amounts from a readily available source, namely the acetone-desiccated powder of bovine pituitary posterior lobes. The formation of artifacts during the isolation procedure may be avoided by destroying the activity of the lysosomal enzyme cathepsin.

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Regulation of Adenylate Deaminase from Ehrlich Ascites-Tumour Cells by a Phosphonate Analogue of Adenosine Triphosphate

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The catalytic activities of many enzymes are modified by AMP and ATP (for review see Atkinson, 1966) and it has been suggested that the AMP/ATP ratio is a major factor in control of metabolic processes (Krebs, 1964). However, this ratio is subject to changes brought about by phosphorylation and by the action of adenylate kinase. For studies of natural and artificial regulation of enzymic activity in crude extracts from cells there is a need for compounds that simulate the regulatory properties of AMP and ATP but are not substrates of kinases that would change the concentrations of these compounds in multi-enzyme systems. The present communication describes the extraction from Ehrlich ascites-tumour cells of an adenylate deaminase (AMP aminohydrolase, EC 3.5.4.6) that is activated by ATP, inhibited by GTP and by high concentrations of ATP, and has an absolute requirement for univalent cations. The catalytic activity of this deaminase is modified by AMP-PCP* (the β -methylene analogue of ATP; Myers, Nakamura & Flesher, 1963) in a manner closely resembling the modification of activity by ATP.

* Abbreviation: AMP-PCP, *O*-adenylyl methylenediphosphonate.

Materials and methods. AMP-PCP was isolated as the barium salt (Myers *et al.* 1963) and converted into the sodium salt by treatment with chelating resin Chelex-100 (Na⁺ form) (Bio-Rad Laboratories, Richmond, Calif., U.S.A.) The ATP content, measured with glucose 6-phosphate, NADP and glucose 6-phosphate dehydrogenase, was less than 1% on a molar basis. Other nucleotides were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A.

Extracts were prepared from ascites-tumour cells as described by Murray (1966); 5mm-glutathione was added to the 50mm-tris-chloride buffer in which the cells were disintegrated. The supernatant obtained by centrifuging for 20min. at 20000g was fractionated at 1° with (NH₄)₂SO₄ and material precipitating between 0.28 and 0.40 saturation (Dixon, 1953) was dissolved in 5mm-glutathione-50mm-tris-chloride buffer, pH 7.3 (2.5ml./ml. of packed cells), and dialysed against three portions of the same solution for a total of 18hr. After centrifuging at 100000g for 30min. the supernatant (about 10mg. of protein/ml.) was used for assays of adenylate deaminase activity. Enzyme kept at -15° for 4 weeks lost no activity. The specific activity in

optimum conditions at 37° was 10–15 μ moles of AMP deaminated/min./mg. of protein, about 15–20 times that of the first extract.

Deaminase activity was measured from the decrease in extinction at 265 $m\mu$ ($\Delta\epsilon 8.8 \times 10^3$; Pabst Laboratories, 1961) in a 1 mm. cell, in a Unicam SP.800 or Zeiss PMQ II spectrophotometer at 37°. Enzyme (0.1 ml.) was added to 0.35 ml. of a solution containing, except where otherwise indicated, tris (30 μ moles), KCl (50 μ moles) and ATP (0.1 μ mole) at 37°; 2 min. later, AMP (0.3 μ mole in 0.05 ml.) was added to start the reaction. Rates were measured from 0.5 to 2.5 min. after mixing and were constant during this period in the standard assay or when other nucleotides or AMP-PCP were present in the assay solution.

The absence of interfering activities (e.g. adenylate kinase or phosphohydrolases + adenosine deaminase) was shown by chromatography of assay mixtures containing 1 μ C of [8- 14 C]AMP. Scanning in a gas-flow counter (Actigraph III; Nuclear-Chicago Inc., Chicago, Ill., U.S.A.) after chromatography in (NH₄)₂SO₄-0.1 M-sodium phosphate buffer (pH 6.8)-propan-1-ol (30:50:1, w/v/v) showed quantitative conversion of AMP (R_F 0.29) into [14 C]IMP (R_F 0.59). When AMP was replaced by adenosine at the same concentration in the standard assay the rate of deamination was less than 2% of that with AMP.

Results and discussion. Adenylate deaminase was one of the first enzymes of nucleotide metabolism shown to have a requirement for a positive modifier (cf. Atkinson, 1966). The deaminase from brain is

activated by ATP (Muntz, 1953; Weil-Malherbe & Green, 1955) and by NaCl (Askari, 1964), and inhibited by GTP (Setlow, Burger & Lowenstein, 1966). Adenylate deaminase from erythrocytes is activated by ATP (Askari & Franklin, 1965; Razin & Mager, 1966) and by univalent cations (Askari & Franklin, 1965). The regulatory function of the deaminase in purine nucleotide interconversion has been discussed by Setlow *et al.* (1966).

In the presence of 0.6 mM-AMP the enzyme from Ehrlich ascites-tumour cells was activated to the same extent (approx. tenfold) by 0.3–0.5 mM-ATP or -AMP-PCP (Fig. 1); concentrations of activators giving half-maximal activation were 0.07 mM and 0.11 mM respectively. Both ATP and AMP-PCP at concentrations greater than 0.5 mM gave rates lower than the maximum. Activation was decreased to half its maximum value at 0.9 mM-ATP or -AMP-PCP (Fig. 1). In the absence of ATP, GTP (up to 0.8 mM) neither stimulated nor inhibited the deaminase, but at all concentrations of ATP or AMP-PCP studied GTP inhibited the activated enzyme. Fig. 1 shows the effect on rate of deamination when ATP and GTP concentrations are increased simultaneously. Similar results were obtained on simultaneous variation of the concentrations of AMP-PCP and GTP. At constant ATP concentration (0.16 mM- or 0.8 mM-ATP) 0.1 mM-GTP suppressed half the extra activity induced by ATP.

From experiments where AMP concentration was varied in the range 0.05–1.0 mM in the absence of activators and in the presence of 0.175 mM-ATP or 0.24 mM-AMP-PCP it was evident that the activators increased the maximum rate (about threefold) and decreased the concentration of AMP required for half-maximal activity (from 0.54 mM to 0.26 mM and 0.30 mM respectively).

The AMP-PCP used in these experiments is clearly very similar to ATP as a regulator of adenylate deaminase, both in its activating effect at low concentrations and its inhibitory effect at high concentrations. It seems likely that this and the numerous other phosphate analogues of nucleotides that are now available (Myers *et al.* 1963; Myers, 1966; Hershey & Monro, 1966) will be valuable reagents in studies of regulatory properties of enzymes and of multi-enzyme systems.

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Fig. 1. Effect of ATP and AMP-PCP on the activity of adenylate deaminase from Ehrlich ascites-tumour cells. Assays were carried out as described in the text in the presence of various concentrations of ATP (○) or AMP-PCP (●). Assays containing ATP and 0.63 mol.prop. of GTP are also shown (△). The final pH was 7.3.

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