STIMULATION OF MONOACYLGLYCEROPHOSPHATE FORMATION BY Z PROTEIN*

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SUMMARY: Z protein has been purified from 110,000 x g rat liver supernatant using Sephadex G-100 and DEAE Sephadex. Z protein obtained in this manner was superior to albumin in stimulating the esterification of sn-glycerol-3-phosphate in the presence of palmityl-CoA and rat liver microsomes. These observations constitute direct evidence for the possible role of Z protein in fatty acid metabolism.

INTRODUCTION: Z is a 12,000 M.W. protein present in the supernatant fractions of rat liver, kidney, myocardium, skeletal muscle, intestinal epithelium and adipose tissue. This protein, initially discovered by virtue of its ability to bind bilirubin and BSP (1), was thought to be involved in the cellular uptake of these and possibly other organic anions. Recently, it was discovered that Z protein has a high affinity for long chain fatty acids in vitro (2,3), and that following an I.V. injection of (^{3}H) oleate, fatty acid radioactivity is recovered on hepatic Z protein. In order to determine whether Z was involved in the hepatic uptake of long chain fatty acids, experiments were performed using flavaspidic acid, a specific inhibitor of organic anion binding to Z protein (1). In the perfused rat liver, inhibition of fatty acid binding to Z by flavaspidic acid yielded two significant observations; fatty acid uptake by the liver was unchanged or actually augmented, while fatty acid esterification was significantly reduced (4). We concluded from these results that while binding of long chain fatty acid to Z was

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not an obligatory step in fatty acid uptake, Z might be involved in some other aspect of fatty acid metabolism. In a recent communication we reported on in vitro studies which showed that Z protein has a high affinity for the CoA derivatives of long chain fatty acids (5). In the case of palmitic acid, the CoA and carnitine derivatives were bound more strongly than the free fatty acid alone. On the basis of these findings we have proceeded to study the effect of Z protein on various enzymatic reactions involving long chain fatty acids and their CoA derivatives. In this communication we will describe experiments which compared the effect of Z protein and albumin on the formation of monoacylglycerophosphate from sn-glycerol-3-phosphate.

MATERIALS: (^{14}C) sn-Glycerol-3-P (80 mCi/mmole) was purchased from New England Nuclear Corp. Palmityl-CoA was purchased from P & L Biochemical Co. and unlabelled sn-glycerol-3-P from Sigma Chemical Co. Silica Gel G was obtained from Brinkman Instruments and Sephadex G-100 and DEAE Sephadex A-25 from Pharmacia.

METHODS:

Preparation of rat liver microsomes - Microsomes were isolated from rat liver, washed and resedimented as described by Fallon and Lamb (6). Microsomes were stored at 4° C and were used within 15 hours of preparation.

Acyl-CoA-sn-glycerol-3-phosphate acyltransferase - The assay used was that of Lamb and Fallon (7). The assay medium consisted of 70 mM Tris-HCl (pH 6.5), 115 mM dithiothreitol, 40 nmoles of palmityl-CoA, 12.5 or 40.0 nmoles fatty acid-poor bovine albumin (Fraction V, Pentex, Inc.) or Z protein, 60 nmoles of (^{14}C) sn-glycerol-3-phosphate and 0.3 mg of microsomal protein in a total volume of 0.34 ml. The reaction was initiated by the addition of microsomes and stopped after a 15-min incubation at 37° C by the addition of 10 ml of chloroform-methanol (2:1). Lipids were extracted and washed by the method of Folch et al (8). The

chloroform-methanol extracts were evaporated at 37° C under N_2, and the lipids were dissolved in 10 ml of chloroform. The radioactivity incorporated into monoacylglycerophosphate and diacylglycerophosphate were determined by the TLC methods of Lamb and Fallon (7). Samples and standards were chromatographed in adjacent lanes, and radioactivity was determined by scraping and counting equal segments in 12 ml of Omniflor (New England Nuclear Corp.) in a Packard Liquid Scintillation Counter (Model 3375). Quench correction was by external standardization. Protein concentrations were determined by the Lowry method (9).

Preparation of "pure" Z protein - Z protein derived from rat liver supernatant was obtained as described earlier (5). The "Z region" obtained by chromatography of 110,000 x g liver supernatant on Sephadex G-100 was found to contain Z protein, as well as three protein bands of M.W. > 12,000 (Fig. 1A), which were removed by subsequent chromatography on DEAE Sephadex A-25 (Pharmacia) using an NaCl gradient (150-600 mM) in 20 mM Tris (pH 7.8) (Fig 1B). The identity of Z protein was established as previously described (2).

