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TECHNICAL PROGRESS REPORT

MASTER

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TITLE: "Degradation of Lecithins and Physiological Functions in
Membranes of Microsomes and Mitochondria"

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PUBLICATIONS:

1. COO-1513-22. "Glycerolphosphorylcholine Diesterase:
Inhibition by Nucleotides", J.J. Baldwin and W. E.
Cornatzer, Proceedings of the Society for
Experimental Biology and Medicine; 131, 271-275 (1969).

Glycerolphosphorylcholine diesterase (glycerolphosphorylcholine glycerophosphohydrolase, EC 3.1.4.2), which catalyzes the following reaction: $\text{glycerolphosphorylcholine} + \text{H}_2\text{O} \rightarrow \text{L-}\alpha\text{-glycerolphosphate} + \text{choline}$ is found at the end of the phosphatidylcholine degradative scheme. Glycerolphosphorylcholine, unlike lysolecithin is not capable of being acylated to form lecithin. Consistent with this data is our inability to demonstrate the synthesis of glycerolphosphorylcholine from L- α -glycerolphosphate and choline. It therefore appears in view of available evidence that the sole purpose of glycerolphosphorylcholine diesterase is to hydrolyze glycerolphosphorylcholine.

The rate at which this enzyme operates with respect to the other degradative enzymes in the metabolism of lecithin in mammals could conceivably exert an influence on the rate of metabolism of lecithin due to its unique position in the degradative scheme.

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Studies designed to test the effects of various biosynthetic and degradative intermediates in lecithin metabolism on glycerylphosphorylcholine diesterase in our laboratory showed ATP to be a strong inhibitor of the enzyme. The present communication reports the results obtained from studies on the nucleotide inhibition of glycerylphosphorylcholine diesterase from rat kidney.

Glycerylphosphorylcholine diesterase (glycerolphosphorylcholine glycerophosphohydrolase, EC 3.1.4.2) from rat kidney was inhibited selectively by purine triphosphate nucleotides and inorganic pyrophosphate. Classical competitive inhibition for all compounds tested was observed. Of the purine nucleotides ATP, GTP, and ITP showed the greatest amount of inhibition, producing K_i values of approximately 1.5 mM. The purine diphosphates showed a lesser degree of inhibition, producing K_i values of approximately 20 mM and the corresponding purine monophosphates gave very little inhibition, producing K_i values of approximately 125 mM. In the case of the pyrimidine nucleotides, both the cytidine and uridine triphosphates were not as effective as the purine triphosphates. The K_i values obtained with the pyrimidine triphosphates were approximately seven times greater than for the purine triphosphates. Inhibition by ATP was found to be pH dependent in the range studied, (pH 7.0-10.2) with the greatest amount of inhibition observed at pH 8.6. Michaelis-Menton kinetics were observed with all of the nucleotides studied. However, when considering inorganic pyrophosphate, sigmodial type kinetics were observed. Inorganic phosphate was without effect on the diesterase; 5'-AMP and cyclic AMP did not reverse ATP inhibition; $MgCl_2$ alleviated the inhibition produced by ATP.

2. COO-1513-23. "Glycerylphosphorylcholine Diesterase:

Effects of Metal-Binding Agents", J.J. Baldwin,
Phyllis Lanes and W. E. Cornatzer, Archives of
Biochemistry and Biophysics, 133, 224-232 (1969).

During the initial characterization of rat kidney glycerylphosphorylcholine diesterase (J.J. Baldwin and W.E. Cornatzer, *Biochimica et Biophysica Acta*, 164, 195-204, 1968; C00-1513-16) we observed a high degree of selectivity of the inhibition produced by several metal-binding agents. This communication reports studies carried out to investigate the effects of several metal-binding agents and the effect of metal ions on glycerylphosphorylcholine diesterase. Results suggest that the metal may be located at or near the active site of the enzyme.

