THE BINDING OF LONG CHAIN FATTY ACID CoA TO Z, A CYTOPLASMIC PROTEIN PRESENT IN LIVER AND OTHER TISSUES OF THE RAT*

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SUMMARY: A cytoplasmic protein (Z protein) has been shown to have a high affinity for the CoA derivatives of long chain fatty acids. Palmityl-CoA was reversibly bound to Z by a single class of high affinity binding sites with an apparent Kd $\approx 2.4 \times 10^{-7}$ moles at 4° C. Bromopalmitin, an inhibitor of fatty acid metabolism, as well as fasting, reduced the binding of palmityl-CoA to Z, while chlorphenoxy-isobutyrate enhanced the binding. Z protein in other tissues also showed a high affinity for palmityl-CoA.

INTRODUCTION: Z is a 12,000 M.W. protein present in the supernatant fractions of rat liver, kidney, skeletal muscle, intestinal mucosa and adipose tissue. Z binds organic anions, particularly bilirubin, cholecystographic agents and long chain fatty acids both *in vitro* and *in vivo* (1,2,3). In view of the fact that Z is believed to be one of the mediators of hepatic bilirubin uptake (4), it was proposed that binding to Z influenced fatty acid uptake by restricting the back diffusion of these molecules. We have tested this hypothesis using flavaspidic acid, a specific inhibitor of organic anion binding to Z protein (1). *In vivo*, inhibition of fatty acid binding to Z yielded two significant observations; fatty acid uptake by the liver was

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unchanged, while fatty acid esterification was significantly reduced (5). These results implied that binding of fatty acid to Z was not an obligatory step in fatty acid uptake and raised the possibility that Z protein may be involved in some other aspect of fatty acid metabolism. We speculated whether Z might interact with the activated CoA derivatives of fatty acids which enter into the various enzyme reactions within the cell. This report describes *in vitro* studies of the capacity of Z protein to bind the CoA derivatives of fatty acids.

MATERIALS: [^{14}C]palmityl-CoA and [^{3}H]palmitic acid were purchased from New England Nuclear Corp., Boston, Mass. Unlabelled fatty acids as well as their CoA and carnitine derivatives were obtained from Sigma Chemical Co., and P & L Biochemical Co., and β-flavaspidic acid-N-methyl-glucaminate was from Dr. Ahö, Turku, Finland.

METHODS: Male Sprague-Dawley rats (200-300 gm) were anesthetized with ether. Their livers were removed, rinsed and perfused with isotonic saline, blotted, weighed and homogenized in 0.5 N saline (identical results were obtained with 0.25 M sucrose in 0.01 M phosphate buffer) to yield a 33% homogenate (W/V). A supernatant fraction was prepared by centrifugation at 110,000 x g for 90 minutes at 2° C in a Model L Spinco ultracentrifuge. Aliquots of supernatant representing 2 g of liver were mixed with various amounts of [^{14}C]palmityl-CoA and other ligands (dissolved in 50 μl of Na acetate buffer 0.1 M pH 5.8). The mixture after equilibrating for 30 minutes at 4° C was subjected to upward flow chromato-

graphy on Sephadex G-100 (2.5 x 43 cm) using 0.5 N saline as the eluting medium. The presence of Z protein in the "Z fraction" of liver supernatant was established by electrophoretic and immunological methods described in an earlier communication (1).

Protein concentrations in the eluted fractions were determined by absorbance at 280 mμ and by the Lowry method (6). Radioactivity in each fraction was determined by counting 1 ml aliquots in 12 ml of Aquasol (New England Nuclear) in a Packard Liquid Scintillation counter (Model 3375). Quench correction was by external standardization.

RESULTS: Binding of [^{3}H]palmitic acid and [^{14}C]palmityl-CoA (4 x 10^{-9} moles) to the proteins in a 110,000 x g liver supernatant after chromatography on Sephadex G-100 is shown in Figure 1. 7.8% of palmitic acid and 46.4% of

