PRELIMINARY NOTES

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5'-Nucleotidase activities in the soluble fraction of rat liver

Recently we investigated the dephosphorylation of the pyrimidine ribo- and deoxyribonucleoside 5'-monophosphates in the soluble fraction of rat liver homogenates[1]. It was found that the dephosphorylation of the deoxynucleotides was optimal at slightly acid pH and, furthermore, that acid phosphatase activity measured with β-glycerophosphate as substrate, was also present in the soluble cell fraction. Since it was generally believed that nucleotidases were not present in the soluble fraction of rat liver, we suggested that the dephosphorylation of dTMP, dUMP, and dCMP at neutral or slightly acid pH was due to the acid phosphatase activity. A slight difference between the variation patterns of β-glycerophosphate dephosphorylation and the dephosphorylation of the deoxynucleotides during liver regeneration was explained by assuming the presence of two or more acid phosphatases with different affinities for the substrates, and with slightly different variation patterns in regenerating liver. In recent studies of nucleotide dephosphorylation during liver carcinogenesis (unpublished experiments) we observed again a significant difference between the above variation patterns. For example, after 80 days the increase in dTMP and dUMP dephosphorylation was 70% whereas β-glycerophosphate dephosphorylation was only increased by 25%. It thus became of interest to explore further the enzymes responsible for the dephosphorylation of the deoxynucleotides. The present communication shows that at least two enzymes are responsible for the dephosphorylation of the deoxynucleotides in the soluble cell sap at acid pH, one of which is a specific 5'-nucleotidase (EC 3.1.3.5), the other a non-specific acid phosphatase (EC 3.1.3.2).

The 105 000 × *g* supernatant of rat liver homogenized for 0.5 min (ref. 1) was diluted with water and 0.1 M Tris–maleate buffer (pH 6.3) to a final concentration of 10 mg protein per ml 0.02 M buffer. Satd. $(NH_4)_2SO_4$ soln. (pH 6.3, containing 0.002 M EDTA) was added, and each of the protein fractions, separated by increasing the salt concentration stepwise by 5% from 30% up to 55% satn., was analyzed for dephosphorylating activity at pH 6.0 with dTMP and β-glycerophosphate as substrates under the incubation conditions which were optimal for dTMP dephosphorylation in the soluble fraction of rat liver homogenates[1]. The protein fraction which separated between 39% and 49% satn. with $(NH_4)_2SO_4$ showed the highest activity with both substrates, but the activity with β-glycerophosphate was only 16% of that with dTMP. This fraction was dissolved in 0.005 M Tris–HCl buffer (pH 7.5) containing 0.001 M EDTA and 0.001 M β-mercaptoethanol, and dialyzed against the same buffer. The dialyzed sample was applied to a DEAE-cellulose column equilibrated with the above buffer, and eluted with that buffer using a linear gradient of NaCl increasing from 0 to 0.5 M. 10-ml fractions were tested for dephosphorylating activity at pH 6.0 with dTMP as substrate. An activity peak appeared when the NaCl gradient reached 0.25 M. The eluted peak sample was also tested with UMP and β-glycerophosphate as substrates under the same incubation conditions. The results are shown in Table I. It can be seen that the enzyme is more active with UMP than with dTMP as substrate. It has no activity towards β-glycerophosphate. Further analysis of the $(NH_4)_2SO_4$ fractions

TABLE I

SUBSTRATE SPECIFICITY OF SOLUBLE 5'-NUCLEOTIDASE FROM RAT LIVER

The enzyme preparation was obtained by $(NH_4)_2SO_4$ fractionation of the 105 000 × *g* supernatant of rat liver homogenate, followed by chromatography on DEAE-cellulose, as described in the text. Dephosphorylating activity was measured at pH 6.0 with 0.02 M substrate, 0.05 M $MgCl_2$, and 0.05 M Tris–maleate buffer. Total vol., 0.5 ml; incubation time, 20 min; temp., 37°. Specific activity is μmoles phosphate released per mg of protein. Protein was determined by the method of LOWRY *et al.*[2].

