Molecular and Cellular Biochemistry **120**: 89–94, 1993.
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Review

Interaction of fatty acids, acyl-CoA derivatives and retinoids with microsomal membranes: effect of cytosolic proteins

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Received 8 May 1992; accepted 7 October 1992

Abstract

This paper reviews characteristics of microsomal membrane structure; long chain fatty acids, acyl CoA derivatives, retinoids and the microsomal formation of acyl CoA derivatives and retinyl esters. It is analyzed how the movement of these molecules at the intracellular level is affected by their respective binding proteins (Fatty acid binding protein, acyl CoA binding protein and cellular retinol binding protein). Studies with model systems using these hydrophobic ligands and the lipid-binding or transfer proteins are also described. This topic is of interest especially because in the esterification of retinol the three substrates and the three binding proteins may interact. (Mol Cell Biochem **120:** 89–94, 1993)

Key words: fatty acid binding protein, microsomes, long chain fatty acids, acyl-CoA derivatives, retinoids, liver

Abbreviations: FABP(s) – Fatty Acid Binding Protein(s), CRBP – Cellular Retinol Binding Protein, ACBP – Acyl-CoA-Binding Protein

Introduction

During recent years a number of reviews on the significance of the lipid binding proteins have been published [1–5] with each having a section devoted to aspects of the present topic. In this review I have summarized some data on the interaction of long chain fatty acids, their CoA derivatives and retinoids with microsomal membranes and the possible significance of their respective intracellular binding proteins with particular reference to the liver, since this organ play a major role in long chain fatty acids and vitamin A metabolism.

Our laboratory have, for some time, been interested in understanding the interaction of hydrophobic molecules (long chain fatty acids-retinoids) with natural and artificial membranes in an aqueous environment, in order to interpret the mechanisms by which these compounds move at the intracellular level. An understanding of these requires a simultaneous knowledge of the

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membrane structure, characteristics of the hydrophobic compounds and properties of cytosolic proteins that are involved in the process.

Most of the enzymes required for lipid biosynthesis in animal cells are localized on the rough and smooth endoplasmic reticulum [6]. Since long chain fatty acids as well as retinol are used mainly by the endoplasmic reticulum for the synthesis of Acyl-CoA derivatives and retinyl esters respectively [7, 8], it is my interest to review what is known about the factors that control the interaction of long chain fatty acids, acyl-CoA derivatives and retinoids with microsomal membranes and how the transcytosolic movement of these compounds is affected by specific binding proteins.

Characteristics of long chain fatty acids, acyl-CoA derivatives and retinoids

Long chain fatty acids as well as retinoids have in common the property of being very little soluble in water. However they fulfill different roles in the physiology of most animals. Whereas fatty acids are the most important substrates for energy production of the organism, form the hydrophobic interior of all biological membranes and are precursors of essential organic molecules such as prostaglandins, leukotrienes and tromboxane, retinoids (Vitamin A) are required for the maintenance of certain tissues (mainly epithelia), regulating the rate of proliferation and direction of differentiation of various cells within those tissues. And also, they provide the cromophore for the visual systems of all animals.

Acyl-CoA derivatives are the precursors of many reactions of lipid metabolism. These amphiphilic compounds, like other detergents, form molecular solutions only at low concentration. As concentration is increased, the critical micelle concentration (CMC) is reached and association into micelles begins. Intracellular concentrations of long chain acyl-CoA have been reported to vary between 110 and 152 μM in rat liver [9]. It is not clear if intracellular acyl-CoA exists transiently as monomers or micelles, bound to soluble proteins or associated with membranes. The intracellular protein ACBP that bind long chain acyl-CoA(s) may prevent micelle formation.

Microsomal membrane structure

Microsomes have been defined as an heterogeneous fraction of submicroscopic vesicles, 20–200 nm diameter, formed during disruption of the cell by the resealing of fragments of the endoplasmic reticulum (and to some extent of the plasma membrane). These vesicles are usually prepared by differential centrifugation of disrupted, homogenized cells; following sedimentation of larger fragments, microsomes are sedimented at 100000 g [10]. Microsomes that are of similar compositions as regards concentrations and activities of certain microsomal enzymes to that obtained by ultracentrifugation can be prepared by Sepharose gel filtration chromatography of postmitochondrial supernatant [11]. In experiments designed to study the interaction of microsomes with soluble proteins, these microsomes are preferable to those prepared by centrifugation since they are not contaminated with cytosolic proteins.

