A molecular model for the retinol binding protein–transthyretin complex

Johan Åqvist† and Orlando Tapia‡

†Department of Molecular Biology, and ‡Department of Physical Chemistry, Uppsala University, Uppsala, Sweden

A three-dimensional model for the complex between human serum retinol binding protein and transthyretin (formerly named prealbumin) is presented. The model was obtained by interactive rigid-body computer graphics docking and the characterization of the molecular surfaces in terms of fractal dimension. Available experimental data, as well as results from molecular dynamics calculations, support the proposed model.

Keywords: protein-protein interaction, surface fractality, retinol binding protein

INTRODUCTION

The molecular details of complexes between proteins have been the subject of many crystallographic studies. The specific microscopic interactions between biomacromolecules that give rise to stable complexes are clearly elucidated by the three-dimensional (3D) structures of such complexes. Prediction of the detailed structure of a protein–protein complex, given only the individual molecular structures, is, however, a very difficult task (usually referred to as *the protein docking problem*). Little is known about actual "recognition mechanisms" and the spatial range of specific interactions. The specificity of short-distance contacts originates basically from electrostatic and van der Waals' forces. This is reflected by a high degree of complementarity both with respect to charge distribution and shape between the interacting surfaces.

Several attempts to design more or less automated docking procedures have been reported.[1–4] One is, however, always faced with the problem of having to search through a large number of orientations, which must be evaluated according to some predetermined strategy. Such a search strategy may take into account physical, chemical and geometric properties, as well as any other relevant information. As has been suggested before,[4] the geometric characterization of the molecular surfaces may be the first and most important tool for proceeding along these lines.

It has recently been documented that protein surfaces possess fractal-like properties and may be characterized by their (fractal) dimension D.[5–7] The fractal dimension may be thought of as a measure of the degree of roughness of the surface. Typical values of D for a protein surface appear to lie around 2.2, and D also has been found to vary between different parts of the surface.[7] Furthermore, a correlation between surface regions participating in intermolecular (protein–protein) interactions, such as subunit interfaces, and regions of high surface dimension or fractality has been reported for several proteins.[5,7] The fact that the interactions across the interface of a stable protein–protein complex are usually quite specific may be one explanation for the observed correlation, since (shape) specificity obviously is achieved more easily with rough rather than flat surfaces. For a given volume of space, the total surface interaction area between two molecules also can be made larger if the dimension of the interacting surfaces is large.[7]

In this paper, we present a 3D model for the complex between human serum retinol binding protein (RBP) and transthyretin (TTR, formerly referred to as thyroxine-binding prealbumin). The model was obtained by interactive computer graphics surface docking based on the calculated residue fractal indices[7] for the two molecules.

Retinol-binding protein is the carrier protein for vitamin A. It transports vitamin A alcohol (retinol) from its storage site in the liver to the target cells.[8] In the plasma a complex is formed between holo-RBP and the thyroxine-binding transthyretin tetramer. This complex is then recognized by a cell surface receptor, which delivers the vitamin to the cell. Since RBP also probably interacts with one or more intracellular proteins when receiving the retinol in the liver hepatocytes, this means that it is involved in at least four different molecular interactions. Both RBP and TTR have been crystallized and their structure determined by X-ray crystallography to high resolution.[9–11] No 3D structure is available for the complex, however. The model we propose here is discussed in relation to available experimental data, as well as molecular dynamics calculations on the RBP system.[12,13]

Color Plates for this article are on page 111.

Address reprint requests to Dr. Åqvist at the Dept. of Molecular Biology, Uppsala Biomedical Centre, Box 590, S-75124 Uppsala, Sweden.
Received 19 August 1991; accepted 16 October 1991

RESULTS AND DISCUSSION

The docking procedure employed here is based on the calculation of the residue fractal indices, as described by Åqvist and Tapia.[7] One set of molecular surfaces for each of the relevant molecules, viz., RBP and TTR tetramer, is calculated using Connolly's MS program.[14] The members in each set correspond to different values of the MS probe size. From these surfaces the fractal dimensions are calculated and a "fractal index" is assigned to each protein residue by a weighted average procedure that maps the surface D values onto the individual residues.[7,15] The molecular graphics program HYDRA[16] was used for interactive rigid-body surface docking and for visualizing fractality by color coding. The regions of highest fractality[7] on RBP and the transthyretin tetramer were selected and displayed with their standard MS surfaces (obtained with a probe radius of 1.4 Å). On RBP, the largest area of high D corresponds to a region around the loops at the β-barrel entrance.[7] The TTR tetramer shows four large symmetry-related stretches with high D values.[7] A zone fully including one of these and part of the adjacent twofold symmetry related high-D region was selected. The molecules were then rotated and translated by "trial and error" on an E&S PS330 graphics screen to examine whether it is possible to obtain a reasonable fit between the two surfaces.

