Short Communications

The nucleoside phosphorylase of pig liver and calf spleen

We have had occasion to prepare nucleoside phosphorylase from pig liver and wish to report on the presence of a contaminant in the material from this source which interferes with the use of the enzyme in the synthesis of inosine from hypoxanthine.

The phosphorolytic cleavage of inosine by enzymes from liver was first demonstrated by KALCKAR[1,2], who showed that the reaction was reversible, the equilibrium favouring the synthesis of inosine:

$$\text{inosine} + \text{phosphate} \rightleftharpoons \text{hypoxanthine} + \alpha\text{-ribose 1-phosphate}$$

For the enzymic testing of synthetic ribose 1-phosphate, we have prepared an enzyme from pig liver following KALCKAR's method of extraction. The enzyme preparation was highly active in bringing about the phosphorolysis of inosine, but confusing results were obtained on incubation of the enzyme with hypoxanthine and either synthetic or natural ribose 1-phosphate. The reaction was followed using KALCKAR's method: after incubation of the enzyme with hypoxanthine and ribose 1-phosphate for varying periods of time, the enzyme was inactivated by heat, and free hypoxanthine was determined by incubation with xanthine oxidase, which has no action on inosine. The uric acid formed was measured by observation of the increase in absorbancy at 290 mμ (Δ_{290}) during the incubation with xanthine oxidase. Synthesis of inosine is indicated by a decrease in the value of Δ_{290} with increasing times of the initial incubation. Using the pig-liver preparation, it was observed that, although the value of Δ_{290} decreased with increase in the time of incubation with the enzyme, the initial value of the absorbancy at zero time after addition of xanthine oxidase increased with increasing time of the initial incubation with the pig-liver enzyme. Since the molar extinction coefficients of hypoxanthine and inosine are approximately equal and extremely low at 290 mμ, the initial absorbancy should remain constant. The progressive increase in the initial value of the absorbancy at 290 mμ suggested that the decrease in Δ_{290} was not due to the formation of inosine. This was proved to be the case by adding orthophosphate and a further quantity of the pig-liver enzyme preparation to a solution of hypoxanthine and ribose 1-phosphate which had been successively incubated with pig-liver enzyme, heated at 100° for 4 min, and then incubated with xanthine oxidase until the absorbancy at 290 mμ remained constant. No further increase in absorbancy at 290 mμ was observed, indicating that no phosphorolysis of inosine was taking place. Careful removal of orthophosphate from both enzyme preparation and ribose 1-phosphate produced no effect on the results obtained. Incubation of hypoxanthine with the pig-liver preparation without addition of ribose 1-phosphate also resulted in an increase in absorbancy at 290 mμ, suggesting that the enzyme was contaminated with xanthine oxidase. This is not unexpected since the xanthine oxidase of pig-liver can be precipitated with ammonium sulphate in the same concentration range as that used by KALCKAR for the preparation of the nucleoside phosphorylase[3].

Since it was clearly undesirable to add an inhibitor of xanthine oxidase during the first incubation, and since further purification of the nucleoside phosphorylase could not be achieved by several of the usual procedures, attention was turned to the nucleoside phosphorylase of calf spleen[4]. This enzyme is highly active in the phosphorolysis of inosine, but had not been tested for its ability to synthesise inosine from hypoxanthine and ribose 1-phosphate[5]. Using the same techniques as with the pig-liver enzyme, experiments showed that the enzyme from calf spleen contained no xanthine oxidase. By varying the concentrations of the components of the incubation mixture, conditions were found in which the formation of inosine readily took place.

To 1.34 mg of the cyclohexylammonium salt of ribose 1-phosphate in 0.5 ml water, 2 drops of a solution of ammoniacal magnesium citrate were added, and the solution was shaken for 10 min. After removal of the precipitate by centrifuging, the supernatant solution was adjusted to pH 7.5 by addition of succinic acid solution, and was incubated with 0.37 mg hypoxanthine and 2 ml of calf-spleen nucleoside phosphorylase (total activity 650 units as measured by the method of PRICE, OTEY AND PLESNER[4]) in 0.1 *M* glycine–acetate buffer at pH 7.5, the volume being made up to 5 ml with buffer. 1-ml samples were withdrawn at appropriate times, heated in a boiling-water bath for 4 min, cooled, diluted to 5 ml with buffer and centrifuged. To a 2-ml sample of the supernatant 0.002 ml xanthine oxidase solution (approx. 200 units/l[6]) was added, the solution was diluted to 5 ml with glycine–acetate buffer and the absorbancy was measured immediately in a cuvette with a light path of 1 cm and at various intervals until the value remained constant. For various time intervals for the initial incubation, the following values for A_{290} were obtained: 0.53 (1 min); 0.23 (5 min); 0.04 (20 min); 0.04 (40 min). We consider that the nucleoside phosphorylase of calf spleen is satisfactory for the demonstration of the reversal of the phosphorolysis of inosine under these conditions.

It is known[7] that xanthine oxidase levels are affected by the nutritional level of animals. It seems likely either that in KALCKAR's experiments, the xanthine oxidase of the liver used was fortuitously low, or that in our experiments the level of this enzyme was unusually high. Whichever is the case, we regard it desirable to record our results which suggest that mammalian liver may not be a suitable source of nucleoside phosphorylase when it is desired to observe the reversal of phosphorolysis.

Gifts of natural ribose 1-phosphate from Dr. H. KLENOW and xanthine oxidase from Professor F. BERGEL, F.R.S. and Dr. R. C. BRAY are gratefully acknowledged. The authors are indebted to the British Empire Cancer Campaign for financial support.

Department of Chemistry, The University, Manchester (Great Britain) G. R. BARKER
I. C. GILLAM

1 H. M. KALCKAR, *J. Biol. Chem.*, 158 (1945) 723.
2 H. M. KALCKAR, *J. Biol. Chem.*, 167 (1947) 477.
3 H. S. CORRAN, J. G. DEWAN, A. H. GORDON AND D. E. GREEN, *Biochem. J.*, 33 (1939) 1694.
4 V. E. PRICE, M. C. OTEY AND P. PLESNER, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. II, Academic Press Inc., New York, 1957, p. 448.
5 V. E. PRICE, private communication.
6 P. G. AVIS, F. BERGEL AND R. C. BRAY, *J. Chem. Soc.*, (1955) 1100.
7 E. C. DE RENZO, *Advances in Enzymology*, 17 (1956) 293.

Received November 30th, 1959