Cholate-independent Retinyl Ester Hydrolysis

STIMULATION BY Apo-CELLULAR RETINOL-BINDING PROTEIN*

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Apo-cellular retinol-binding protein (apoCRBP) activated the hydrolysis of endogenous retinyl esters in rat liver microsomes by a cholate independent retinyl ester hydrolase. A Michaelis-Menten relationship was observed between the apoCRBP concentration and the rate of retinol formation, with half-maximum stimulation at 2.6 ± 0.6 μM (mean ± S.D., n = 5). Two other retinol-binding proteins, bovine serum albumin and β-lactoglobulin, acceptors for the rapid and spontaneous hydration of retinol from membranes, had no effect up to 90 μM. These data suggest activation of the hydrolase by apoCRBP directly, rather than by facilitating removal of retinol from membranes. The hydrolase responding was the cholate-independent/cholate-inhibited retinyl ester hydrolase as shown by: 60% inhibition of the apoCRBP effect by 3 mM cholate; apoCRBP enhancement of retinyl ester hydrolysis in liver microsomes that had no detectable cholate-enhanced activity; inhibition of cholate-dependent, but not apoCRBP-stimulated retinyl ester hydrolysis by rabbit anti-rat cholesteryl esterase. Compared to the rate (mean ± S.D. of [n] different preparations) supported by 5 μM apoCRBP in liver microsomes of 6.7 ± 3.7 pmol/min/mg protein [10], microsomes from rat lung, kidney, and testes had endogenous retinyl ester hydrolysis rates of 1.8 ± 0.3 [5], 0.5 ± 0.2 [3], and 0.3 ± 0.2 [5] pmol/min/mg protein, respectively. N-Ethylmaleimide and N-tosyl-L-phenylalanine chloromethyl ketone were potent inhibitors of apoCRBP-stimulated hydrolysis with IC50 values of 0.25 and 0.15 mM, respectively, but phenylmethylsulfonyl fluoride and diisopropylfluorophosphate were less effective with IC50 values of 1 mM, indicating the importance of imidazole and sulfhydryl groups to the activity. These data provide evidence of a physiological role for the cholate-independent hydrolase in retinoid metabolism and suggest that apoCRBP is a signal for retinyl ester mobilization.

Retinoic acid is an endogenous transcription modulator that is critical to embryonic development and the maintenance of normal differentiated phenotypes in adults (1, 2). In situ synthesis of retinoic acid occurs in a spectrum of vitamin A-dependent tissues from retinol, which is the principal, but not exclusive, metabolic precursor to retinoic acid (3–5). Liver is one retinoid target tissue that converts retinol into retinoic acid and it is also the quantitatively major locus of retinoid storage (for a review see Ref. 6). In the liver retinol occurs largely in two forms: bound to retinoid binding proteins, such as CRBP, and as esters of long chain fatty acids (7). Hydrolysis of hepatic retinyl esters provides substrate for in situ retinoic acid synthesis and is the first step in the mobilization and distribution of retinol to extrahepatic retinoid target tissues.

Initial work with retinyl ester hydrolysis reported a specific requirement for cholate, as opposed to a general requirement for detergent, and maximum activity at neutral pH (8). Since then studies have concentrated on this neutral, cholate-dependent retinyl ester hydrolysis activity (9–14). A requirement for cholate is problematic because liver is not the only site of retinyl ester storage and hydrolysis. Retinyl esters are stored in and mobilized from tissues that do not contain cholate (15, 16). Moreover, the cholate-dependent retinyl ester hydrolyase activity has higher affinity and specific activity for cholesteryl esters and triacylglycerol, even after partial purification (10, 13). These findings, and the demonstration that nonspecific antipancreatic cholesteryl esterase inhibits cholate-dependent retinyl ester hydrolysis (17), demonstrate that the cholate-dependent activity and pancreatic cholesteryl ester hydrolyase are closely related or identical. Recently, neutral, cholate-independent retinyl ester hydrolysis activity was demonstrated in liver and in extrahepatic tissues (17–19). When partially purified, this microsomal activity did not hydrolyze cholesteryl esters and had higher specific activity for retinyl esters than it did for triacylglycerol (18, 19), implying that this activity is the one physiologically important to retinyl ester hydrolysis.

