With all the glycogen β -dextrins, the yield of maltose and maltotriose indicated a high proportion of A-chains that was equal to (or approached) that calculated for multiply branched molecules containing equal numbers of A- and B-chains, and exceeded that calculated for a molecule with twice as many B-chains as A-chains. The above results probably represent minimum values. First, although the digests still contained active pullulanase at the end of 48 hr., each substrate molecule contains several thousand A-chain 'stubs', and there can be no guarantee that all of these are debranched. Secondly, it is possible that the glycogen molecules contain a small proportion of 'buried' A-chains (French, 1957), which would not be readily susceptible to β -amylolysis and if subsequently debranched would not yield maltose or maltotriose. It is significant that the molar ratio of maltose to maltotriose was approximately unity, which is in accord with a random arrangement of equal numbers of chains containing either an even or odd number of glucose residues.

The results for the proportion of A-chains in potato amylopectin and waxy sorghum starch confirm the findings of Peat *et al.* (1956b) for waxy

maize starch, and suggest that in general amylopectins and glycogens (including phytoglycogens) do not differ significantly in degree of multiple branching.

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Purification of 5'-Nucleotidase from Human Liver

By C. S. SONG and OSCAR BODANSKY

*Division of Biochemistry, Sloan-Kettering Institute for Cancer Research,
 New York, N.Y. 10021, U.S.A.*

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The activity of 5'-nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5) has been found to be elevated in the serum of patients with hepatobiliary diseases (Dixon & Purdom, 1954; Campbell, 1962; Schwartz & Bodansky, 1965). The study of this enzyme in the human liver, however, has been seriously limited because of the presence in this organ of non-specific phosphatase of an approximately equal activity (Reis, 1951). The purpose of the present communication is to report a method for the purification of 5'-nucleotidase from human liver that resulted in a practically complete elimination of non-specific phosphatase activities.

All the steps in the purification procedure, unless otherwise indicated, were carried out at 0-4°. The methods of assay for 5'-nucleotidase and for non-specific phosphatase were reported by Bodansky & Schwartz (1963).

A piece of human liver (97 g.), which appeared normal on gross pathological examination, was obtained 6-12 hr. after death. It was minced with scissors and homogenized for 2 min. in 4 vol. (388 ml.) of water in a Waring Blendor turning at full speed. The homogenate was centrifuged for 5 min. at 600 g. The sedimented material was discarded and supernatant was centrifuged for 30 min. at 35000 g. The resulting pellet, corresponding to approx. 70 g. of the original liver, was resuspended in water (140 ml.) in a glass homogenizer. The suspension was then divided into 20 ml. fractions, placed in glass cups immersed in a container of ice-water and subjected to ultrasonic oscillation for 3 min. at 20000 cyc./sec. in an MSE Ultrasonic Power Unit.

The ultrasonically treated suspension was heated for 20 min. at 58°, cooled in a container of ice and

mixed with an equal volume of 6% (w/v) sodium deoxycholate in 20mm-sodium diethylbarbiturate buffer, pH 7.5. The mixture was homogenized for 5min. in a Waring Blendor turning at a slow speed to minimize foaming.

The blended mixture was centrifuged for 60min. at 78000g. The pellet was discarded. The supernatant was added, dropwise with mixing, to an equal volume (210ml.) of ethanol maintained at -5° to -10° . Stirring was continued for 10min. and the resulting precipitate was collected by centrifuging for 30min. at 30000g in a Lourdes refrigerated centrifuge at -5° .

The pellet was resuspended in 20mm-sodium diethylbarbiturate buffer, pH 7.5 (60ml.), and dialysed for 24hr. against three changes of water. The dialysed suspension was added to 4mm-sodium diethylbarbiturate buffer, pH 7.5 (750ml.), so that the final concentration of protein was approx. 0.2mg./ml. This diluted suspension was then heated for 20min. at 62° . No precipitate was formed.

The resulting preparation was a slightly-turbid particulate suspension with an approximately tenfold increase in specific activity of 5'-nucleotidase over the initial homogenate. The yield of the enzyme was about 15%. It showed no decrease in activity during 3 months' storage at -38° .

The enzyme preparation was highly specific for nucleoside 5'-phosphates in its hydrolytic action. It hydrolysed all of the four nucleoside 5'-phosphates studied (AMP, GMP, CMP and UMP) and NMN. At pH 7.1 and at 5mm-substrate concentration the relative rates of hydrolysis were: AMP, 100; GMP, 52; CMP, 105; UMP, 104; NMN, 32. In contrast, the relative rates of hydrolysis of β -glycerophosphate, glucose 6-phosphate, adenosine 2'-phosphate and adenosine 3'-phosphate were negligible, ranging from 0 to 1.

Fig. 1 shows the effect of pH on the activity of purified human liver 5'-nucleotidase. In the absence of added metal ions, it exhibited a pH optimum in the range pH 7.1–7.5. In the presence of 10mm-Mg²⁺, however, the enzyme was activated and a second pH optimum appeared at about pH 9.3–9.5. Such a double pH optimum was observed with AMP, CMP or UMP as substrate and with tris-chloride or sodium diethylbarbiturate as buffer for the reaction mixtures. The pH-activity curves for human-liver 5'-nucleotidase therefore closely resembled those observed for the 5'-nucleotidase purified from bull seminal plasma (Bodansky & Schwartz, 1963; Levin & Bodansky, 1966) and rat liver (C. S. Song & O. Bodansky, unpublished work).

The effect of metal ions on purified human-liver 5'-nucleotidase was generally similar to that reported for 5'-nucleotidase of human aorta (Ahmed & Reis, 1958) and bull seminal plasma (Levin & Bodansky, 1966). At pH 7.5 and in the presence of

Fig. 1. pH-activity curves for human-liver 5'-nucleotidase. The reaction mixtures contained (final concentrations): AMP (5mm), tris-chloride (80mm) and enzyme (64μg. of protein/ml.). The reactions were carried out at 37° in the presence (●) and in the absence (○) of 10mm-Mg²⁺.

80mm-tris-chloride buffer, human-liver 5'-nucleotidase was activated 44% by 0.1mm-Mn²⁺ and 33% by 1mm-Mn²⁺. The inhibition of the enzyme by 0.1mm-Zn²⁺ was 11%. The inhibitions by 1mm-Zn²⁺ and 1mm-Ni²⁺ were 99 and 24% respectively.

The Michaelis constant (K_m) was determined with AMP as a substrate according to the method described by Levin & Bodansky (1966). At pH 7.1 and in the presence of 80mm-tris-chloride and 10mm-Mg²⁺, the mean value of three determinations was 3.9×10^{-5} M. K_m (AMP) values previously reported for 5'-nucleotidases of various sources are: 2×10^{-5} M for a crude homogenate of dog cerebrum (Waked & Kerr, 1955); 1.0×10^{-5} – 1.2×10^{-5} M for rat-liver microsomes (Segal & Brenner, 1960); 2.1×10^{-5} M for bull seminal plasma (Levin & Bodansky, 1966).

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