The result of this exercise is shown in Color Plate 1 where it can be seen that the selected surface patches do possess a high degree of shape complementarity. The surface shown for transthyretin may be described as a Y-shaped canyon. The first of these branches is limited by the βF–βG bend (around SER 100) of one subunit (subunit 1) on one side, and the loop at the C-terminal of the α-helix (around GLY 83) from the second subunit (subunit 2) in the same dimer on the other side. This valley buries the loop around ASN 65 of RBP. The second branch is bounded by the C-terminal residues (around ANS 124) of the first subunit (subunit 1) and the βA–βB loop (around residues 21–26) of the adjacent subunit (subunit 3) from the second TTR dimer. This valley accommodates the loop around SER 95 of RBP. Finally, the third branch of the canyon is made up by the loop at the C-terminal of the α-helix of subunit 2 and the same loop from subunit 3. In this cleft the C-terminal residues 173–175 of RBP are buried. The last residues of RBP (176–182) are not visible in the electron density.[9] Judging from the present model they probably would not be part of the interface, but rather would be exposed to the solvent.

The residues from RBP involved in intermolecular contacts with TTR are 56, 58, 63–73, 91–97 and 173–175. On TTR the interacting region comprises residues 99–101, 103 and 122–125 from subunit 1; residues 20, 21, 82–86 and 114 from subunit 2; and residues 21, 23, 24, 78 and 81–84 from subunit 3. The interactions are dominated by hydrophobic contacts, but there are also several hydrogen bonding possibilities across the interface (Table 1). Furthermore, ASP 72 of RBP and ARG 21 (from subunit 3) of transthyretin are sufficiently close to allow for the formation of a salt link. Color Plate 2 shows a stereo view of the protein–protein interface, where the loop around ASN 65 of RBP is seen from the front. It can be noted that TRP 67 on RBP becomes completely buried by residues from two TTR monomers that belong to the same dimer.

Table 1. Possible hydrogen bonding interactions between RBP and TTR

RBP	TTR (subunit)	Distance (Å)
ASP 68 O	SER 85 N (2)	2.9
LYS 58 NZ	LEU 82 O (3)	3.5
TRP 67 NE1	SER 85 OG (2)	3.6
TRP 67 NE1	TYR 114 OH (2)	3.9
ASP 72 CG	ARG 21 CZ (3)	4.0†
ASP 175 OD1	ARG 21 NE (2)	4.0†
TYR 173 OH	GLY 83 N (2)	4.1
TYR 173 OH	ILE 84 N (2)	4.9

†Possible salt link

The credibility of the present model is sustained by several experimental and theoretical studies, of which we will here give a brief account.[8,17]

It has been shown with fluorescence polarization[18] and equilibrium dialysis[19] that the formation of the RBP–TTR complex does not interfere with the binding of thyroxine to TTR. Our model is compatible with this result, since the RBP binding site on transthyretin is situated near the twofold axis (Color Plate 3), which is perpendicular to the axis along the thyroxine-binding channel. There are also indications that the complex formation stabilizes the retinol–RBP interaction.[20–22] This may point towards the β-barrel entrance on RBP being involved in TTR binding,[10,13] since a change in mobility of the entrance loops would affect the retinol–RBP interaction. Furthermore, reconstitution experiments of apo-RBP with retinol analogues[23] have shown differences in the affinity for transthyretin. While complexes of retinal isomers and retinyl acetate with RBP did not bind TTR, retinoic acid-RBP was found to be bound to TTR under the same conditions. Assuming that these retinoids bind to RBP in a similar manner to that of retinol, the different end groups of the isoprene tail would be close to the β-barrel entrance loops. The different transthyretin affinities for the retinoid–RBP complexes therefore also point towards this part of RBP being involved in the interaction with TTR, assuming that no other conformational changes are induced by the binding of the retinol analogues.

