Cellular Retinoid-Binding Proteins: Limited Proteolysis Reveals a Conformational Change upon Ligand Binding†

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ABSTRACT: Intracellular retinoid-binding proteins are small, tightly folded, compact proteins, which appear to be involved in the delivery of retinoids to microsomal metabolic enzymes, among other potential roles. Recently, it has been demonstrated that two of these binding proteins, cellular retinol-binding protein (CRBP) and cellular retinol-binding protein type II [CRBP(II)], interact with the same microsomal enzyme but in different manners, depending on the absence or presence of ligand [Herr, F. M., & Ong, D. E. (1992) *Biochemistry 31*, 6748–6755]. The structural components of the binding proteins responsible for these differential interactions are presently unknown. In addition, it is not clear how the ligand is able to gain entry into the solvent-inaccessible interior binding cavity. Limited proteolysis of the apo and holo forms of CRBP and CRBP(II) was used to probe the conformational differences between the different states of these two proteins in solution. It was found that the apo forms of both proteins were significantly more susceptible to proteolysis, and probably adopted a more open conformation, than the holo forms. The initial cleavage site of endoproteinase Arg-C in the apo forms occurred at a conserved arginine residue near a possible site of ligand entry. Similar results were obtained by limited proteolysis of cellular retinoic acid-binding protein and heart fatty acid-binding protein, indicating that a common ligand-induced conformational change may occur for other members of this family of intracellular binding proteins. Additionally, it was found that the relative susceptibility of holo-CRBP and holo-CRBP(II) to proteolysis was related to their affinities for ligand, with holo-CRBP, which has a significantly lower K_d than that for holo-CRBP(II), being the more resistant to proteolysis. The results of the limited proteolysis experiments, and the implications for protein–protein recognition and ligand entry mechanisms, can be related to the known X-ray structures of CRBP and CRBP(II).

The cellular retinoid-binding proteins are members of a family of small intracellular proteins that bind retinoids and fatty acids (Sundelin et al., 1985; Matarese et al., 1990). Members of this family appear to be involved in the trafficking of compounds within the cell (Ong et al., 1987; Kakkad & Ong, 1988; Yost et al., 1988; MacDonald et al., 1990; Waggoner & Bernlohr, 1991; Veerkamp et al., 1991; Napoli et al., 1991; Napoli, 1993; Sha et al., 1993; Herr & Ong, 1992). Retinoid-binding proteins are found in a wide variety of tissues and can constitute up to 1% of total cellular protein (Ong, 1985). Two of these proteins, cellular retinol-binding protein (CRBP[1]) and cellular retinol-binding protein type II [CRBP(II)], have been shown to have the ability to sequester their bound ligands from metabolism by certain enzymes, while permitting metabolism by other, presumably physiologically significant, enzymes (Herr & Ong, 1992; Napoli et al., 1991). Studies indicate that several of the latter group of enzymes, in particular a microsomal retinal reductase and a microsomal retinol esterifying enzyme, lecithin–retinol acyltransferase (LRAT), can discriminate between the apo and holo forms of the binding proteins (Herr & Ong, 1992).

Currently, the crystal structures for eight members of this family have been reported, all sharing the same basic structural motif [e.g., Xu et al. (1993) and Cowan et al. (1993)]. The motif comprises 10 antiparallel β-strands (a–j), which form a flattened barrel. The barrel is closed at one end by the N-terminal β-strand and at the other end by a helix–turn–helix structure (helices A and B). The ligand-binding site lies within a solvent-inaccessible cavity inside of the β-barrel. Structural determination of apo- and holo-CRBP(II) at a resolution of 2.0 Å showed no significant structural differences between the apo- and holoprotein forms (Winter et al., 1993). Additionally, no opening in the apoprotein could be seen that was large enough to admit a ligand. Thus, from structural analysis, it is not clear how enzymes discriminate between the apo and holo forms of the retinoid-binding proteins, nor is the mechanism by which ligands gain access to the binding sites apparent.

In the structural determination of apo- and holo-ALBP (aP2 or 422), another member of this protein family, at a resolution of 1.6 Å, it was observed that the helix–turn–helix motif of this protein was conformationally altered in the apo versus the holo form (Xu et al., 1993). Although the α-helix closest to the N-terminus (helix A) was anchored to the barrel structure by hydrogen bonds and salt links, the adjacent helix (B) was able to twist away from the barrel in the apoprotein form. The conformational positions of two residues at the C-terminal end of helix B were ambiguous, indicating that these amino acids are relatively mobile. The authors suggest that these residues may act as a hinge, allowing the rotation of helix B and thus exposing the solvent-inaccessible ligand-binding site. Should such a conformational change also occur in CRBP and CRBP(II), the helix–turn–helix motif might provide a likely area for discrimination by enzymes between the apo and holo forms.