Liver microsomes contain great variety of phospholipids in a high concentration, in washed membranes the protein, phospholipid ratio is around 2 [12]. Microsomes contain the following species of phospholipids expressed as percent: phosphatidylcholine 55, phosphatidylethanolamine 19, phosphatidylserine 10, phosphatidylinositol 10 and sphingomyelin 6 [13]. Due to the presence of these molecules the microsomes have a high net negative surface charge density [14]. Microsomal membranes also contain cholesterol, which make up 7% of total lipids [13]. The fatty acid composition of the bulk lipids of rat liver microsomes is so highly unsaturated that they are very fluid at physiological temperatures and do not gelify till temperatures are far below 10° C [15].

The degree of unsaturation of phospholipid acylchain is determined, mainly, at the level of membrane biogenesis, where the adequate fatty acids are being selected on the process of phospholipid biosynthesis. The acyl-CoA desaturation system located on the membrane of the endoplasmic reticulum can manipulate the number of double bonds in the available pool of fatty acids [16]. Whether or not the lipid binding proteins can induce a net change in the overall degree of unsaturation is still a point of controversy.

Microsomal formation of acyl-CoA derivatives and retinyl esters

Fatty acids of endogenous or exogenous origin can be found in the cytoplasmic environment of the cell. Ockner *et al.* [17] demonstrated that in rat liver, 60% of the cytosolic long-chain fatty acids, is associated with the FABP-containing fraction. Fifty to seventy percent of these endogenous fatty acids are unsaturated: oleate, linoleate and arachidonate. The first step for the synthesis of different types of lipids is the activation of the fatty acid to acyl-CoA. The enzyme responsible for this synthesis is an acyl-CoA synthetase [acid: CoA ligase (AMP) EC 6.2.3] present in microsomes and has been extensively studied [18, 19].

Vitamin A is stored mainly in liver as retinyl ester. In several vertebrate species examined, retinyl palmitate, stearate and oleate accounted for approximately 90% of all retinyl esters found in the liver [20]. The enzyme acyl-CoA: retinol acyltransferase (ARAT), EC 2.3.1.76) responsible of retinol esterification is found mainly bound to liver microsomes. Retinyl ester synthesis is a two-substrate reaction requiring an acyl donor and retinol. An endogenous acyl donor is present in microsomes, giving retinyl ester on addition of retinol alone [21].

Cytosolic proteins which bind long chain fatty acids, acyl-CoA derivatives and retinoids

The utilization by the cell of long chain fatty acids and retinoids appears to be mediated by specific cellular binding proteins. The properties of these proteins are given in Table 1. Two small (14–15 KDa) proteins capable of binding fatty acids and retinol and therefore named fatty acid binding protein (FABP) and cellular retinol binding protein (CRBP), has been analyzed. Recently the sequence homology of these two binding proteins has been reported [22]. They have structural similarities, it is, however unlikely that CRBP may play a role in the intracellular movement of retinyl esters since this protein has strict binding specificity toward retinol, and in addition its cellular content is too low to operate the movement of retinyl esters.

Table 1. Properties of proteins that bind long chain fatty acids, acyl-CoA derivatives and retinol

Protein	Ligand(s)	Mr (KDa)	Content nmol/g w.w.	Kd (μM)	Reference
FABP	fatty acids	14.2	200	0.5–1.0	33
	retinyl esters			1.4	30
ACBP	acyl-CoA	10.0	28	0.1–0.2	31
CRBP	all-trans retinol	14.6	3.4	0.016	34–35
			1.2		36
CRBP II	all-trans retinol	16.0			23

Besides cellular retinol binding protein (CRBP) other related proteins have been described, i.e. CRBP II and cellular retinoic-binding protein (CRABP). Although the latter does not seem present in liver, CRBP II is an intracellular retinol binding protein presumed to be involved in retinol esterification, expressed primarily, if not solely, in the villus-associated enterocytes of the small intestine of adult rats [23]and transiently in liver hepatocytes of neonates [24].

These cytosolic non-enzymic proteins involved in the metabolism of lipophilic compounds are thought to act as intracellular transport proteins and may effect the specific transfer of long chain fatty acids or retinol to the enzyme site of the microsomal bound enzymes. Thus (FABP) modulate, *in vitro*, the activity of hepatic microsomal acyl-CoA synthetase [25], acyl-CoA: glycerol-3-phosphate acyltransferase [25, 26], phosphatidate phosphohydrolase [27], diacylglycerol acyltransferase [28] and stearoyl-CoA desaturase [29].

It has been demonstrated that the intracellular transport and storage of retinyl esters is conducted by FABP. By using anti-FABP serum, it was shown that FABP was localized in the parenchymal cells and with particularly high concentration in the perisinusoidal cells, probably fat-storing cells [30].

Acyl-CoA-binding protein (ACBP) is a 10 KDa cytosolic protein which binds medium- and long-chain acyl-CoA derivatives with high affinity, thus the apparent Kd for acyl-CoA binding to bovine and rat ACBP was determined to be 0.14 μM and 0.13 μM respectively, for oleoyl-CoA, and 0.22 μM and 0.28 μM for palmitoyl-CoA [31]. ACBP does not bind fatty acids.