Horwitz and Heller[24] have shown that in the RBP–TTR complex three out of four tryptophan residues are protected from labeling with 2-hydroxy-5-nitrobenzyl bromide. In free RBP, on the other hand, two TRP residues can be labeled. Since TRP 24 and TRP 105 are buried in the interior of RBP, this leaves TRP 67 and TRP 91, both solvent accessible, as the best candidates for labeling.[10,13] In the present docking model (Color Plate 2), TRP 67 is buried by the TTR tetramer, which is in agreement with the observation that the complex formation results in an additional tryptophan becoming protected from labeling. It has also been demonstrated that iodination of isolated RBP decreases its affinity for TTR, while iodination followed by dissociation of the complex yields iodinated RBP with full affinity for TTR.[25] This suggests that one or more tyrosine residues on RBP are involved in the intermolecular interaction. Furthermore, acetylation experiments using N-acetylimidazole

indicate that acetylation of lysyl groups on RBP prevents normal binding to transthyretin.[26] The docking model has both a tyrosine (TYR 173) and a lysine (LYS 58) of RBP participating in the complex interface.

The interacting surface regions involve the largest continuous stretch of solvent accessible hydrophobic residues both on RBP and TTR.[10] In particular, the protruding β-barrel entrance loops (βC–βD and βE–βF) on RBP are very hydrophobic, which suggests a possible interaction with something other than solvent. This would also be consistent with the observed dissociation of the RBP–TTR complex at low ionic strength.[27] The hydrophobicity of the complex interface is also elicited by electrostatic calculations.[15] Most of the interface region shows low absolute values of the electrostatic potential, except around ASP 72 of RBP and ARG 21 of TTR, which in the model are close enough to each other to allow the formation of a salt bridge. On the other hand, there is a high degree of charge complementarity between acidic and basic residues in the region surrounding the "primary" interface. This is illustrated in Color Plate 4, which highlights the charged groups surrounding the interface region. It is also worth noticing that potential energy minimization of the present model, in addition to producing a general tightening of the interface, also indicates that two additional salt links may be formed between GLU 44 of RBP and LYS 80 (subunit 3) of TTR, and between LYS 99 and GLU 51 (subunit 3). These charged residues, however, are all situated on the solvent exposed surface of the complex and their possible ionic interactions are probably not as crucial for the complex formation as that between ASP 72 and ARG 21.

The interacting residues are also highly conserved in both RBP and transthyretin among the human, rat and rabbit species.[28,29] This seems to be a requirement since RBP and TTR from different species have been found to cross react with each other.[29,30] In fact, all residues on RBP that interact closely with TTR are conserved between the human and rabbit sequences. On TTR, the interacting surface region shows three mutations: ILE 26 → Val, TYR 114 → Phe and THR 123 → Ser. These mutations may not be crucial for the binding, however, since the changes are fairly small and can be easily accommodated by the present model. It has also been found[29,31] that RBP competes with the binding of four out of 12 rabbit antihuman transthyretin F_{ab} fragments on the TTR tetramer. Therefore, it was suggested that the RBP binding site on TTR may be situated close to an antigenic site.[29] Both ILE 26 and THR 123 were proposed to be parts of antigenic sites on TTR.[29] These two residues are close (~5 Å) to residues from RBP (cf. Color Plate 2) in our model while the rest of the proposed antigenic sites are exposed on the surface of the complex. If the proposed antigenic sites on TTR are correct, then the binding of RBP in our model, which allows two RBP molecules to bind simultaneously to the TTR tetramer, would interfere with four F_{ab} fragments. In general, RBP has accumulated very few mutations among the known sequences, which may reflect its participation in several molecular interactions. There is also a fairly large continuous conserved region that involves the N-terminal loop and the C-terminal part of the α-helix (see below).

Another interesting piece of information concerning amino acid mutations comes from a study of amyloid fibrils from patients who died from familial amyloidotic polyneuropathy type II.[32] It was found that the fibril subunit protein, which is derived from plasma transthyretin, contained an amino acid substitution at position 84, where the normal isoleucine residue had been replaced by serine. Furthermore, it was found that the affected individuals had unusually low serum levels of RBP. Since the formation of the serum RBP–transthyretin complex prevents RBP from being filtered through the kidney glomeruli, the observed low serum RBP level indicates that ILE 84 → Ser mutation may break up the RBP–TTR complex. As discussed above, ILE 84 is right in the middle of the interface in our model and is also present in two copies, one from each of subunits 2 and 3. A substitution of this residue would thus be expected to affect the affinity for RBP drastically, in accordance with the experimental observation.