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[1] Abbreviations: CRBP, cellular retinol-binding protein; CRBP(II), cellular retinol-binding protein type II; CRABP, cellular retinoic acid-binding protein; H-FABP, heart fatty acid-binding protein; LRAT, lecithin–retinol acyltransferase; ALBP, adipocyte lipid-binding protein.

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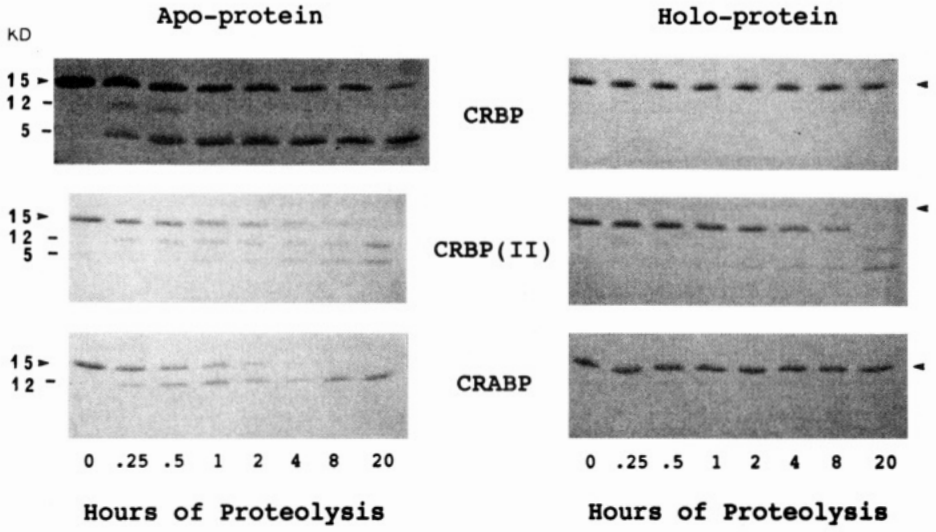

FIGURE 1: Time course of proteolysis of the apo- and holo-binding proteins by endoproteinase Arg-C. Binding proteins (0.5 mg/mL) were digested with endoproteinase Arg-C using a ratio of protein to protease of 20:1 (w/w). Aliquots taken at the indicated times were analyzed by SDS–gel electrophoresis, followed by Coomassie blue staining as described in Experimental Procedures. Undigested binding proteins, which migrated at approximately 15 kDa in molecular mass (standards not shown), are indicated with arrows. The approximate molecular masses of the major proteolytic fragments are indicated.

In this study, limited proteolysis of native apo and holo forms of the intracellular binding proteins, CRBP, CRBP-(II), CRABP, and H-FABP, was used as a probe of tertiary structure. Generally, the apo forms of each of the four proteins were found to be more sensitive to proteolysis than were the holo forms. Sequencing of clostripain (Arg-C)-generated proteolytic fragments showed that the apoproteins were initially cleaved at a conserved arginine residue located within helix B. These results suggest that the apo forms of the retinoid-binding proteins adopt a more open, protease-accessible structure than do the respective holo forms. The results of the proteolysis experiments interpreted in terms of the tertiary structures of the proteins suggest a possible site of ligand entry, as well as a potential protein–protein recognition site.