The present study was undertaken to further explore a role for the retinol binding protein, CRBP (20), in retinol metabolism and especially to determine whether CRBP modifies cholate-independent retinyl ester hydrolysis activity. The results will show that apo-CRBP activates cholate-independent but not cholate-dependent retinyl ester hydrolysis. These data strengthen the evidence that the cholate-independent hydrolysis is physiologically significant to retinol mobilization, provide new insight into the function of CRBP, and suggest a mechanism for signaling mobilization of retinyl ester stores to provide retinol for retinoic acid synthesis.

EXPERIMENTAL PROCEDURES

Materials—BSA, β-lactoglobulin, and most other chemicals were purchased from Sigma. Bovine adrenal apoCRBP was expressed in

The abbreviations used are: CRBP, cellular retinol binding protein; CRABP, cellular retinoic acid binding protein; BSA, bovine serum albumin; DIFP, diisopropylfluorophosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high performance liquid chromatography; NEM, N-ethylmaleimide; TPCK, N-tosyl-L-phenylalanine chloromethyl ketone; PMSF, phenylmethylsulfonyl fluoride.

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Escherichia coli with the expression vector pET-3a/CRABP (21). The two independently raised rabbit antibodies to rat pancreatic esterase were obtained from two investigators: Dr. David Y. Hui, The University of Cincinnati College of Medicine (22), and Dr. Linda L. Gallo, George Washington University Medical School (23).

Preparation of CRBP—CRBP was expressed in E. coli with the vector pMONCRBP, a gift from Dr. Marc Levin, Washington University School of Medicine, and was purified as described up to including disrupting the cells (24). One-half of the 100,000 x g supernatant was saturated with radiolabeled retinol. The two portions (~500 mg each) were eluted from individual Sepharose G-50 columns (5 x 100 cm) with buffer A (20 mM Heps, 150 mM KCl, 2 mM dithiothreitol, 1 mM EDTA, pH 7.5). The amount of apoCRBP recovered was verified by saturating an aliquot of it with retinol, separating bound from free with Sephadex G-75 (1 x 5 cm column) eluted with buffer A, and determining the A430/A260 (25). To determine whether the various hydrolase inhibitors affected the integrity of CRBP, 5 μM holocRBP was incubated with the highest concentration of inhibitor used, and the UV of the complex was obtained. Zinc eradicated the UV spectrum. The other inhibitors had no effect.

Preparation of Microsomes—Tissues were obtained from male Sprague-Dawley rats that had been starved overnight and were sacrificed by decapitation. The tissues were rinsed in ice-cold saline; kidneys and testes were decapsulated, and tissues were homogenized (1 g/4 ml) in buffer B (10 mM Heps, 250 mM sucrose, 1 mM EDTA, 2 mM dithiothreitol, pH 7.5). Microsomes were prepared by differential centrifugation as described (26). The microsomes were homogenized in buffer B (20 mg protein/ml) and were stored in small aliquots at -80 °C. Protein was measured by the dye-binding method described under "Experimental Procedures." The absorbance scale (y axis) was equivalent to 0.005 absorbance units at full scale.

Retinyl Ester Hydrolysis Assay—Assays were done at 37 °C in buffer A for 30 min with 1 mg of microsomal protein in a final volume of 0.2 ml, unless noted otherwise. The pH range of maximum activity was between 7.5 and 9, and therefore a pH of 8.0 was used routinely. Assays were done in duplicate, and generally, duplicates were within 10% of their averages. To quench the reaction 0.3 ml of buffer B and 1 ml of 0.025 N KOH/ethanol were added sequentially to the incubation mixture (27). Neutral retinoids were extracted with 2.5 ml of hexane. The hexane was evaporated under a stream of nitrogen and the residue was dissolved in 0.1 ml of hexane and analyzed by HPLC.

HPLC Analyses—Retinol was quantified as reported with the following modifications (27, 28). The normal-phase HPLC column was a DuPont Zorbax-Si1 Reliance Cartridge (0.4 x 4 cm) from which retinol eluted in 5 min at a flow rate of 2 ml/min with 5% aceton/hexane. Absorbance was monitored with a Waters model 484 Tunable Absorbance Detector set at 325 nm. Retinol was quantified by comparing its integrated peak area to those of known quantities of standards. A standard curve with 8 points from 200 to 1600 pmol of retinol (axis) versus peak area (y axis) produced a straight line (r > 0.99) with a slope of 0.0067. Overall retinol recovery was >75% from incubation through HPLC.

