X-ray Structures of the Leucine-binding Protein Illustrate Conformational Changes and the Basis of Ligand Specificity*

Ulrika Magnusson‡, Branka Salopek-Sondi§, Linda A. Luck§, and Sherry L. Mowbray¶**

From the ‡Department of Cell and Molecular Biology, Uppsala University, Biomedical Center, Box 596, S-751 24 Uppsala, Sweden, the §Department of Chemistry, Clarkson University, Potsdam, New York 13699, and the ¶Department of Molecular Biosciences, Division of Structural Biology, Swedish University of Agricultural Sciences, Biomedical Center, Box 590, S-751 24 Uppsala, Sweden.

The periplasmic leucine-binding protein is the primary receptor for the leucine transport system in Escherichia coli. We report here the structure of an open ligand-free form solved by molecular replacement and refined at 1.5-Å resolution. In addition, two closed ligand-bound structures of the same protein are presented, a phenylalanine-bound form at 1.8 Å and a leucine-bound structure at a nominal resolution of 2.4 Å. These structures show the basis of this protein’s ligand specificity, as well as illustrating the conformational changes that are associated with ligand binding. Comparison with earlier structures provides further information about solution conformations, as well as the different specificity of the closely related leucine/isoleucine/valine-binding protein.

Binding proteins act as primary receptors in the ABC systems of bacterial transport (1). The importance of these systems is highlighted by the observation that their components form the largest group of paralogous genes in bacterial and archaeal genomes, 2% or more of all genes present (2). The binding proteins are characterized by a two-domain architecture, with a connecting hinge; open forms are thought to be most common in the absence of ligand. When the cognate ligand binds, a closed form of the binding protein is stabilized that can then act together with the membrane-bound permease and a cytoplasmic ATPase to mediate transport of the ligand across the cell membrane. The conformational changes associated with ligand binding have often been referred to as classic examples of hinge motion (3).

As is typical for the members of its class, the leucine-binding protein (LBP) binds strongly and specifically to its selected ligands. The $K_D$ of LBP for leucine is 0.40 μM and that for phenylalanine is 0.18 μM; other amino acids, such as isoleucine and valine, are not thought to bind with appreciable affinity (4–7). A related leucine/isoleucine/valine-binding protein (LIV, ~80% amino acid sequence identity) can also bind leucine, with a $K_D$ of 0.40 μM. In contrast to LBP, LIV does not bind phenylalanine but does recognize isoleucine ($K_D$ 0.40 μM) and valine ($K_D$ 0.7 μM) (5). Furthermore, LIV interacts weakly with threonine, serine, and alanine (8). LBP and LIV use the same permease system to facilitate entry of bound ligands into the cell. An open ligand-free structure of LBP was solved previously (9), together with the equivalent form of LIV (10). Small-angle x-ray scattering studies indicate that closure of LIV (and presumably LBP) is indeed associated with ligand binding (11), but the only complex structure reported to date represents leucine bound to one domain of the open LIV protein (10). In the absence of structures in the closed ligand-bound state, however, the differences in LBP/LIV specificity have been difficult to understand.

We report here the structure of an open ligand-free form of LBP with a different conformation than that reported previously. Complexes with leucine and phenylalanine as seen in the closed protein further show the basis of ligand binding and specificity, as well as documenting the conformational changes that occur in association with their binding.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—Plasmid pKSty (5) kindly provided by the Oxender laboratory was transformed into Escherichia coli BL21(DE3). Cells were grown to $A_{600}$ 0.7 in LB medium supplemented with ampicillin (100 μg/ml). Protein production was induced by addition of 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) with subsequent incubation at 37 °C for 4 h. Cells were harvested and standard sucrose osmotic shock procedures were used to release periplasmic proteins. Protein purification was performed on DEAE-Sephacel column using a 0–0.25 M NaCl gradient in 10 mM Tris-HCl buffer, pH 7.5. Purified protein, typically 75 mg/liter cell culture, was dialyzed against several changes of 10 mM potassium phosphate buffer, pH 6.9, 0.02% NaN₃, and stored at −20 °C until use. Purity was analyzed by SDS-PAGE.