EXPERIMENTAL PROCEDURES

Materials. The apo forms of CRBP and CRBP(II) were isolated from transformed *Escherichia coli* clones, as reported previously (Stump et al., 1991; Li et al., 1987). The CRABP coding sequence was cloned into the prokaryotic expression vector pT7 (Tabor & Richardson, 1985) after the introduction of an *Nde*I site 5′ and a *Hin*dIII site 3′ to the coding sequence of bovine CRABP (Nilsson et al., 1988) using PCR technology. The sequences of the primers were 5′-CCCCCC**CATATG**C-CCAACTTCGCTGGCACC-3′ and 5′-CCCCCC-**AAGCTT**TCATTCCCGAACATAAATCCTCG-3′. The plasmid was transformed into the cell line BL21(DE3)/pLysS. CRABP production was induced in log-phase cells with 1 mM IPTG (U.S. Biochemical) for 3 h at 37 °C. Cells were pelleted, resuspended in TEK buffer (10 mM Tris (pH 7.4), 1 mM EDTA, and 100 mM KCl), and lysed by sonication. The lysate was centrifuged at 30 000*g* for 30 min. The supernatant was applied to a 5 × 51 cm Sephadex G-75 column equilibrated in 20 mM imidazole acetate (pH 6.4) and 1 mM 2-mercaptoethanol at a flow rate of 1.5 mL/min, with the collection of 10-mL fractions. Fractions 68–84 were concentrated to 10 mL by ultrafiltration on a YM3 membrane (Amicon) and applied to a 15 cm × 20 mm DEAE PW column (TosoHaas) equlibrated in the imidazole acetate buffer. A gradient from 20 to 200 mM imidazole acetate and 1 mM 2-mercaptoethanol was applied at 3.0 mL/min for 80 min, and the eluted fractions were monitored for absorbance at 280 mn. Purity was assessed by SDS–PAGE. Functionality was assessed by ability to bind *all-trans*-retinoic acid. Holo forms of the binding proteins were generated by the addition of a 2-fold excess of the appropriate ligand, followed by gel-filtration chromatography. Both the apo and holo forms of H-FABP were kindly provided by Fiona Herr and Judith Storch (Department of Nutrition, Rutgers University).

Proteolysis of Binding Proteins. Digestion of native proteins at either 0.2, 0.5, or 1.0 mg/mL (as indicated) with endoproteinases was carried out at 37 °C for the times shown. Unless otherwise indicated, the ratio of binding protein to protease was 20:1 (w/w). The following buffers were used. For endoproteinase Arg-C (clostripain), endoproteinase Asp-N, *Staph* V8 protease, proteinase K, and elastase, (Sigma) digestions were performed in 20 mM Tris-HCl (pH 7.8), 50 mM NaCl, and 1 mM DTT. Digestions with α-chymotrypsin and thermolysin (Calbiochem) were carried out in the same buffer, with 5 mM $CaCl_2$. Papain (Calbiochem) digestions were done in 20 mM Tris-HCl (pH 7.4), 50 mM NaCl, and 1mM DTT. Pepsin (Calbiochem) digestions were performed in 50 mM sodium acetate (pH 4.0) and 1 mM DTT. Protease reactions were terminated by boiling the samples for 5 min in Laemmli sample buffer (Laemmli, 1970).

To generate a time course of proteolytic processing of binding proteins, both apo and holo forms of CRBP, CRBP(II), CRABP, and H-FABP (0.5 mg/mL) were treated with Arg-C as described above. At the indicated times, aliquots of the reaction mixtures were taken and analyzed by SDS–polyacrylamide gel electrophoresis (Laemmli, 1970) followed by Coomassie staining. For the generation of thermolysin fragments of binding proteins used for sequencing, 1 mg/ mL solutions of binding proteins (in the buffer mixture described above) were digested for 30 s at 37 °C with a 1:20 (w/w) ratio of thermolysin.

Sequencing of Proteolytic Fragments. Following proteolysis, protein samples were run on 20% SDS–polyacrylamide gels (Laemmli, 1970) and electrophoretically transferred to Immobilon (Millipore) PVDF membranes (Ruff-Jamison & Glenney, 1993). Proteolytic fragments transferred to the membranes were visualized by staining in Coomassie brilliant blue R-250 (Bio-Rad). Automated Edman degradation

Table 1: Degree of Proteolysis of Binding Proteins with Various Proteases[a]

	CRBP		CRBP(II)		CRABP		H-FABP	
protease	apo	holo	apo	holo	apo	holo	apo	holo
Asp-N	0	0	0	0	0	0	nt[b]	nt
elastase	0	0	0	0	0	0	nt	nt
V8	0	0	1	0	0	0	0	0
chymotrypsin	1	0	1	0	2	1	3	0
thermolysin	1	0	1	0	3	1	3	0
papain	2	1	2	1	3	1	3	1
Arg-C	2	0	3	1	3	0	1	0
proteinase K	2	1	3	2	3	2	3	2

[a] Relative rates of proteolysis are presented as a scale from 0 to 3, with 0 equaling no detectable proteolysis, 1 representing relatively low levels of proteolysis (below 50% digestion), 2 representing moderate levels of proteolysis (greater than 50% digestion), and 3 equaling essentially complete proteolysis. Reactions were performed with 0.5 mg/mL binding protein at a 20:1 (w/w) protein to protease ratio for 60 min at 37 °C. [b] nt: not tested.