It has been determined that rates of hydratation of fatty acids bound to lipid vesicles appear to be rapid enough to account for intracellular movement between compartments in the absence of carrier proteins. Increasing fatty acid chain length diminishes the rate of

hydratation whereas increasing unsaturation increases this rate [32]. However, the estimated concentration of FABP is at least an order of magnitud greater than that of monomeric long chain fatty acids, and its likely that transport in the bound state may constitute the more efficient mechanism of transport for these hydrophobic molecules [25].

Studies in model systems

a) Long chain fatty acids

Although movement of long chain fatty acids can occur spontaneously, it has been postulated that intracellular movement is facilitated by FABP.

The possible function of FABP to act as a long chain fatty acid carrier protein has been investigated in model system by many laboratories. Thus, liver FABP interacts with long chain fatty acid that desorb from phospholipid bilayers and promotes movement to a membrane-bound enzyme (acyl-CoA synthetase associated with rat liver microsomes), suggesting that FABP may act intracellularly by increasing net desorption of long chain fatty acid from cell membranes [37]. When mitochondrias were separated from vesicles in an equilibrium dialysis cell, a stimulating effect of FABP on oleic acid transfer could be demonstrated [38]. These results indicate that the FABP-long chain fatty acid complex may function as an intermediate in the transfer of long chain fatty acids between membranes.

Liver fatty acid binding protein can interact with different kinds of membranes increasing specifically the desorption of fatty acids. The cytosolic proteins can act as acceptors for fatty acids but not for phospholipids of microsomal origin. These experiments were confirmed using liposomes made of egg yolk phosphatidylcholine, containing both (C^{14}) labeled phospholipids and (1-C^{14}) palmitic acid [39].

b) Acyl-CoA derivatives

Both rat ACBP and bovine ACBP were able to extract [1-^{14}C]hexadecanoyl-CoA and cis-9-[1-^{14}C]octadecenoyl-CoA from multilamellar liposomes, in addition bovine ACBP almost completely prevented acyl-CoA binding to heat denatured microsomal membranes when added in equimolar amounts to the acyl-CoA derivatives [31]. Results from the same laboratory indicated that ACBP binds acyl-CoA derivatives incorporated in multilamellar liposomes with much higher affinity than either bovine cardiac or hepatic FABP. Considering that the concentration of ACBP in liver is 2-4-fold the acyl CoA concentration, it must be expected that ACBP, and not FABP, is the transport protein for acyl-CoA derivatives in the liver.

c) Retinoids

The transfer of retinoic acid and retinyl palmitate between single unilamellar vesicles was studied by resonance energy transfer. The retinoic acid transfer spontaneously between single unilamellar vesicles with a first order rate constant of $9.6\,s^{-1}$ at 15° C and pH 7.4, but not spontaneous transfer of retinyl palmitate was observed over 60 min [40]. In our laboratory we have conducted experiments in order to explore the effect of mouse liver cytosolic proteins enriched in (FABP) or (CRBP) on the removal of palmitic acid and retinoids from microsomal membranes. When the soluble proteins were incubated with mouse liver microsomes containing (C^{14})palmitic acid or (H_3) retinoids, it was observed that (FABP) removed selectively the fatty acid and retinyl esters, whereas the retinol was mainly removed by (CRBP) [41]. These results are in accordance with those of Fukai *et al.* [30] who have demonstrated from binding analysis that FABP has a relatively high affinity ($Kd = 1.4 \times 10^{-6}\,M$) to retinyl palmitate, while binding of retinyl palmitate to CRBP was scarcely detectable. When mouse liver cytosol was fractionated by 70% ammonium sulphate, a precipitate and a soluble fraction were obtained. The soluble fraction containing FABP was enriched in endogenous retinyl esters, and was able to remove this retinoid as well as palmitic acid from microsomal membranes [42].

Conclusions

Long chain fatty acids and retinoids have in common the physical property of extreme hydrophobicity. We now know specific cytosolic transport proteins which 'solubilize', and deliver these important molecules to their sites of metabolism. Despite the advances made in our knowledge of long chain fatty acids, acyl CoA and retinoid binding proteins, much remains to be learned. Since many of the metabolic events in which are involved long chain fatty acids and retinoids, occur associ-

ated with the endoplasmic reticulum, future research on the interaction of these small but abundantand closely regulated intracellular proteins must be expanded to include more studies of interaction between microsomes, long chain fatty acids, retinoids and acyl CoA derivatives to determine precisely how these molecules flow in the intracellular space of the cell.

Acknowledgement

I wish to thank Miss Gabriela Romero for help in the corrections of the english version of the manuscript.

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