There has been some discussion of how many binding sites for RBP are on the transthyretin tetramer.[18,30,33,34] Although *in vivo* only one molecule of RBP is bound per TTR tetramer, it is clear that TTR possesses multiple binding sites for RBP. The general picture is that human TTR can simultaneously bind two molecules of human RBP, although there presumably are four binding sites on the tetramer, reflecting negative cooperative binding.[30,34] The C_α drawing (Color Plate 3) illustrates how this notion is fulfilled by the present model; while two RBP molecules can bind on opposite sides of the tetramer, the binding of four RBP molecules would be sterically hindered because each of the binding sites is too close to one of the twofold symmetry axes of the tetramer.

Finally, it has been demonstrated by molecular dynamics (MD) calculations[12,13,15] on both the apo- and holo-forms of RBP that the β-barrel entrance loops undergo fairly large conformational changes upon removal of the retinol molecule from the holo-structure. It has also been shown experimentally that apo-RBP has reduced, or no, affinity for transthyretin.[17,35,36] From the present model of the RBP–TTR complex it is clear that conformational differences in the entrance loops, such as those observed for apo-RBP in the MD simulations, would affect the binding to transthyretin. Conformational differences between the holo- and apo-forms were also suggested by Newcomer et al.,[37] since apo-RBP did not crystallize under the same conditions as holo-RBP. However, a different crystal form of RBP has been reported by Ottonello et al.,[38] and in this form apo- and holo-RBP were found to give isomorphous crystals.[39] The 3D structures of holo- and apo-RBP in this crystal form have not yet been determined, but will certainly be of great interest once they are solved.

As mentioned above, there is also a rather large continuous region with conserved residues on RBP that is made up mainly from the N-terminal loop and the C-terminal of the α-helix. This is also, besides the β-barrel entrance loop region, a part of the molecular surface that has higher-than-average values of the fractal dimension D. Furthermore, the MD calculations[12,13,15] indicated that, in addition to producing conformational changes in the barrel entrance region, the removal of retinol also triggered a conformational change (mainly manifested as a tilting of the helix) in this part of the molecule. It is thus tempting to guess that this part of the molecule may also be involved in some intermolecular interaction. We have examined the possibility of docking

this region with the TTR tetramer, but without success. Therefore, if it does interact with some other molecule, and this molecule is not TTR, the cell surface RBP receptor might be a reasonable guess.

Although the present docking study was based just on the geometrical concept of surface fractality and the actual shapes of the molecular surfaces, the plausibility of the resulting complex conformation is sustained or at least compatible with the available experimental and theoretical data. This latter type of information may be useful as a guide when attempting to design automated docking procedures in that it can help to reduce the number of geometric possibilities that have to be examined. The question is how and at what stage it should be incorporated into the algorithm. The fact that, in particular, sidechains are likely to change their conformation when a complex is formed is also a problem that must be considered. It seems clear, however, that the geometrical properties of the interacting molecular surfaces, as quantified, e.g., by Connolly's shape function[4] or by fractal indices,[7] can provide a useful basis for the design of more sophisticated approaches to the protein docking problem.

ACKNOWLEDGEMENTS

We wish to thank Dr. T.A. Jones for useful discussions and for providing the RBP coordinates and Dr. R.E. Hubbard for making his HYDRA program available to us. This work was supported by a grant from the Swedish Natural Science Research Council.