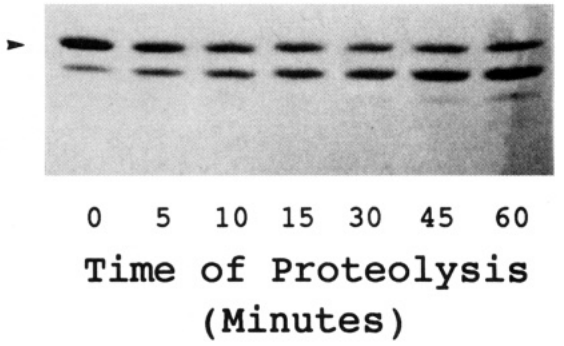

FIGURE 2: Time course of proteolysis of high concentrations of apo-CRBP(II) by endoproteinase Arg-C. apo-CRBP(II) at a concentration of 1 mg/mL was digested with endoproteinase Arg-C using a ratio of protein to protease of 100:1 (w/w). Aliquots taken at the indicated times were analyzed by SDS–gel electrophoresis, followed by Coomassie staining as described in Experimental Procedures. Undigested CRBP(II) is indicated with an arrow.

(Applied Biosystems) sequencing of transferred protein bands was performed by Dr. Thomas J. Lukas in the Population Center core sequencing facility at Vanderbilt University.

Molecular Modeling. Molecular modeling was done on a Silicon Graphics Personal Iris with Biosym Insight and O version 5.7 (Jones et al., 1991).

RESULTS

Limited Proteolysis of Apo- and Holoproteins. Using a battery of enzymes, proteolysis was performed on native apo and holo forms of the retinoid-binding proteins, CRBP, CRBP-(II), and CRABP, as well as the heart fatty acid-binding protein (H-FABP). In general, the apo forms of the binding proteins were proteolyzed more rapidly than the holo forms (Table 1). Two of the proteinases used, elastase and endoproteinase Asp-N, did not appear to cleave either the apo or holo forms of any of the retinoid-binding proteins. The proteinases Arg-C, papain, thermolysin, and proteinase K all initially generated a fragment of approximately 12 kDa from the apo-binding proteins, while V8 generated such a fragment only with apo-CRBP(II) (data not shown). This common pattern of proteolysis suggested that the four binding proteins might have a common site, which was readily accessible to these proteases. Of these, Arg-C showed the greatest level of selectivity between the apo and holo forms of the retinoid-binding proteins (Table 1). Although it readily cleaved the apoproteins, it was unable to cleave the holoproteins, with the exception of CRBP(II). For this reason, endoproteinase Arg-C was used to identify regions of the binding proteins that changed conformation upon ligand binding.

An extended time course of proteolysis by Arg-C revealed that the holo forms of CRBP and CRABP remained largely protease-resistant up to 20 h, while holo-CRBP(II) was essentially degraded over this prolonged reaction time (Figure 1). It appeared that the fragments generated by the proteolysis of CRBP(II) in the apo and holo forms were equivalent; however, they were generated more rapidly with apoprotein. The first fragment generated from apo-CRBP(II), migrating at a molecular mass of 12 kDa, was subsequently cleaved to give a smaller fragment. For CRABP the 12-kDa fragment remained stable. However, for CRBP, the initial 12-kDa fragment was rapidly cleaved to give the prominent smaller fragment.

Arg-C Digestion of High Concentrations of apo-CRBP-(II). To determine whether a larger fragment might be serving as the precursor of the 12-kDa fragment, a higher protein: protease ratio was monitored at shorter time intervals. At the shortest time examined (5 min), only the 12-kDa fragment was detected (Figure 2). No proteolytic fragments of higher molecular mass were visible at any of the time points examined. From this it was concluded that the 12-kDa fragment was most likely the product of the initial cleavage event. The appearance of the smaller fragment was not readily apparent until about 30 min under these conditions.