Substrate	*Specific activity*	*Relative activity*
5'-UMP	2.3*	100
5'-dTMP	1.4	61
β-Glycerophosphate	0	0

* Corresponds to a 10-fold purification from the 105 000 × *g* supernatant.

with UMP as substrate has now indicated that it is preferable to use the fraction which separates between 34% and 40% satn. for preparation of the 5'-nucleotidase. Preliminary studies of this fraction indicate that the enzyme dephosphorylates all the purine and pyrimidine ribo- and deoxyribonucleotide 5'-monophosphates. It shows its lowest activity with dCMP, but also has a relatively low activity with dAMP as substrate (measured at pH 6.0). With 2'- or 3'-nucleotides no activity could be detected.

For purification of the acid phosphatase the 39–49% $(NH_4)_2SO_4$ fraction was prepared as described above, the precipitate was dissolved in 0.005 M Tris–maleate buffer (pH 6.5) containing 0.001 M EDTA and 0.001 M β-mercaptoethanol, and dialyzed overnight against 0.005 M acetate buffer (pH 5.0). The precipitate which was formed during dialysis, was centrifuged and discarded, and the supernatant used for measurement of dephosphorylating activity (Table II). It can be seen that the enzyme dephosphorylates dTMP and β-glycerophosphate almost equally well and may thus be termed a non-specific acid phosphatase. It is interesting to note that its activity with UMP as substrate is only 18% of that with dTMP. Preliminary results obtained with the $(NH_4)_2SO_4$ fraction further indicate that the activity of the acid phosphatase towards all the other purine and pyrimidine ribo- and deoxyribonucleotides is also low

TABLE II

DEPHOSPHORYLATING ACTIVITY OF SOLUBLE ACID PHOSPHATASE FROM RAT LIVER

The enzyme preparation was purified from the 105 000 × *g* supernatant of rat liver homogenate, as described in the text. Dephosphorylating activity was measured at pH 6.0 under the conditions described in Table I.

Substrate	*Specific activity*	*Relative activity*
5'-dTMP	0.495	100
β-Glycerophosphate	0.417*	84
5'-UMP	0.089	18

* Corresponds to a 7-fold purification from the 105 000 × *g* supernatant.

except for the dephosphorylation of dUMP and dGMP which occupies an intermediate position.

At the moment it is not clear whether or not the present nucleotidase is identical with the 5'-nucleotidase described in the foregoing communication[1], and which was measured at pH 9.5. In view of the finding of LEVIN AND BODANSKY[3] of a double pH optimum of 5'-nucleotidase of bull seminal plasma, the present enzyme may also exert activity at both pH 6 and pH 9.5. On the other hand, PILCHER AND SCOTT[4] have recently reported resolution of seminal-plasma 5'-nucleotidase into 3 active components. The acid phosphatase may or may not be a set of isoenzymes.

The activities of our 5'-nucleotidase toward the different substrates indicate that the enzyme is different from the nucleotidase with pH optimum 5.0 which has recently been purified from rat liver lysosomes[5]. This is in accordance with our previous conclusion[1] that the enzymes in the soluble fraction of rat liver homogenates which dephosphorylated dTMP were localized in the soluble space of the intact cell. Further studies are in progress to explore the enzymes in the soluble liver fraction which dephosphorylate the ribo- and deoxyribonucleotides.

In summary, the experiments have shown that the dephosphorylation of dTMP at pH 6.0 in the soluble fraction of rat liver homogenates (*cf.* ref. 1) is due to two enzymes, one of which is a specific 5'-nucleotidase, the other a non-specific acid phosphatase. The 5'-nucleotidase dephosphorylates all the purine and pyrimidine ribo- and deoxyribonucleotide 5'-monophosphates. The acid phosphatase has a high activity with dTMP as substrate, whereas the activity toward most of the other ribo- and deoxyribonucleotides is low.

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