Kinetic Data—Kinetic data were fitted to Michaelis-Menten plots with the microcomputer program "Enzfitter" (29).

RESULTS

Stimulation of Endogenous Retinyl Ester Hydrolysis by ApoCRBP—Unesterified retinol in rat liver microsomes was readily measured by normal-phase HPLC (Fig. 1). Incubation of the microsomes at 37 °C, without adding retinyl ester as substrate, resulted in a 3-fold increase in free retinol, reflecting the temperature-dependent hydrolysis of endogenous retinyl esters. Including 5 μM apoCRBP in the 37 °C incubation produced a nearly 4-fold additional increase in unesterified retinol, showing that apoCRBP stimulates the hydrolysis of endogenous membrane retinyl esters.

Different microsomal preparations had different background retinol concentrations (pmol/mg protein), but with any specific preparation the background retinol concentration was constant regardless of the amount of protein used, i.e. the retinol level was proportional to the amount of protein (e.g. Fig. 2, bottom panel). The increase in free retinol relative to this background was directly proportional to the amount of microsomal protein incubated at 37 °C in the absence or presence of apoCRBP over a wide range of microsomal protein. The temperature-dependent hydrolysis of endogenous retinyl esters in the absence of apoCRBP was rapid and limited: the maximum increase was observed at zero time (Fig. 2, top panel). Including apoCRBP in the incubation produced an additional increase in retinol that depended on the microsomal protein.

![Fig. 1. HPLC analyses of retinol in rat liver microsomes and retinol hydrolyzed from endogenous retinyl esters.](image1)

![Fig. 2. Relationship of retinol formation to incubation time and the amount of microsomal protein. Top panel, retinol formation from endogenous retinyl esters versus time in the absence (filled circles) and presence of 8 μM apoCRBP (open circles). Incubations were done at 37 °C with 1 mg of microsomal protein. Bottom panel, retinol formation versus microsomal protein at 4 °C (triangles), 37 °C (filled circles), 37 °C plus 5 μM apoCRBP (open circles). Incubations were done for 30 min.](image2)
bation time, in contrast to apoCRBP-independent hydrolysis. ApoCRBP sustained the rate of retinyl ester hydrolysis for at least 30 min, after which retinol accumulation ended, even though CRBP was only 50% occupied. The reaction did not stop as a result of retinyl ester depletion. The retinol released typically represented not more than 10% of the total esters present, which ranged from 8 to 11 nmol/mg of microsomal protein, depending on the specific liver microsomal preparation.

A hyperbolic relationship was observed between retinol formation and the apoCRBP concentration, showing that the apoCRBP-stimulated rate of hydrolysis was apoCRBP concentration-dependent and saturable kinetically (Fig. 3). The average concentration of apoCRBP that produced a half-maximum stimulation of retinyl ester hydrolysis was 2.6 ± 0.6 μM (± S.D., n = 5). At the end of the experiment shown, the CRBP was 73% occupied with ligand at the lowest CRBP concentration, assuming the "background" retinol was bound as well as the newly generated retinol, and was less than 4% occupied at the highest concentration. At 10 μM apoCRBP, in the five experiments done, from 8 to 47% of the CRBP was bound with retinol, assuming that the background free retinol was also bound. (If the free retinol in the microsomes were in a CRBP-inaccessible pool, then the occupancy would range from about 6 to 32%.) Saturation of apoCRBP with ligand, therefore, was not responsible for attenuating the rate of retinyl ester hydrolysis.

Velocities stimulated by 5 μM apoCRBP were determined with 10 different microsomal preparations. The range of these was 2.7 to 14 pmol/min/mg protein with a mean (± S.D.) of 6.7 ± 3.7 pmol/min/mg. Four individual livers from a group with a mean of 4.9 ± 1.2 pmol/min/mg protein were assayed to obtain the animal to animal variation. The range was 3.7 to 6.8 pmol/min/mg.

To determine whether holoCRBP interferes with apoCRBP, the rates of retinol hydrolysis over concentration ranges of apoCRBP similar to those in Fig. 3 were determined in the presence of 0, 0.5, 1, and 3 μM holoCRBP. No inhibition was noted.