N-Terminal Amino Acid Sequences of the Proteolytic Fragments. The N-terminal sequences of the 12-kDa polypeptides generated by Arg-C were as follows: apo-CRBP, KIANL; apo-CRBP(II), KIAVR; apo-CRABP, KVAVA;

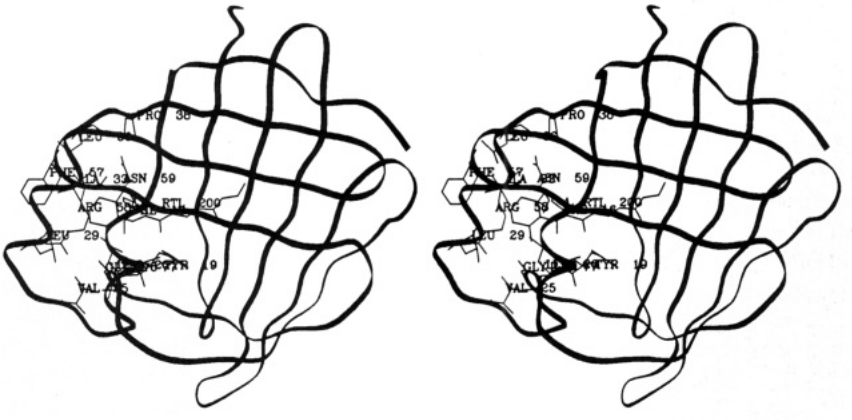

FIGURE 3: Ribbon drawing of the CRBP structure. The amino acids that are shown in later figures are drawn in. Helix B is the left-most helix. The horizontal strands are a, b, c, d, and e, from top to bottom.

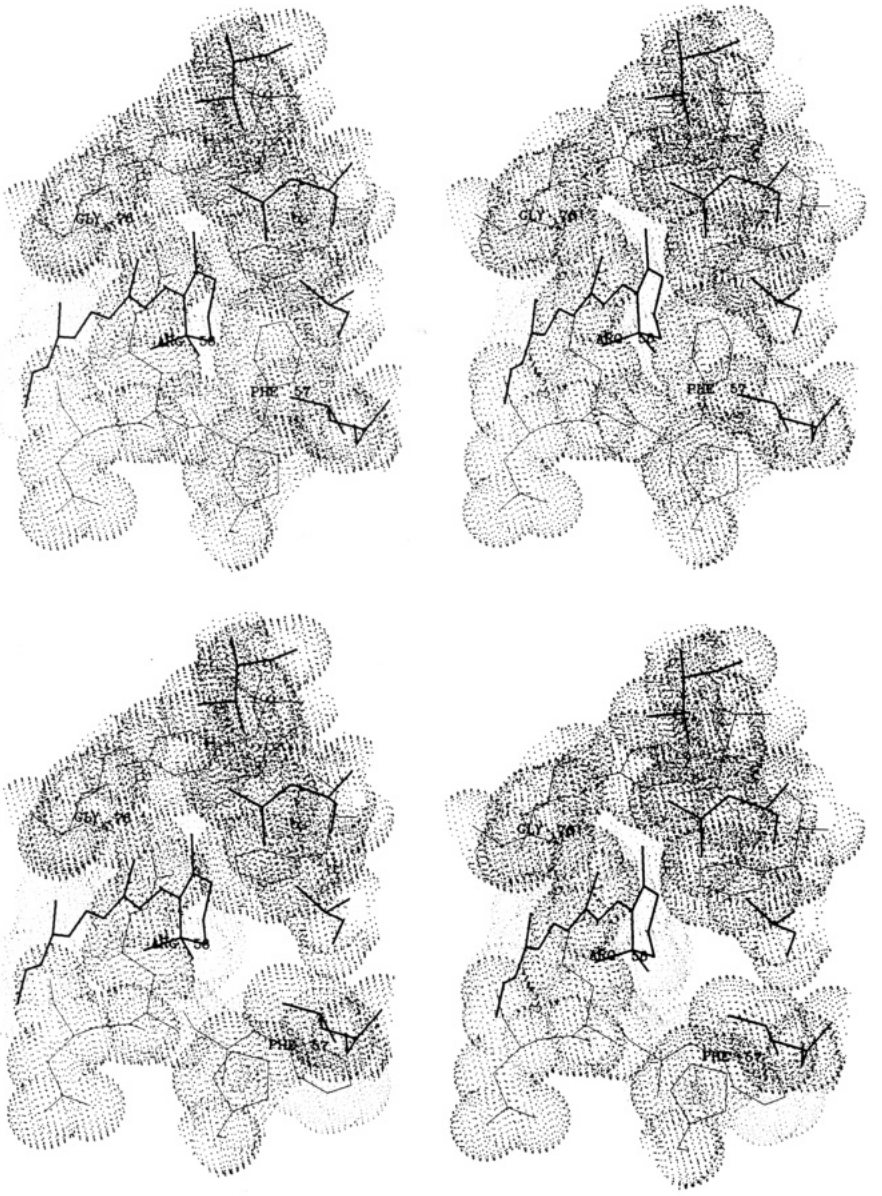

FIGURE 4: (a, top) Putative entrance end of the retinol-binding site. The van der Waals surfaces are indicated with higher dot density for the protein and lower dot density for the ligand. The retinol and side chains that come from helix B are indicated in heavy lines (from the X-ray coordinates of Cowan et al. (1993)]. (b, bottom) The same as above except that the side chain on Phe-57 has been rotated into an alternate rotamer than it has been observed to adopt in the holo X-ray strcuture.