Crystallization and Data Collection—All crystals were obtained using the hanging drop-vapor diffusion method (12), with a protein concentration of 6 mg/ml in 20 mM Tris-HCl, pH 8.0. For the open form (LBP-apo), 2 μl of protein were mixed with 2 μl of mother liquor containing 20% polyethylene glycol (PEG) 5000, 100 mM sodium acetate, pH 4.6, 0.2 mM ammonium sulfate, and 10 mM CoCl₂. For the complex with phenylalanine (LBP-Phe), the protein was mixed with the amino acid (final concentration 1.5 mM) and incubated at 4 °C for 24 h. 2 μl of this solution were then mixed with 20% PEG 4000, 100 mM sodium cacodylate buffer, pH 6.5, and 0.1 μM ammonium sulfate. For the form with leucine bound (LBP-Leu), the protein was incubated with 1.2 mM ligand for 24 h. The protein was then mixed with an equal volume of 20% PEG 4000, 100 mM sodium citrate, pH 5.6, and 20% 2-propanol. All crystals grew within 1 week and had a size of ~0.5 × 0.01 × 0.01 mm³.
Table I

Data collection statistics

|               | LBP-apo | LBP-Phe | LBP-Leu |
|---------------|---------|---------|---------|
| Space group   | C2      | P21     | P1      |
| No. of molecules in the asymmetric unit | 1       | 2       | 4       |
| Cell (Å and °) | 91.4, 79.2, 65.2 (90.0, 133.6, 90.0) | 32.62–1.53 (1.56–1.53) | 35.00–1.80 (1.83–1.80) |
| Resolution range (Å, highest shell) | 68.2, 78.3, 70.0 (90.0, 102.7, 90.0) | 38.00–2.00 (2.03–2.00) |
| Average multiplicity | 3.2 (2.6) | 3.1 (1.9) | 2.8 (1.1) |
| R-merge (%)    | 4.6 (17.7) | 9.5 (45.1) | 16.4 (43.1) |
| Completeness   | 95.7 (93.5) | 96.2 (74.0) | 79.1 (22.7) |
| % data with I/σ > 3 | 81.0 (51.7) | 71.0 (42.1) | 47.3 (5.3) |
| I/σ           | 25.4 (4.9) | 10.6 (1.5) | 4.6 (1.4) |
| Solvent content (%) | 52.2 ($V_m = 2.3$) | 51 ($V_m = 2.5$) | 48.1 ($V_m = 2.4$) |

* As reported by SCALEPACK.

Prior to freezing in liquid nitrogen, all crystals were soaked in mother liquor containing 20% glycerol. Data were collected using frozen crystals at beamline 1711 at MAX-lab, Lund Sweden, processed using DENZO and SCALEPACK (13), and merged using programs of the CCP4 package (14). Statistics for the x-ray data are summarized in Table I.

Structure Solution and Refinement—To obtain the new LBP-apo structure, the individual domains (domain 1 including residues 1–120 and 251–329 and domain 2 including residues 121–250 and excluding the residues of the loop between 330–350) of the apo crystal structure (Protein Data Bank, 15, 16) entry 2LBP (9) were used as search models in AMoRe (17) (resolution range 15 to 4 Å). The searches yielded one clear solution for the N-terminal domain with a correlation coefficient of 38.6% after refined fitting and another for the C-terminal domain with a correlation coefficient of 30.1%. After combination of the two domains, rebuilding using the graphics program O (18), refinement (carried out using the program REFMAC5 (19) as implemented in CCP4 (14)), and addition of the 330–345 segment and waters, the final model had an R-factor of 18.9% and an R-free of 21.6% (Table II).

During the rebuilding process, clear electron density supported the introduction of the amino acid substitutions suggested by the updated gene sequence (20).

For the LBP-Phe structure, the same search models and resolution range were used in AMoRe. Two complete molecules were located in the asymmetric unit, and following rigid body fitting that allowed the domains to move independently in CNS (21), the correlation coefficient was 61.5%. The ligand was not included in the calculation of the first electron density maps but clear, positive density was visible between the two structural domains. After refinement and rebuilding, including the addition of ligand and water, the final R-factor was 18.9% and R-free was 22.2% (Table II).

The program MOLREP (22) was used for the molecular replacement solution of LBP-Leu. A complete protein molecule from the refined LBP-Phe structure was used as search model. Four clear peaks were found after the rotation search, giving a final correlation coefficient of 38.6% after refined fitting and another for the C-terminal domain with a correlation coefficient of 30.1%. After combination of the two domains, rebuilding using the graphics program O (18), refinement (carried out using the program REFMAC5 (19) as implemented in CCP4 (14)), and addition of the 330–345 segment and waters, the final model had an R-factor of 18.9% and an R-free of 21.6% (Table II).