REFERENCES

1 Wodak, S.J. and Janin, J. *J. Mol. Biol.* 1978, **124**, 323–342
2 Rashin, A.A. and Yudman, B.H. *FEBS Lett.* 1979, **101**, 6–9
3 Krigbaum, W.R. and Komoriya, A. *Biochim. Biophys. Acta* 1979, **576**, 229–246
4 Connolly, M.L. *Biopolymers* 1986, **25**, 1229–1247
5 Lewis, M. and Rees, D.C. *Science* 1985, **230**, 1163–1165
6 Pfeiffer, P., Welz, U. and Wipperman, H. *Chem. Phys. Lett.* 1985, **113**, 535–540
7 Åqvist, J. and Tapia, O. *J. Mol. Graph.* 1987, **5**, 30–34
8 Rask, L., Anunndi, H., Bohme, J., Eriksson, U., Fredriksson, A., Nilsson, S.F., Ronne, H., Vahlquist, A. and Peterson, P.A. *Scand. J. Clin. Lab. Invest.* 1980, **40**, *Suppl.* **154**, 45–61
9 Newcomer, M.E., Jones, T.A., Åqvist, J., Sundelin, J., Eriksson, U., Rask, L. and Peterson, P.A. *EMBO J.* 1984, **3**, 1451–1454
10 Cowan, S.W., Newcomer, M.E. and Jones, T.A. *Proteins* 1990, **8**, 44–61
11 Blake, C.C.F., Geisow, M.J., Oatley, S.J., Rérat, B. and Rérat, C. *J. Mol. Biol.* 1978, **121**, 339–356
12 Sandblom, P., Åqvist, J., Jones, T.A., Newcomer, M.E., van Gunsteren, W.F. and Tapia, O. *Biochem. Biophys. Res. Comm.* 1986, **139**, 564–570
13 Åqvist, J., Sandblom, P., Jones, T.A., Newcomer, M.E., van Gunsteren, W.F. and Tapia, O. *J. Mol. Biol.* 1986, **192**, 593–604
14 Connolly, M.L. *J. Appl. Cryst.* 1983, **16**, 548–558
15 Åqvist, J. Ph.D. thesis. Swedish University of Agricultural Sciences, 1987
16 Hubbard, R.E. in *The Representation of Protein Structure, Proc. Computer-Aided Molecular Design Conference*. Oyez, New York (1984), pp. 99–106
17 Goodman, D.S. in *The Retinoids*. Academic Press, New York (1984) vol. 2
18 van Jaarsveld, P.P., Edelhoch, H., Goodman, D.S. and Robbins, J. *J. Biol. Chem.* 1973, **248**, 4698–4705
19 Raz, A. and Goodman, D.S. *J. Biol. Chem.* 1969, **244**, 3230–3237
20 Peterson, P.A. *J. Biol. Chem.* 1971, **246**, 44–49
21 Goodman, D.S. and Raz, A. *J. Lipid Res.* 1972, **13**, 338–347
22 Goodman, D.S. and Leslie, R.B. *Biochim. Biophys. Acta* 1972, **260**, 670–678
23 Horwitz, J. and Heller, J. *J. Biol. Chem.* 1973, **248**, 6317–6324
24 Horwitz, J. and Heller, J. *J. Biol. Chem.* 1974, **249**, 7181–7185
25 Vahlquist, A., Peterson, P.A. and Wibell, L. *Eur. J. Clin. Invest.* 1973, **3**, 352–362
26 Heller, J. and Horwitz, J. *J. Biol. Chem.* 1975, **250**, 3019–3023
27 Peterson, P.A. *J. Biol. Chem.* 1971, **246**, 34–43
28 Sundelin, J., Laurent, B.C., Anundi, H., Trägårdh, L., Larhammar, D., Björck, L., Eriksson, U., Åkerström, B., Jones, T.A., Newcomer, M.E., Peterson, P.A. and Rask, L. *J. Biol. Chem.* 1985, **260**, 6472–6480
29 Sundelin, J., Melhus, H., Das, S., Eriksson, U., Lind, P., Trägårdh, L., Peterson, P.A. and Rask, L. *J. Biol. Chem.* 1985, **260**, 6481–6487
30 Kopelman, M., Cogan, U., Mokady, S. and Shinitzky, M. *Biochim. Biophys. Acta* 1976, **439**, 449–460
31 Vahlquist, A. and Peterson, P.A. *J. Biol. Chem.* 1973, **248**, 4040–4046
32 Dwulet, F.E. and Benson, M.D. *J. Clin. Invest.* 1986, **78**, 880–886
33 Heller, J. and Horwitz, J. *J. Biol. Chem.* 1974, **249**, 5933–5938
34 Trägårdh, L., Anundi, H., Rask, L., Sege, K. and Peterson, P.A. *J. Biol. Chem.* 1980, **255**, 9243–9248
35 Heller, J. and Horwitz, J. *J. Biol. Chem.* 1973, **248**, 6308–6316
36 Peterson, P.A. *Eur. J. Clin. Invest.* 1971, **1**, 437–444
37 Newcomer, M.E., Liljas, A., Eriksson, U., Sundelin, J., Rask, L. and Peterson, P.A. *J. Biol. Chem.* 1984, **259**, 5230–5231
38 Ottonello, S., Mariani, G., Mammi, M., Monaco, H.L., Spadon, P. and Zanotti, G. *J. Mol. Biol.* 1983, **163**, 679–681
39 Monaco, H.L., Zanotti, G., Ottonello, S. and Berni, R. *J. Mol. Biol.* 1984, **178**, 477–479