Three other retinoid binding proteins were tested to determine whether the activation of retinyl ester hydrolysis was specific to apoCRBP. BSA and β-lactoglobulin bind retinol (30, 31) and are acceptors for retinol donated from hepatic membrane preparations during in vitro transfer experiments (32, 33). Under conditions in which 5 μM CRBP caused a 4- to 5-fold stimulation in the rate of retinyl ester hydrolysis, neither BSA nor β-lactoglobulin as high as 90 μM increased retinyl ester hydrolysis (Table I). CRABP is a specific retinoid acid binding protein (34) that shares considerable amino acid sequence similarity with CRBP. ApoCRABP had no effect on retinyl ester hydrolysis, nor did an equimolar mixture of apoCRABP and holoCRABP.

Differences between the ApoCRBP-stimulated and Cholate-dependent Retinyl Ester Hydrolase Activities—A distinction was made recently between cholate-dependent retinyl ester hydrolysis and cholate-independent retinyl ester hydrolysis in liver and other tissues. The latter not only does not require cholate for catalytic activity, but is inhibited by cholate (17-19). The impact of cholate on the ability of apoCRBP to stimulate retinyl ester hydrolysis was tested to determine which of the two activities was sensitive to apoCRBP. The hydrolysis of endogenous retinyl esters in the absence of apoCRBP was not affected by less than 6 mM cholate, but was stimulated by greater than 6 mM cholate (Fig. 4). The maximum increase of about 6-fold was noted at cholate concentrations of 18 mM and greater, consistent with previously reported data using radiolabeled exogenous retinyl palmitate as substrate (9-11, 17). In the absence of cholate, apoCRBP caused a 3-fold stimulation in retinol formation. Cholate concentrations as low as 1 mM, i.e. concentrations that did not stimulate the cholate-dependent activity, inhibited apoCRBP-stimulated retinyl ester hydrolysis. Inhibition was cholate concentration-dependent, with 3 mM cholate causing 60% reduction. The marked reduction in apoCRBP-stimulated retinol formation by the low concentrations of cholate indicate that apoCRBP activates the cholate-independent/cholate inhibited retinyl ester hydrolase.

Cholate-dependent hydrolase activity varies from undetectable to as high as 200 pmol/min/mg protein with exogenous radiolabeled retinyl palmitate as substrate, although the average is closer to 40 pmol/min/mg (17, 19). Six microsomal
preparations were identified that did not respond to 18 mM cholate by hydrolyzing endogenous retinyl esters. Each of these responded to 5 \( \mu \text{M} \) apoCRBP. The average increase in retinol was 3.4-fold (S.D. = ± 1.3), with an average rate of 4.4 pmol/min/mg (±1.1). Three of these were determined whether the apoCRBP-stimulated hydrolysis was inhibited by 18 mM cholate. The inhibition observed was 68, 100, and 100%.

The cholate-dependent retinyl ester hydrolyase activity is inhibited by antibodies to rat pancreatic cholesteryl esterase, whereas the cholate-independent hydrolyase is not (17). To further classify the hydrolyase activity stimulated by apoCRBP, the effects of rabbit anti-rat cholesteryl esterase antisera were tested on the hydrolysis of endogenous retinyl esters stimulated by either cholate or apoCRBP (Fig. 5). ApoCRBP-stimulated retinol formation was unaffected by incubating microsomes with anti-rat pancreatic esterase, whereas 15 \( \mu \text{g} \) of antisera protein (from Dr. David Hui) per mg of microsomes inhibited the cholate-dependent activity by 80%. When the experiment was repeated with 45 \( \mu \text{g} \) of rabbit anti-rat pancreatic esterase antisera (from Dr. Linda Gallo) per mg of microsomal protein, cholate-stimulated hydrolysis was inhibited 100%, but apoCRBP-stimulated hydrolysis was not inhibited at all. Preimmune serum had no effect on hydrolysis stimulated by cholate or apoCRBP.

Characteristics of the ApoCRBP-stimulated Retinyl Hydrolyase Activity—A series of hydrolyase inhibitors had differential effects on apoCRBP-stimulated retinyl ester hydrolysis (Fig. 6). The most potent inhibitors were TPCK and NEM, which caused 50% inhibition at 0.15 and 0.25 \( \mu \text{M} \), respectively. At higher concentrations each produced nearly complete inhibition. TPCK is specific for the imidazole function of the histidine residue in the active site of serine and thiol hydroases, whereas DIFP is considered to be specific for serine residues. These results indicate that the active site of apoCRBP-stimulated hydrolyase relies on a histidine residue and possibly a sulfhydryl residue. Alternatively, the sulfhydryl group inactivated by NEM may not be associated with the active site. Whether or not this hydrolyse is active site serine or thiol-dependent must await further character-