apo-H-FABP, QVASM. These sequences were singular to a specific site for each protein and established that the sites of cleavage were located at a conserved Arg in helix B. This residue corresponds to Arg-30 in CRBP and CRBP(II), Arg-29 in CRABP, and Arg-31 in H-FABP. Interestingly, this residue is conserved in eight of the nine members of this protein family that have been sequenced to date (Jones et al., 1988; Giguère et al., 1990).

To test whether proteolysis by Arg-C reflects a protease-accessible region in the tertiary structure, and not just a limited number of potential cleavage sites for this enzyme, the fragments generated by other enzymes with varied specificities were also sequenced. The N-terminal sequence of the fragment generated from apo-CRBP(II) by thermolysin (which has a broad specificity for aliphatic amino acid side chains) established that the initial cleavage occurred at Ile-32, also in helix B. The N-terminal sequence of the V8 protease-generated fragment of CRBP(II) revealed a cleavage site at Glu-17, which is found in helix A.

Proteolysis of all-trans-Retinal–CRBP. To assess the influence of ligand affinity on protein structure and the rate of proteolysis, CRBP was allowed to bind *all-trans*-retinal, a compound for which it has a 2 order of magnitude lower affinity than for *all-trans*-retinol (Levin et al., 1988; Li et al., 1991). Retinal–CRBP was more resistant to proteolysis than apo-CRBP, but not as resistant as retinol–CRBP. Additionally, the fragments produced were similar to those produced by Arg-C proteolysis of apo-CRBP, with initial production of a 12-kDa fragment, which was rapidly degraded to produce 5- and 2–3-kDa fragments (data not shown).

DISCUSSION

We have used limited proteolysis to probe the conformational changes that occur in CRBP and CRBP(II) following ligand binding. We reasoned that the clamlike structure of the protein must open somewhat to allow the entry of ligand and that the open form of the protein might be more easily cleaved by proteases. We found that the liganded forms of both CRBP and CRBP(II) are resistant to proteolysis by a variety of proteases. However, in the absence of ligand we are able to observe specific cleavage at a peptide bond in helix B. These results suggest that the apoprotein forms adopt a more open

