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Intermediates in the hydrolysis of ATP by human alkaline phosphatase

It is now known that alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) from human and other animal tissues can hydrolyse nucleoside di- and triphosphates as well as the monophosphate esters[1,2]. With a triphosphate such as ATP as the substrate, the reaction could take alternative courses. A stepwise removal of P_i (1) would lead successively to the production of ADP and then AMP, both of which are substrates of the enzyme and would be further broken down, but PP_i would not be formed. Removal of two P_i groups together from ATP (2) would result in the formation of AMP and PP_i, and, although the latter is also a substrate for the enzyme, its rate of hydrolysis is lower than that of AMP or ATP under conditions optimal for hydrolysis of ATP and so it would be present in the reaction mixture before completion was reached. A random attack on the ATP molecule would similarly lead to the release of some PP_i, by a combination of (1) and (2).

(1)

(2)

EATON AND MOSS[2] were able to show that AMP is an intermediate in the action of human alkaline phosphatase on ATP by chromatography of reaction mixtures, but the presence of ADP could not be unequivocally demonstrated by these means. Nucleotide intermediates in the hydrolysis of ATP by purified human intestinal alkaline phosphatase have now been determined enzymically.

The reaction mixtures consisted of 1 ml of 0.1 M Tris–HCl buffer (pH 9.5), 1 ml of 4 mM ATP solution and 0.1 ml of human intestinal alkaline phosphatase solution, purified by the method of Moss *et al.*[3]. (At the final concentration of Tris of less than 0.05 M in the reaction mixtures, transfer of phosphate to molecules of Tris[4] is negligible). After incubation for intervals of 0, 15, 30, 45 and 60 min at 37° the enzyme action was stopped by addition of 0.5 ml 2 M $HClO_4$. The mixtures were placed in an ice-bath before neutralisation with 5 M potassium carbonate. ATP was determined with hexokinase and glucose 6-phosphate dehydrogenase[5] and ADP and AMP by reaction with phosphoenolpyruvate, pyruvate kinase and myokinase[6]. P_i was measured by the method of DELSAL AND MANHOURI[7]. Non-enzymic hydrolysis, which was slight, was corrected for by controls.

The results (Fig. 1) show that ADP is formed in the reaction and that its concentration at first exceeds and then falls below that of AMP. This is consistent with a sequential removal of P_i groups from ATP, forming first ADP and then AMP. From the total concentration of adenine nucleotides present at different stages of the reaction it is possible to calculate the expected amount of P_i assuming that this is the only phosphate product of nucleotide hydrolysis. The measured levels of P_i at each interval lie close to the calculated values and, since any deficit of P_i would indicate the accumulation of PP_i, the observations further support a stepwise removal

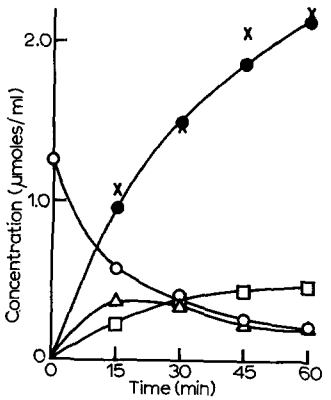

Fig. 1. Concentrations of nucleoside phosphates and P_i during hydrolysis of ATP by human intestinal alkaline phosphatase at pH 9.5. ○, ATP; △, ADP; □, AMP; ●, P_i. From the concentrations of each adenine derivative present at different stages of the reaction (adenosine concentration is obtained by difference), the expected concentration of P_i can be calculated assuming that phosphate is released only as P_i. The values thus obtained are represented by ×.

of P_i groups. During the later stages of the reaction the level of ADP declines, indicating that its rate of removal by conversion to AMP exceeds its rate of production from ATP. This is in agreement with observations on the relative rates of hydrolysis of adenine nucleotides by intestinal phosphatase at this pH value, at which ADP is more rapidly attacked than either ATP or AMP[2].

The successive removal of P_i groups from ADP and ATP by alkaline phosphatase implies that the substrate requirements of this enzyme are for a P_i group in which only one hydroxyl is esterified, though the nature of the moiety combined with the hydroxyl, which may be an organic alcohol or phenol or another phosphate group as in ATP, ADP and PP_i, appears not to be critical. When more than one of the hydroxyl groups of P_i are esterified, *e.g.* as in the inner phosphate groups of ATP and ADP or in organic phosphate diesters, the enzyme is unable to attack these bonds.

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