMP; this view is supported by the simultaneous finding of marked amounts of AMP besides ADP even after very short incubations.

The close correspondence existing between the curve representing ADP formation and that

concerning P suggest that both these compounds are produced at the same time from ATP, according to the following reaction:

$$ATP \rightleftharpoons ADP + P \quad (1)$$

The rapid fall of both these compounds after the first 45 minutes of incubation can easily be explained assuming a development of the reaction in the opposite direction.

This fact is also supported by the curve representing ATP, which indicates a resynthesis of this compound.

The curves of AMP and PP, on the other hand, although comparable in their shape, show a considerable discrepancy when values for PP, experimentally determined, are compared with the amount of PP to be expected stoichiometrically, assuming that all AMP is produced from ATP according to reaction (3).

This difference suggests that AMP is formed from ATP by the simultaneous occurrence of the two reactions:

$$(ATP) \rightleftharpoons ADP \longrightarrow AMP + 2P \quad (2)$$

$$ATP \longrightarrow AMP + PP \quad (3)$$

It appears most probable that the partial resynthesis of ATP, occurring when reaction (1) is reversed, accelerates reactions (2) and (3). This fact explains the remarkable increase in the rate of formation of AMP that can be observed after 90 minutes of incubation.

A reaction of the myokinase type can not be excluded: it is, however, inadequate in itself to account for all features of the phenomena quoted above.

A full report of these findings and of other results concerning the features of the enzymic breakdown of ATP under wider experimental conditions will be given together with a kinetic treatment of the data in a forthcoming paper.

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A SIMPLE METHOD OF ANALYSING PAPER-STRIPS IN ELECTROPHORESIS ON FILTER-PAPER

by

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In paper-electrophoresis, which is becoming more and more valuable for routine analysis of serum proteins, the analysing of the paperstrip may take place in two ways. By the first method[1,2,3,4] the paper is cut into a great number of narrow segments, the dye absorbed by the protein is eluted from each segment and the optical density (O.D.) of the elution fluid is measured. Instead of this laborious method GRASSMAN[5,6] uses a faster one. The paper, made transparent by dipping into a suitable liquid, is laid between two glass plates and passed along a slit illuminated by monochromatic parallel light. The O.D. can then be measured point by point by means of a selenium cell and a galvanometer.

For more than a year we have been using a paper-photometer as shown by Fig. 1. The dry paper-strip lies directly, *i.e.* without glass-plates, between the two slits S_1 and S_2. This avoids the necessity of parallel light and therefore the use of lenses. The strip being stained with bromphenol-blue, a sodium vapour lamp is particularly suitable as a monochromatic light source, with, moreover, the advantage that the light-output is very little influenced by mains variations. If a suitable kind