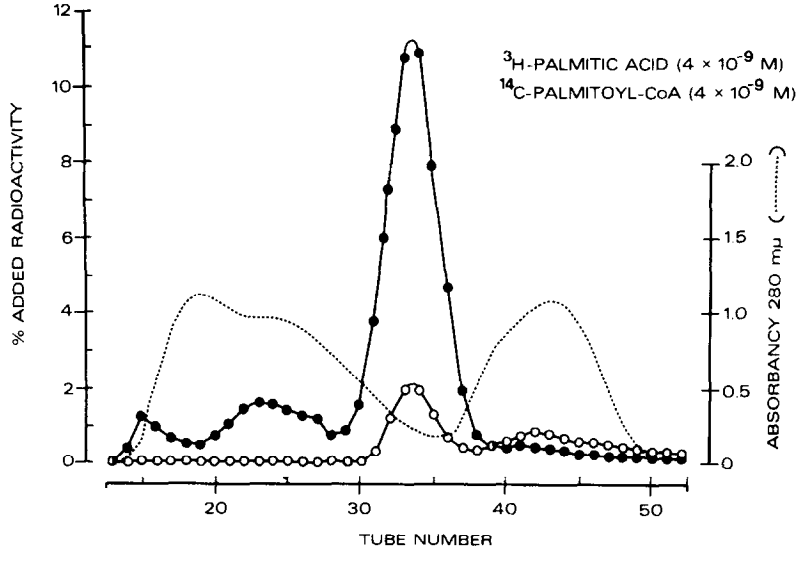

Fig. 1 Binding of [^{3}H]palmitic acid and [^{14}C]palmityl-CoA to the proteins in rat liver supernatant after chromatography on Sephadex G-100. The "Z region" was eluted in tubes 30-38 (the peak of the Z region occurs at x 2.5 the void volume). The open circles represent [^{3}H]palmitic acid and the closed circles represent [^{14}C]palmityl-CoA.

palmityl-CoA radioactivity were recovered in the "Z fraction", indicating that Z protein has a higher affinity for the CoA derivative of palmitic acid than palmitic acid alone. The percentages of [^{14}C]palmityl-CoA bound to high M.W. proteins in the void volume, as well as to albumin (as determined by acrylamide gel electrophoresis) were 2.8% and 10.3% respectively. The binding of [^{14}C]palmityl-CoA to Z was reversible as indicated by the finding that increasing amounts of unlabelled palmityl-CoA effectively displaced the radioactive compound from Z (Figure 2). The relationship between

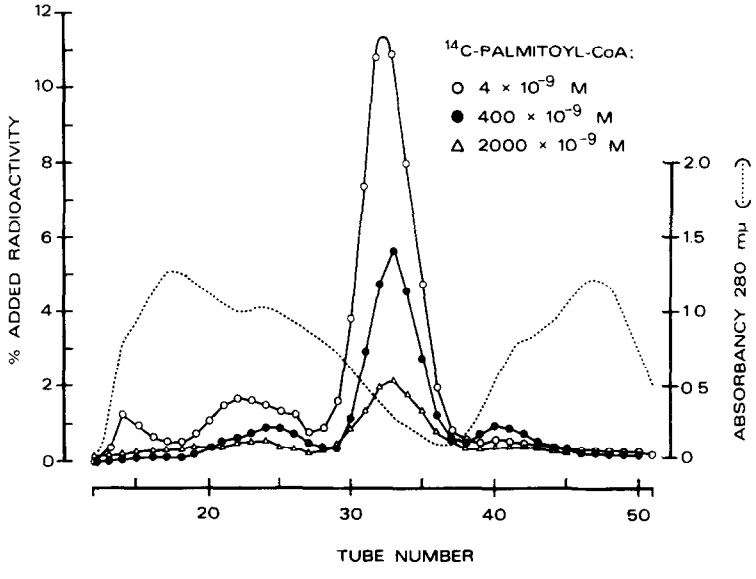

Fig. 2 Effect of increasing concentrations of palmityl-CoA on the binding of [^{14}C]palmityl-CoA (4 x 10^{-9} moles) to the proteins in rat liver supernatant after chromatography on Sephadex G-100. The Z region was eluted in tubes 30-37. Unlabelled palmityl-CoA (dissolved in 50λ of Na acetate buffer) was added at the same time as [^{14}C]palmityl-CoA (also dissolved in 50λ of Na acetate buffer). A total of 100λ of Na acetate buffer was added in each case.

the amount of [^{14}C]palmityl-CoA added to a constant volume of liver supernatant and the amount of palmityl-CoA recovered on Z after chromatography is plotted in Figure 3A. The initial rapid rise in uptake, which tapered off with amounts of palmityl-CoA in excess of 40×10^{-8} moles, indicates that saturation of binding sites had occurred. A plot of the binding data according to Scatchard (7) indicated a single class of high affinity binding sites with an apparent dissociation constant of 2.4×10^{-7} M at 4° C (Figure 3B). The concentration of binding sites, calculated from the results of eight experiments, was approximately 0.5 - 1.0 moles of palmityl-CoA per mole of Z protein.

We have studied the ability of various unlabelled fatty acids and their CoA derivatives (2.0×10^{-6} moles) to displace [^{14}C]palmityl-CoA (0.4×10^{-6} moles) from Z protein. These ligands were added at the same time as [^{14}C]palmityl-CoA to constant amounts of supernatant which were subsequently chromatographed on Sephadex G-100. On the assumption that these compounds share common binding sites, we used this method to determine the relative affinity of Z protein for each of these substances (Table 1). The results indicated that Z protein has a high affinity for the CoA derivatives of C14:0, C16:0, C18:0, C18:1, and C18:2 fatty acids. Oleic acid was tightly bound to Z in agreement with our previous studies (1). Palmityl-carnitine, the activated intermediate of palmitic acid which traverses the mitochondrial membrane, was more tightly bound than palmitic acid alone. The CoA derivatives of short chain fatty acids and CoA alone did not