![](image)

**Fig. 5. Differential effects of rabbit anti-rat cholesteryl esterase antisera on cholate-dependent and apoCRBP-stimulated, cholate-stimulated retinyl ester hydrolysis.** Microsomes (15 mg/0.75 ml buffer B) were incubated with the indicated amount of antisera for 20 h at 4°C. One-mg protein aliquots were then assayed under standard conditions for retinyl formation in the presence of either 18 mM cholate (filled circles) or 5 \( \mu \text{M} \) apoCRBP (open circles). The data for each additive (cholate or apoCRBP) are independently normalized to the activity in the absence of IgG.

*Fig. 6. Inhibition of apoCRBP-stimulated retinol formation.** Retinol was measured in the presence of apoCRBP and DIFP (open circles), NEM (closed circles), TPCK (triangles), or PMSF (squares). Inhibitors were added to the microsomes 2 min before the incubation at 37°C began. These reagents affected the background retinol concentrations, nor did they affect the ability of CRBP to bind retinol.

| Inhibitor (mM) | Activity |
|---------------|----------|
| 0.0           | 1.0      |
| 0.5           | 0.5      |
| 1.0           | 0.0      |
| 250 nM retinoic acid | 0.0      |
| 100 \( \mu \text{M} \) retinyl palmitate | 0.0      |
| 10 to 500 \( \mu \text{M} \) \( \alpha \)-tocopherol | 0.0      |
| 2 mM TPCK     | 0.0      |
| 1 mM ZnCl\(_2\) | 0.0\(^a\) |

*Experiments were done under standard conditions as described in "Materials and Methods" with 5 \( \mu \text{M} \) apoCRBP. The rates are relative to the apoCRBP-stimulated rate in the absence of the reagent indicated. Experiments were done at least twice.

\(^*\)The inhibition with zinc may be due entirely to the ability of zinc to disrupt holoCRBP, as determined by UV analysis.

![Diagram](image)

*Figures and Table*
Retinyl ester

Fig. 7. Summary of CRBP effects on retinol metabolism in the liver. HoloCRBP is substrate for retinyl ester synthesis catalyzed by lecithin:retinol acyltransferase (L-RAT) and is also substrate for an NADP-dependent retinol dehydrogenase that catalyzes the synthesis of retinal as an intermediate in retinoic acid synthesis. The present work shows that cholate-dependent retinyl ester hydrolysis is stimulated by apoCRBP. These enzymes (lecithin:retinol acyltransferase, retinyl ester hydrolyase, retinol dehydrogenase) are microsomal. In contrast to the effect of apoCRBP, neither apoCRABP nor mixtures of apo- and holoCRABP appear to affect retinyl ester hydrolysis in vitro.

pmol of retinol/min/mg protein were much lower than those observed with liver.

DISCUSSION

The hydrolysis of retinyl esters, whether in liver, or in other retinoid target tissues, is fundamental to maintaining retinol homeostasis and the concentration of holoCRBP, a substrate for retinol formation, the initial reaction of retinoic acid synthesis from retinol (36). It seems likely that unique hydrolyases(s), distributed widely throughout retinoid target tissues and capable of responding to specific retinoid needs and/or regulators of retinoid metabolism, would be vital to in situ generation of biologically active retinoids. The "retinyl ester hydrolyase" frequently studied not only has a specific requirement for cholate, but also has greater activity with non-retinoid esters, such as cholesteryl esters, even after partial purification (10, 13). This report shows that apoCRBP activates the hydrolysis of endogenous retinyl esters by the cholate-independent activity that is distributed throughout retinoid target tissues, including liver, testes, kidney and lung (17). This strengthens the case that the cholate-independent retinyl ester hydrolyase is the one physiologically important to retinoid metabolism and provides further insight into the role of CRBP in retinol metabolism.