During the rebuilding process, clear electron density supported the introduction of the amino acid substitutions suggested by the updated gene sequence (20).

For the LBP-Phe structure, the same search models and resolution range were used in AMoRe. Two complete molecules were located in the asymmetric unit, and following rigid body fitting that allowed the domains to move independently in CNS (21), the correlation coefficient was 61.5%. The ligand was not included in the calculation of the first electron density maps but clear, positive density was visible between the two structural domains. After refinement and rebuilding, including the addition of ligand and water, the final R-factor was 18.9% and R-free was 22.2% (Table II).

The program MOLREP (22) was used for the molecular replacement solution of LBP-Leu. A complete protein molecule from the refined LBP-Phe structure was used as search model. Four clear peaks were found after the rotation search, giving a final correlation coefficient of 38.6% after refined fitting and another for the C-terminal domain with a correlation coefficient of 30.1%. After combination of the two domains, rebuilding using the graphics program O (18), refinement (carried out using the program REFMAC5 (19) as implemented in CCP4 (14)), and addition of the 330–345 segment and waters, the final model had an R-factor of 18.9% and an R-free of 21.6% (Table II).

During the rebuilding process, clear electron density supported the introduction of the amino acid substitutions suggested by the updated gene sequence (20).

The structures of LBP bound to leucine and phenylalanine were also solved by molecular replacement (Fig. 1, D and E). In each case, the closed structures have r.m.s.d. values of 0.2–0.3 Å when the Ce atoms of all molecules in the respective asymmetric units are compared. The overall r.m.s.d. values between the two ligand bound forms are only slightly larger, 0.3–0.5 Å. In both closed structures one domain has rotated ~55° with respect to the other when compared with the new apo form (Fig. 1F) and ~40° to the previously solved apo form. The sense of the rotation is the same in each case, i.e. the original apo form represents a conformational intermediate along the pathway between the new apo and the closed forms. The domains remain rather rigid upon closing of the protein: r.m.s.d. values in Ca position are 0.6–0.8 Å for domain 1 and 0.7–0.8 Å for domain 2. In domain 1, two loops on the outside of the protein distant from the ligand-binding site are affected (residues 34–44 and 292–301). In domain 2, residues 188–192 have different conformations, as do 10 residues at the C terminus. The open conformation is stabilized by one new contact point on the outside surface of the hinge. The hydroxyl group of Thr127 interacts with the main-chain oxygen of Ser125, as well as one of the carbohydrate groups of Asp230. The conformation of the new apo form is also stabilized by the crystal packing, as domain 1 from one molecule is placed between the domains in an adjacent molecule, so preventing the protein from closing.

The electron density in the present structures supports the version of LBP found in the genomic sequence (20) rather than that in the earlier report (33). All three changes (Ile230 → Asp, Glu27 → Arg and Ala233 → Asn) are clearly observed in the electron density in all structures. In all of the new structures, the electron density also clearly shows an unusual non-proline cis peptide between residues Gly775 and His776 that was not noted previously; this does not appear to have any functional significance.

| Accession Numbers—Coordinates and structure factors have been deposited with the Protein Data Bank with entry codes 1USG for the apo form, and 1US1 and 1USK for the phenylalanine- and leucine-bound forms, respectively. | RESULTS |

## Overall Structures

LBP is comprised of two domains, each consisting of a central β-sheet flanked by α-helices. The structure of a new open form of LBP was obtained by molecular replacement, using the two domains of the previously reported apo structure (9) separately in the rotational and translational searches (Fig. 1A). This apo form is opened by an additional 14° compared with the previous structure (Fig. 1, B and C). The individual domains of the two open forms are however very similar with r.m.s.d. values of 0.8 Å for domain 1 and 0.4 Å for domain 2 when all Ca are included. The largest differences in domain 1 are in a surface loop containing residues 292–301 and two loops directly involved in ligand binding (residues 12–18 and 99–103). The biggest changes in domain 2 are in a surface helix containing residues 232–238, which is distant from the ligand-binding site. None of these changes appears to be of functional significance.