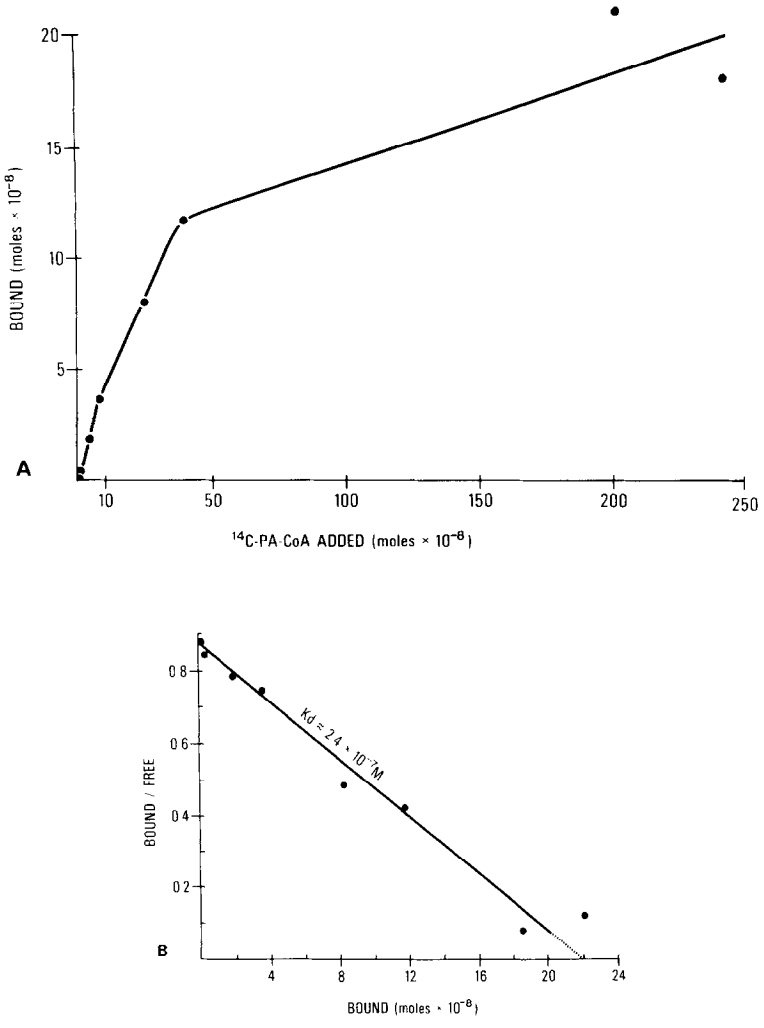

Fig. 3 A. Effect of addition of increasing amounts of palmityl-CoA to liver supernatant (equivalent to 2 g liver) on the amount of palmityl-CoA (PA-CoA) recovered in the "Z fraction" after Sephadex G-100 chromatography.

B. Scatchard plot of [^{14}C]palmityl-CoA bound to the "Z region" of rat liver supernatant. Data were calculated from the results of A.

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bind to any significant degree. Bromopalmitin, an inhibitor of various stages of fatty acid metabolism (8) markedly reduced the binding of palmityl-CoA to Z, while chlorphenoxyisobutyrate significantly enhanced the

TABLE 1

INHIBITION OF ^{14}C-PALMITYL-CoA BINDING TO Z

Substance		% Inhibition	Mean
C 14:0	Myristyl-CoA	69.0, 61.0	65.5
C 16:0	Palmityl-CoA	52.3, 76.0	64.2
C 18:0	Linoleyl-CoA	60.3, 69.6	65.0
C 18:1	Oleyl-CoA	60.9, 59.8	60.3
C 18:0	Stearyl-CoA	50.0, 60.7	55.4
C 18:1	Oleic Acid	57.1	57.1
C 16:0	Palmityl-Carnitine	56.6, 57.0	56.8
C 16:0	Palmitic Acid	15.7	15.7
C 12:0	Lauryl-CoA	0	0
C 3:0	Propionyl-CoA	4.7	4.7
C 3:0	Malonyl-CoA	4.3, 0	2.2
	CoA	0, 0	0

binding. Palmityl-CoA binding to Z was reduced by fasting (24 hours) while Z concentration was not detectably decreased. Z protein present in myocardium, skeletal muscle, and small intestine also showed a high affinity for palmityl-CoA.

DISCUSSION: Although the presence of the carrier molecules for fatty acid-CoA has been suggested (9), we believe that this study represents the first demon-

stration of the binding of fatty acid-CoA to cytoplasmic proteins. Our results indicate that palmityl-CoA is reversibly bound to Z protein by a single class of high affinity binding sites which appear to be specific for long chain fatty acids, their derivatives, and a number of other organic anions. In view of the documented ability of fatty acid-CoA to inhibit various enzyme reactions (9,10) we would wonder whether binding to Z protein might serve a protective function in the cell. It is of interest that the amount of Z protein in rat liver is adequate to bind the acyl-CoA normally present in this tissue (40 - 160 nmoles/g) (11). At present we can only speculate whether Z protein is involved in the intracellular transport of long chain fatty acid-CoA or actually serves as a cofactor in the enzymatic transformations of long chain fatty acids within the cell.

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