Short Communications

Hydrolysis of thiamine phosphates by phosphatases in rat liver

In the normal rat liver the supernatant fraction contains nearly twice as much TDP as the mitochondrial fraction[1-3], and more than 15 times as much thiamine[3]. The nuclear fraction is very poor in TDP[1-3].

Repeated (4–6 times) injection of the rats with CCl_4 decreases the content of the supernatant TDP (see ref. 2, 3). This is also the case in the liver supernatant from rats fed ethanol for several months[4]. An increased dephosphorylation of TDP, induced by the treatments, was suggested to be the cause of the above decrease[3-4].

The enzymic dephosphorylation of TDP has been further investigated, and in this brief note information is given about the enzymic activity in different liver cell fractions, pH optima, and the effect of pretreating the animals with CCl_4.

Liver cell fractions were prepared according to SCHNEIDER AND HOGEBOOM[5]. The nuclear fraction was then resuspended, homogenized, and centrifuged twice more before incubation. The incubation medium contained 15 μmoles Mg^{2+}, 0.5 μmole [^{32}P]TDP, equally labelled in both phosphate groups, or 0.5 μmole [^{32}P]TMP, tissue extract or crude enzyme corresponding to 80 mg liver, and 0.1 *M* Tris–maleate or glycine–NaOH buffer to a final volume of 3 ml. The hydrolysis of the thiamine phosphates was calculated as per cent of the total radioactive phosphate occurring as inorganic phosphate after the incubation.

Incubation of TDP with cell fractions from rat liver. Radioactive TDP was incubated for varying times with supernatant (containing microsomes), mitochondria, or nuclei from normal and CCl_4-treated rats. All fractions were found to contain an enzyme decomposing TDP, the optimum pH being 9.6. In mitochondria also a second optimum at pH 8.6 was found. The supernatant fraction was about ten times as active as the mitochondrial and the nuclear fractions. CCl_4 treatment of the animals strongly increased the enzymic dephosphorylation of TDP in the supernatant (Fig. 1B) and mitochondrial fractions but only slightly in the nuclear fraction.

Paper chromatographic analyses showed that TMP was the main product of the TDP decomposition. Only traces of thiamine could be detected on the chromatograms, irrespective of whether the incubation had been performed at a pH around 7 or as high as 9.6.

Incubation of TMP with cell fractions from rat livers. When radioactive TMP was incubated with the supernatant fraction in the same way as described for TDP the optimum pH for its hydrolysis was found to be 6.1. No significant increase was caused by previous CCl_4 treatment of the rats (Fig. 1A).

Incubation of TDP and TMP with crude enzyme preparations from rat liver. In order to make sure that the pH effect on the rates of decomposition of TDP and

Abbreviations: TDP, thiamine diphosphate; TMP, thiamine monophosphate; Tris, tris(hydroxymethyl)aminomethane.

TMP, respectively, was not caused by a pH dependent release of phosphatase(s) from subcellular particles, crude enzyme preparations were made from normal rat liver in either of two ways: as a $KHCO_3$ extraction of a liver acetone powder, described by GIBSON *et al.*[6] for isolation of nucleoside diphosphatase from mitochondria, and by acetate extraction and treatment with butanol as used by MORTON[7], when isolating intestinal alkaline phosphatase.

In either case we found the preparations to be very active against TDP, transforming it into TMP and with only a slightly altered pH optimum as compared with that of the cell fractions (Fig. 1D).

With the acetone powder extract an obvious hydrolysis of TMP was obtained with an optimum pH at 5.8 (Fig. 1C), whereas the activity against TMP was almost negligable with the second preparation.

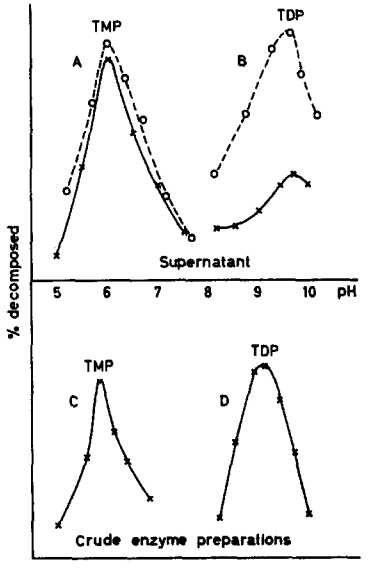

Fig. 1. Effect of pH on the activity of phosphatases hydrolysing TDP and TMP. A and B, effect of pH when TDP and TMP are incubated with the supernatant fraction from rat liver; C and D, influence of pH when incubated with crude enzyme preparations from rat liver. Continuous lines, enzymes from normal rats; lines of short dashes, enzymes from CCl_4-treated rats. Additions: see text; time, 30 min; temp. 37°. The reaction was stopped by addition of H_2SO_4 to a final concn. of 0.5 *M*, and inorganic phosphate separated from organic according to ERNSTER *et al.*[8].

The facts that both crude enzyme preparations hydrolysed TDP into TMP, whereas only one transformed TMP into thiamine, and that TDP and TMP are hydrolyzed at a maximum rate at widely different pH's strongly indicate that the two steps are catalyzed by different phosphatases.

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