Glycerylphosphorylcholine diesterase (L-3-glycerylphosphorylcholine glycerophosphohydrolase, EC 3.1.4.2) has been studied with respect to metal ion involvement. The effects of a number of metal-binding agents have been investigated by preincubation and instantaneous inhibition techniques. A high degree of selectivity of the metal-binding agent has been observed, with EDTA showing the greatest amount of inhibition. Structural analogs of EDTA have however, been found to be as effective as EDTA. The inhibition produced by EDDA has been determined to be competitive inhibition with respect to substrate. Heat and urea potentiate the inhibition seen with EDTA. Zn^{2+} ions have been found to alleviate the EDTA-produced instantaneous inhibition and were observed to produce noncompetitive inhibition with respect to glycerylphosphorylcholine. Dialysis against 0.01 M EDTA, pH 7.1, and water has been found to completely inactivate the enzyme, whereas addition of several cations to the EDTA-dialyzed enzyme were observed to bring about restoration of activity. Of 26 cations tested, Zn^{2+} and Sr^{2+} reactivated the diesterase 100% and 109%, respectively. Zn^{2+} ions, when titrated with the enzyme obtained after dialysis with EDTA and water were found to restore the activity in a sigmoidal manner. The binding constant for Zn^{2+} was calculated to be approximately $3 \times 10^{-6}\text{M}$. The substrate, glycerylphosphorylcholine, was observed to reverse the instantaneous inhibition produced by EDTA, possibly by protecting the metal from the EDTA.

These observations suggest that glycerylphosphorylcholine diesterase may be a metalloenzyme, and a mechanism is proposed which suggests the inclusion of two molecules of Zn^{2+} per molecule of enzyme.

3. COO-1513-24. "Biosynthesis of Liver Microsomal Phosphatidyl Cholines during the Development of Choline Deficiency",
D.J. Rytter and W.E. Cornatzer, Submitted to Proceedings
of the Society of Experimental Biology and Medicine -
(one copy enclosed).

Phosphatidylcholine biosynthesis in liver microsomal is known to occur by two major different pathways; Kennedy pathway involves cytidine diphosphocholine and α - β -diglyceride to form phosphatidylcholine. The Greenberg pathway involves the methylation of phosphatidyl ethanolamine from adenosyl methionine to form phosphatidyl choline. The specificity of the incorporation of 1,2- ^{14}C -choline and 1,2- ^{14}C -ethanolamine into these phosphatidyl choline fractions as a means of measuring these two biosynthetic pathways of lecithin synthesis has been determined in this laboratory. Phosphatidyl choline of fraction one and two are chiefly incorporated from 1,2- ^{14}C -ethanolamine and provides a lecithin rich in polyunsaturated fatty acids. The phosphatidyl cholines of fractions 3 and 4 are mainly synthesized by the 1,2- ^{14}C -choline pathway. The biosynthesis of the phosphatidyl choline fractions have not been determined in microsomes from choline deficient animals with a fatty liver. Female albino rats of Sprague-Dawley strain weighing 176 ± 19 g were divided into two groups. Group I served as control and was fed Purina Laboratory Chow. The animals of Group II were fed 5% casein - 5% fat diet plus 1% guanidoacetic acid for 7 days.

The incorporation of 1,2-¹⁴C-choline and 1,2-¹⁴C-ethanolamine into the total phospholipid-P, total lecithin P and the phosphatidyl choline-P fractions of liver microsomes at 1,2,3, and 4 hours following intraperitoneal injection of the isotopic compounds in choline deficient animals was studied. The incorporation of 1,2-¹⁴C-choline into the total phospholipid-P and lecithin-P of liver microsomes was greater in the choline deficient than in the control animals. The greatest incorporation occurred in lecithin fractions 3 and 4 in the choline deficient animals. The incorporation data would support the idea that the biosynthesis of lecithin by the CDP-choline- α - β -diglyceride is not deficient in the production of a fatty liver. The greatest incorporation of 1,2-¹⁴C-ethanolamines is in the lecithin fractions 1 and 2 in both control and choline deficient animals. It was apparent from the data of the specific activities of the lecithin fractions of the liver microsomes of the choline deficient are much smaller than the control animals. This observation would suggest an impairment in the formation of phosphatidyl choline from phosphatidyl ethanolamine in choline deficiency.