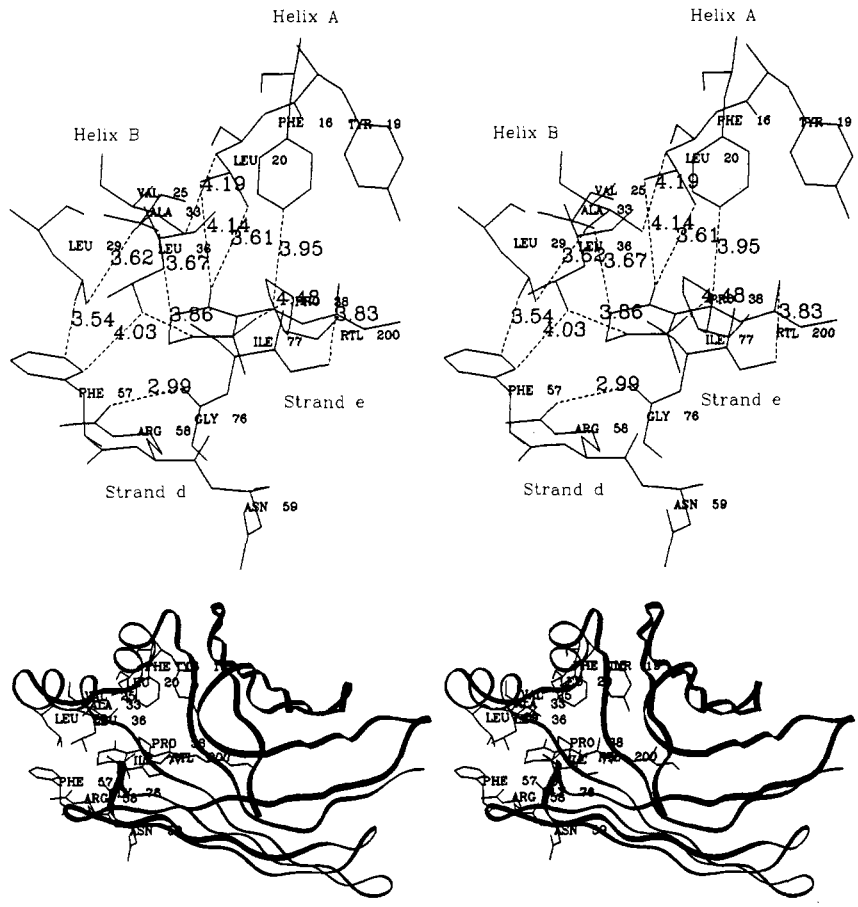

FIGURE 5: (a, top) Amino acids of CRBP at the β-ionone ring end of the ligand-binding site. Some of the distances between atoms in van der Waals contact are included. (b, bottom) Same view as in a with the ribbons drawn in such that the view of the binding site can be seen in the context of the secondary structural elements.

conformation (or series of conformations) in solution than do the holoproteins. Digestion of the apo-binding proteins with the proteases Arg-C, papain, proteinase K, and [in the case of CRBP(II)] thermolysin all generated a fragment of approximately 12 kDa. This implies that all of these proteases cleave the binding proteins at a similar site within the α-helical cap. Apparently, residues within this region of the protein are more accessible to proteases than are residues within the β-barrel.

The crystal structure of apo-CRBP(II) at a resolution of 2 Å is virtually unchanged from that of holo-CRBP(II) (Winter et al., 1993). Consequently, the crystal structure of the apoprotein gives little clue as to how the retinol, so inaccessible to exterior solvent molecules when buried in the β-barrel, is able to get in and out of the protein. However, there may exist in solution multiple conformations of apoprotein, including a more open state that can allow the entry and exit of ligand.

One can interpret the results of proteolysis in terms of the X-ray structure of CRBP. The retinol in CRBP is in a solvent-inaccessible cavity sandwiched between two layers of β-sheet (Figure 3). The hydroxyl group of the retinol is innermost, and the β-ionone ring of the ligand makes several contacts with Phe-57 and helix B of the helix–turn–helix motif, which cap the entrance to the β-barrel. Phe-57 is a highly conserved amino acid in this family of proteins and has been suggested to form part of the portal for ligand entry in other proteins in this family. For example, in ALBP, in the apo and holo forms the side chain of the corresponding phenylalanine is found in different positions. In the apo form it is found blocking the internal binding cavity, and in the holo it is rotated away from the bulk of the protein to allow room for the tail of the fatty acid ligand. However, in CRBP, Phe-57 makes van der Waals contacts with the β-ionone ring of the retinol and with a side chain from helix B (Figure 4a). This conformation is equivalent to that which is observed for the corresponding Phe in apo-ALBP. If we model Phe-57 into its most preferred conformation, it rotates away from the bulk of the protein to create an opening (Figure 4b). This opening, although a portal of sufficient size to allow water molecules into the binding cavity, is still not large enough to admit the ligand. Additional conformational changes must occur.

In Figure 5a are drawn the amino acids at the β-ionone ring end of the binding site and the ring end of the retinol. Some of the distances between atoms consistent with good van der Waals contacts are included. The protein–ligand contacts provided by amino acid side chains from helix A and helix B constitute a majority of the protein–ligand contacts in the holoprotein. In Figure 5b, it may be seen that the β-ionone ring of the ligand is at the putative entrance end of the binding cavity, where it makes van der Waals contacts in a cluster of hydrophobic amino acid side chains, three of which (Leu-29, Ala-33, and Leu-36) come from helix B and one of which (Phe-57) comes from the turn between β-strands c and d. There are no hydrogen bonds that connect these elements of secondary structure. (Although there appears to be a gap between strands d and e from Figure 3, this gap is filled by amino acid side chains. In addition, there are side chain–main chain hydrogen bonds between d and e that would limit the flexibility of this region. In Figure 5a, the hydrogen bond between Arg-58 of strand d and Gly-76 of strand e is shown.) In the absence of retinol, the favorable van der Waals contacts

that the β-ionone ring makes with the three amino acids from helix B and Phe-57 in the hairpin turn are lost. Perhaps these contacts help to secure the helix "cap", while in the absence of ligand, the helix–turn–helix segment is able to move enough to provide access to the binding site. Movement of the helices as rigid rods would not disrupt the intrahelical hydrogen bonds, which stabilize them. The pivot point for this movement could be the "hinge" region at the end of helix B, as was described for ALBP (Xu et al., 1993). Furthermore, the side chain of Phe-57 is able to rotate to adopt the conformation that is most favorable without moving other side chains, resulting in possible solvent access to the binding cavity.

