Thiamine Phosphatases in Liver, Kidney, and Brain of the Rat

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In previous papers [1,2] it has been shown that thiamine diphosphate * is hydrolyzed by liver fractions to TMP and TMP to thiamine with different optimal rates, *viz.* at pH 9.3 and 6.0, respectively. This fact together with the findings that the TDP splitting capacity could be separated from that of TMP at the extraction of a liver homogenate in a special way, indicates that the two steps are catalyzed by different enzymes.

In the present communication the occurrence of the enzymes hydrolyzing TDP to TMP (TDPase) and TMP to thiamine (TMPase) are studied for liver, kidney, and brain.

Experimental. Whistar rats were decapitated, brain, liver, and kidneys rapidly removed, and put in icecold 0.02 M $KHCO_3$, cut in pieces with a pair of scissors, washed, and homogenized in a Waring blendor for 2 min in acetone at −15°C. After filtration on a Büchner funnel the powder was suspended in cold acetone, rehomogenized, collected by filtration, weighed, and suspended in 20 vol. of 0.02 M $KHCO_3$ at 0°C. After 25 min. extraction at 0°C with continuous stirring the sample was centrifuged, and the clear supernatant used as source of enzyme.

0.25−0.5 ml of the supernatants was used for incubation together with 1 ml 0.1 M Tris-maleate buffer (pH 5−7.5) or 0.1 M Tris-buffer (pH 7.5−10), 0.2 ml 0.1 M $MgCl_2$, 0.5 $TD^{32}P$, containing 1 μmole TDP and giving about 2×10^3 counts/min, and water to 2.5 ml. To find out if the Tris-maleate and the Tris-buffer acted differently on the enzymes, an incubation at pH 7.5 was made with either of the two buffers. No significant difference was found. After 20 min incubation at 37°C with continuous shaking, the samples were cooled to 0°C, 5 M H_2SO_4 added to a final concentration of 0.5 M, proteins removed by centrifugation, and 1 ml samples taken for determination of inorganic and organic radioactive phosphate according to Ernster *et al.*[3]

$TD^{32}P$ and $TM^{32}P$ were prepared according to Viscontini *et al.*[4] with the addition that radioactive inorganic phosphate was also present during the synthesis. The compounds were then separated by paper chromatography [5].

Results and discussion. A comparison of the TDPase and TMPase activities in the different rat organs at pH 6, 7 and 9 gave the following proportions between liver, brain, and kidney. The figures are mean values of four experiments.

TDPase	pH 7	1.5 : 1 : 3
	pH 9	2.2 : 1 : 4.7
TMPase	pH 6	2.2 : 1 : 1.9
	pH 7	1.6 : 1 : 3.3

Figs. 1 and 2 show the activities of TDPase and TMPase at different pH values. There, the percentages of hydrolyzed TDP are not comparable from one tissue to the other, the tissues being taken from different individuals and also different amounts of enzyme having been used.

Of the tissues investigated kidney is most active in hydrolyzing TDP to TMP. From Fig. 2 it is seen, however, that kidney and brain, but not liver, have two optima for hydrolyzing TMP, one at pH 6 and another at pH about 9, while liver shows one only at pH 6. The figures for TDPase activity in kidney at pH 9, given above, could therefore be expected not only to show TDPase activity but also TMPase activity as the TMP formed by the first enzyme might be further hydrolyzed to thiamine by the second enzyme. This might also be the case with enzymes prepared from brain, although to a smaller extent. Paper chromatographic analysis of the samples after finished incubations, however, shows that a surprisingly small amount of the TMP formed has been further hydrolyzed. This may very truly be ascribed to the short incubation times (20 min) used in these experiments.

In none of the tissues examined TDPase and TMPase are dependent in the same manner upon pH. With the liver enzymes the two substrates TDP and TMP are hydrolyzed with optimal rate at distinctly different pH, with the kidney enzyme one peak is common to the two substrates, while the brain enzyme gives two common

* Abbreviations: TDP: thiamine diphosphate, TMP: thiamine monophosphate, TDPase: Thiamine diphosphatase, TMPase: thiamine monophosphatase.

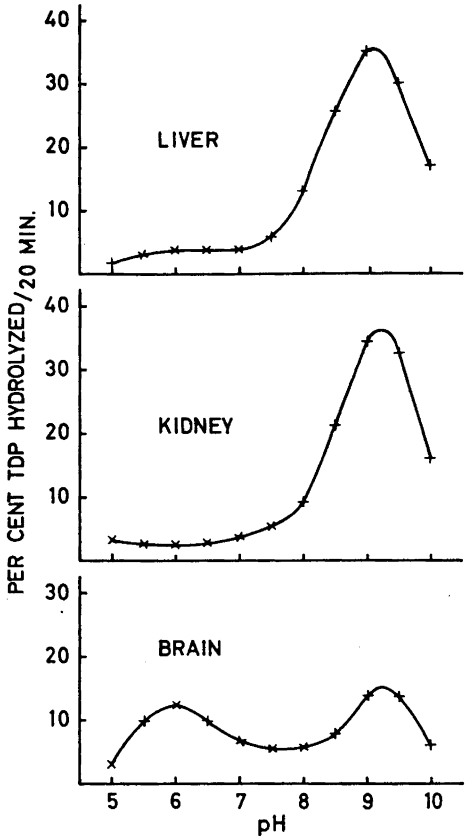

Fig. 1. Enzymatic hydrolysis of thiamine diphosphate to thiamine monophosphate. Incubations were performed as desribed in Experimental.

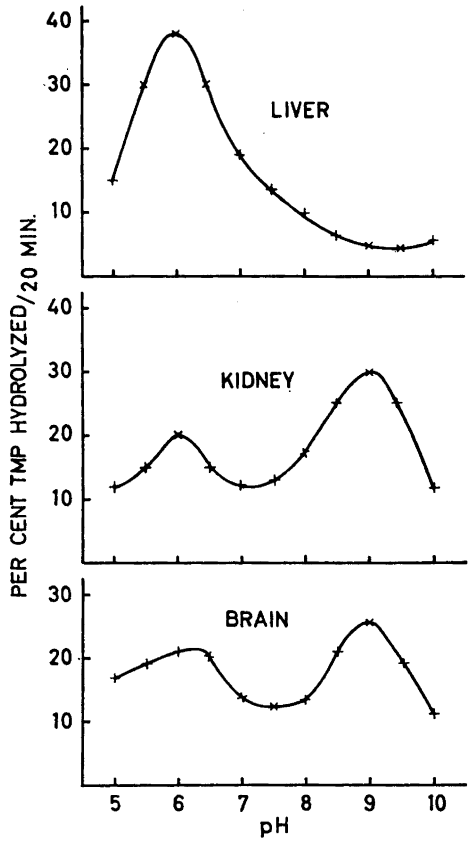

Fig. 2. Enzymatic hydrolysis of thiamine monophosphate to thiamine. Incubations were performed as described in Experimental.

peaks. With the last enzyme the peaks with TDP as a substrate are not very distinct, that is, TDP is hydrolyzed at moderate speed over a broad pH range. This is in agreement with findings by Naidoo and Pratt [6] in their histochemical investigation of a thiamine pyrophosphatase in nervous tissue from chicken.

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