The mechanism of apoCRBP-stimulated retinyl ester hydrolysis is not likely to be sequestration of retinol. Although retinol is sparingly soluble in aqueous media, it spontaneously and rapidly (t½ = 1.1 s) hydrates from hepatic membranes and diffuses through the aqueous phase to bind with acceptors such as BSA (32, 33). If the mechanism of apoCRBP action were merely to remove retinol from the microsomal membranes, then other proteins that are retinol acceptors, such as BSA and β-lactoglobulin (30, 31), should have had the same effect as CRBP. The failure of BSA and β-lactoglobulin to stimulate retinol formation by microsomes suggests that the mechanism of the apoCRBP effect is more specific than facilitating removal of retinol from the microsomes. Stimulation of hydrolysis by interaction of apoCRBP with a hydrolase, to facilitate removal of retinol from the active site or to stimulate the enzyme at a site distal to the active site, rather than indirectly through removing retinol from the membrane and relieving feedback inhibition, is suggested by these factors: rapid and spontaneous retinol hydration from membranes; enhancement of retinol formation specifically by apoCRBP; a hyperbolic relationship between the apoCRBP concentration and the rate of retinol formation.

CRABP binds retinoic acid specifically and does not bind retinol (25, 34), but shares considerable sequence homology with CRBP and belongs to the same superfamily of lipid binding proteins (37, 38). It is possible that CRABP has external topology similar to that of CRBP. The ratio apo/ holo-CRABP is also a logical possibility as a signal of retinoic acid status and might moderate retinyl ester hydrolysis to modulate availability of substrate for retinoic acid formation. Our results do not support this supposition because apoCRABP and 1:1 mixtures of apoCRABP and holoCRABP, a ratio close to the physiological one, do not activate retinyl ester hydrolysis.

The distinction between the cholate-dependent and cholate-independent hydrolyses rests on their dissimilar responses to cholate and to antibodies raised against cholesteryl esterase, and on the ability of apoCRBP to enhance retinol formation in microsomes that do not respond to cholate. The apoCRBP-stimulated hydrolyse was inhibited by concentrations of cholate (less than 6 mM) that did not influence the cholate-dependent hydrolyase, distinguishing both the nature of the responses to cholate and the concentrations of cholate that elicit the responses. The apoCRBP-stimulated hydrolyase also was present in and inhibited by cholate in microsomal preparations that did not respond to cholate with increased retinol formation, i.e. from which the cholate-dependent enzyme was absent. Inhibition of only the cholate-dependent activity by polyclonal antibodies to cholesteryl esterase suggests that cholate-independent retinyl ester hydrolyase is fundamentally distinct from the family of lipases that are involved in lipid digestion and absorption.

Another difference between the cholate-dependent and the cholate-independent hydrolyses are their responses to α-tocopherol. Dietary supplementation with α-tocopherol spares hepatic retinyl esters, whereas α-tocopherol-deficient diets result in retinyl ester depletion (11). The mechanism is unclear, but it does not appear to be related to the antioxidant properties of α-tocopherol, because sparing of retinyl esters does not occur in animals dosed with the antioxidant diphenylphenylene diamine, DPPD, the most effective synthetic α-tocopherol substitute known (39). Apparent insight into this phenomenon was provided with the report that cholate-dependent hydrolysis of retinyl palmitate by rat liver microsomes is inhibited 50% in vitro by 100 μM α-tocopherol (11). The lack of response of the apoCRBP-stimulated hydrolyase to α-tocopherol would seem to exclude the cholate-independent hydrolyase as a site of tocopherol action and to counter the notion that interference with retinyl ester hydrolysis is a primary mechanism of the sparing action of α-tocopherol in vivo.

Evidence is increasing that the progression of intermediates through metabolic pathways occurs by direct transfer via interactions among proteins, rather than by diffusion through the aqueous phase (40). For retinol metabolism, this could involve CRBP as substrate in several reactions, including retinol esterification, oxidative catabolism and dehydrogenation. In support of this concept, retinol bound to CRBP is recognized for esterification by lecithin:retinol acyltransferase (41, 42), and is protected from esterification by acyl-CoA:retinol acyltransferase (43). Similarly, holoCRBP is substrate for a microsomal NADP-dependent retinol dehydrogenase, which produces retanal for retinoic acid synthesis, and is
protected from dehydrogenation by NAD-dependent microsomal and cytosolic dehydrogenases (36). The current results show that apoCRBP may be a signal of retinol depletion that activates retinyl ester hydrolysis. It is possible that the reactions in retinoic acid synthesis from retinyl ester hydrolysis through at least retinal formation proceed via CRBP-bound retinol. This emerging central role of CRBP in retinyl ester/retinol balance and retinoic acid synthesis is summarized in Fig. 7.

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