The suggestion of a conformational change that requires the movement of a helical cap is not without precedent. A helical lid that moves to allow the entry of a hydrophobic substrate into a binding site has been described for a fungal lipase (Brzozowski et al., 1991; Derewenda et al., 1992). Arg-30, the side chain at which proteolysis occurs in the apo form of CRBP, is at the amino terminus of helix B, just one residue removed from Leu-29, which in the holoprotein makes van der Waals contact with C4 of the retinol and with Phe-57. Cleavage at Arg-30 in the apo form suggests that, in the absence of retinol, the loss of the van der Waals interaction allows helix B to move away from the c–d turn, so that the peptide bond at Arg-30 is accessible to protease. Additionally, some unwinding of the amino-terminal end of helix B may have to occur in the apoprotein to increase the accessibility of the Arg-30 peptide bond to protease.

That similar ligand-induced conformational changes occurred in the structures of other members of this protein family is evidenced by the fact that Arg-C cleaved at the same peptide bond in CRBP(II), CRABP, and H-FABP. Of CRBP and CRBP(II) in the the apo and holo forms, holo-CRBP was by far the most resistant to protease, followed by holo-CRBP-(II), apo-CRBP (which remained largely protease-resistant), and finally apo-CRBP(II), which was readily cleaved. One of the questions that can be addressed is why apo-CRBP was more resistant to proteases than apo-CRBP(II). The answer may lie in the composition of the amino acid side chains involved in the interaction of the β-barrel and the α-helical cap. In CRBP(II) the analogous residue to Leu-29 of CRBP is a threonine. The favorable hydrophobic interaction between Leu-29 and Phe-57, which can occur in the absence or presence of ligand, is not present in CRBP(II). Helix B has lost a favorable interaction with a side chain from the c–d loop that the leaves the helix more susceptible to protease.

The protease sensitivity was affected by the affinity of ligand for the binding protein. holo-CRBP, which has a higher affinity for retinol than does CRBP(II) (Li et al., 1991), remained more resistant to protease than holo-CRBP(II). However, retinal–CRBP was cleaved more readily than holo-(retinol)–CRBP, consistent with the fact that the K_d for retinal–CRBP is higher than that for retinol–CRBP. A higher mobility of the helical cap may contribute to a lower affinity for ligand.

As stated above, the apoproteins CRBP and CRBP(II) were both cleaved by proteases more readily than their holo forms, but apo-CRBP was considerably more stable than apo-CRBP-(II). This difference in the conformations of the proteins may be of physiological relevance. It has been demonstrated that CRBP and CRBP(II) can provide a ligand to metabolizing enzymes. Herr and Ong (1992) have observed that both CRBP and CRBP(II) will provide retinol to LRAT for esterification. apo-CRBP was shown to competitively inhibit the esterification of retinol by the enzyme LRAT, in an assay in which retinol was provided in the form holo-CRBP. apo-CRBP(II) did not, in contrast, inhibit the esterification by LRAT of CRBP-delivered retinol. Like the proteases then, LRAT discriminates between apo and holo CRBP(II), but less so between the apo- and holo-forms of CRBP. The differential proteolysis observed here suggests how this discrimination might be accomplished. Since apo-CRBP(II) was clearly more susceptible to proteolysis than holo-CRBP(II), one can speculate that apo-CRBP(II) no longer has an intact LRAT recognition site. In contrast, apo-CRBP, which remained largely protease-resistant, still maintains the recognition site for the enzyme. Protein–protein recognition is often mediated by favorable van der Waals interactions between largely hydrophobic surfaces. Helix B of CRBP has an exposed hydrophobic face (Cowan et al., 1993). The hydrophobic face is within a region where three elements of secondary structure come together to close off the ligand-binding cavity. This area of the protein would be a likely recognition site for LRAT. In apo-CRBP-(II), in which the helices can move away from the barrel more freely, the site is no longer intact. As a result, apo-CRBP(II) does not compete with holo-CRBP(II) for binding to LRAT.

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