RESULTS: The incorporation of (^{14}C) sn-glycerol-3-phosphate into monoacylglycerophosphate was studied under three experimental conditions, as seen in Table I. In all cases, more than 90% of the radioactivity corresponded to the region containing monoacylglycerophosphate on the Silicic acid chromatogram. In the absence of added protein, 0.13 ± 0.04 nmoles of sn-glycerol-3-phosphate were esterified. The addition of 12.5 and 40.0 nmoles of albumin to the assay medium enhanced esterification by a factor of 2.0 and 5.4 respectively. It should be noted that 40 nmoles of albumin have been previously shown to provide maximal stimulation in this assay system (7). The important observation of these experiments, however, was that equimolar Z protein resulted in much greater stimulation of esterification than did albumin, at the two concentrations studied. These differences were found to be significant at $p < 0.05$.

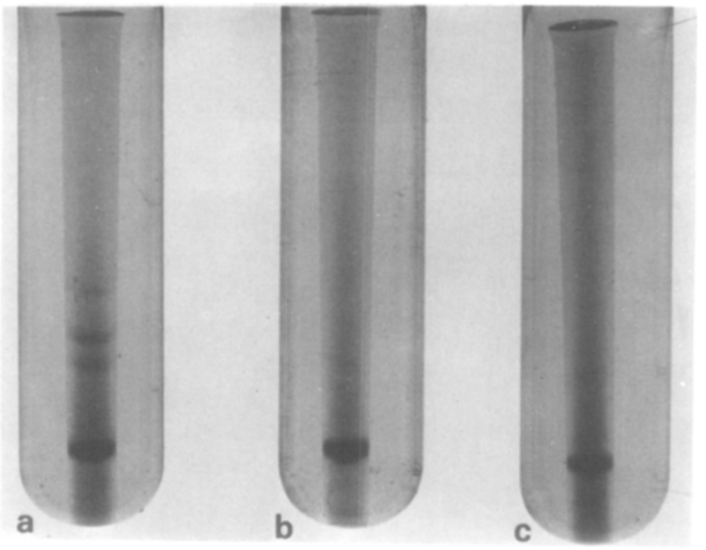

Fig. 1. SDS polyacrylamide gel electrophores of

A) "Z region" obtained by Sephadex G-100 chromatography of 110,000 x g liver supernatant
B) Purification of (A) using DEAE Sephadex as described in Methods
C) Ribonuclease standard M.W. 12,000.

Gel electrophoresis was performed using 7.5% BIS Acrylamide 10 mM Tris-HCl buffer (pH 8) according to the method of Fairbanks et al (20)

DISCUSSION: It is well known that the incorporation of glycerol-3-phosphate into phospholipid by various cell-free systems is enhanced by the addition of boiled microsomes or albumin (10), as well as heat labile proteins in the 104,000 x g soluble fraction (11). On the basis of these observations, it has been proposed (12) that in the intact cell, a protein-bound acyl group rather than acyl-CoA, serves as the acylating agent and that by this mechanism specificity could be exerted in either the formation of the acyl protein complex, or in the transfer of the acyl group from the protein to glycerol-3-phosphate. To date, this hypothesis has been tested in cell-free preparations using acyl carrier protein (ACP) isolated from bacteria, with essentially negative results (13,14). The present study provides new evidence in support of this hypothesis by de-

TABLE I

EFFECT OF Z PROTEIN ON INCORPORATION OF (^{14}C)SN-GLYCEROL-3-PHOSPHATE INTO MONOACYLGLYCEROPHOSPHATE

PROTEIN	NMOLES	INCORPORATION OF (^{14}C)SN-GLYCEROL-3-PHOSPHATE (nmoles)	Z/ALBUMIN
Albumin (6)*	40.0	0.70 ± 0.05	6.66
"Z" (4)	40.0	4.66 ± 0.25	
Albumin (4)	12.5	0.26 ± 0.09	9.78
"Z" (6)	12.5	2.54 ± 0.29	
None (4)	-	0.13 ± 0.04	

*Number of Determinations.

monstrating that Z, a 12,000 M.W. protein present in the supernatant fraction of liver, muscle, small intestine, kidney and adipose tissue (2), significantly enhances the esterification of glycerol-3-phosphate in the presence of palmityl CoA.

Furthermore, the superiority of Z protein over albumin in our assay system (Table I) appears to be highly significant, in view of the fact that albumin is present in trace amounts in the supernatant fraction of liver (15), in contrast to Z which constitutes $\sim$ 2% of liver supernatant proteins (20-25 mg/g liver protein) (16). On the basis of these observations it would appear that Z protein might be the more "physiological" protein for use in these enzyme assays.

At present one can only speculate on the possible role of Z protein in other aspects of fatty acid metabolism. It is of note that the substrate specificity of 1-acylglycerol-3-phosphate acyltransferase for var-

ious forms of acyl-CoA is analogous to the relative binding affinity of these compounds for Z, namely: oleyl CoA > linoleyl CoA $\simeq$ palmityl CoA > myristyl CoA > stearyl CoA > lauryl CoA (17). It remains to be determined whether Z protein is the answer to the paradox that the positional specificity manifested in the intact cell is different from that observed with the cell-free preparations currently used (12).

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