The structures of LBP bound to leucine and phenylalanine were also solved by molecular replacement (Fig. 1, D and E). In each case, the closed structures have r.m.s.d. values of 0.2–0.3 Å when the Ce atoms of all molecules in the respective asymmetric units are compared. The overall r.m.s.d. values between the two ligand bound forms are only slightly larger, 0.3–0.5 Å. In both closed structures one domain has rotated ~55° with respect to the other when compared with the new apo form (Fig. 1F) and ~40° to the previously solved apo form. The sense of the rotation is the same in each case, i.e. the original apo form represents a conformational intermediate along the pathway between the new apo and the closed forms. The domains remain rather rigid upon closing of the protein: r.m.s.d. values in Ca position are 0.6–0.8 Å for domain 1 and 0.7–0.8 Å for domain 2. In domain 1, two loops on the outside of the protein distant from the ligand-binding site are affected (residues 34–44 and 292–302). In domain 2, residues 188–192 have different conformations, as do 10 residues at the C terminus. The open conformation is stabilized by one new contact point on the outside surface of the hinge. The hydroxyl group of Thr127 interacts with the main-chain oxygen of Ser125, as well as one of the carbohydrate groups of Asp230. The conformation of the new apo form is also stabilized by the crystal packing, as domain 1 from one molecule is placed between the domains in an adjacent molecule, so preventing the protein from closing.

The electron density in the present structures supports the version of LBP found in the genomic sequence (20) rather than that in the earlier report (33). All three changes (Ile230 → Asp, Glu27 → Arg and Ala233 → Asn) are clearly observed in the electron density in all structures. In all of the new structures, the electron density also clearly shows an unusual non-proline cis peptide between residues Gly775 and His776 that was not noted previously; this does not appear to have any functional significance.
The Hinge—As seen in most other periplasmic binding proteins of this class, the two domains of LBP are connected by a three-stranded hinge. The hinge segments are here designated connection I (residues 117–121), connection II (residues 248–252) and connection III (residues 325–331). Connections I and III form “direct” links between the β-sheets of the two domains, while connection II is interrupted by a helix (residues 253–268). On opening and closing, most of the changes in main-chain torsion angles occur in connections I and III; these two connections are contributed by domain 1. When the protein is open, one water molecule (W1) forms hydrogen bonds with Ala118 –N and Leu326 –N. A second water (W2) hydrogen bonds with Leu120 –N and Leu326 –O as well as another water molecule (W3), which in turn hydrogen bonds to Leu230 –O and Phe329 –N. When the protein closes, W1 retains its interactions with domain 1, but the other two waters adjust to the new structural setting. W2 loses its hydrogen bond with Leu120 but gains one with W1. W3 also moves, losing its interactions to 326–O and 329–N, and gaining new ones with W2, Leu120 –N and Phe329 –O. The electron density for W2 was comparatively weak in two of the four molecules in the LBP-Leu structure but was strong for both molecules in the LBP-Phe structure; this may reflect the weaker and less complete crystallographic data for the leucine complex.

Ligand Binding—Ligands bind to LBP in a cleft formed between the two domains of the closed proteins. Phenylalanine and leucine are bound in very similar fashion (Fig. 3, A and B), with both hydrogen-bonding and non-polar contributions. The amino group of the ligand in each case forms hydrogen bonds with Gly100, Thr102, and Glu226. The carboxylate groups of a ligand form hydrogen bonds with Ser79, Thr102, and Tyr202. The distances between the polar atoms are very similar for all structures with ligand bound (Fig. 3). Most of these interactions are contributed by domain 1.

The non-polar groups of the ligand-binding site are all aromatic. Trp18, Tyr202, and Tyr276 make van der Waals contacts with the hydrophobic side chains of the ligands, while Tyr150 stacks on their planar main-chain groups (Fig. 4). When ligand is bound, the side chain of Tyr276 (along with the associated 272–276 loop) moves away from the cleft, thus providing the space required. This motion is exaggerated slightly to accommodate the larger side chain of the phenylalanine ligand in all of the available structures (Fig. 4B). More van der Waals interactions are found for the phenylalanine ligand, which may be correlated to its tighter binding (13 inter-atomic distances < 4 Å are found for leucine, as compared with 33 for phenylalanine). The side chain of Trp18 also changes its conformation.
upon ligand binding, rotating almost 130° in all ligand-bound structures (Fig. 4A); again the change makes room for the ligand. Crystal packing probably explains some minor differences in the observed conformer of this residue in the two apo structures.

**Comparison with LIV**—The LBP structures are very similar to that of LIV, as expected from the ~80% amino acid sequence identity. Compared with the previously reported apo LBP structure, the available apo-LIV structure is opened by ~2°, again demonstrating the variations possible in the opening angle. Comparing domain 1 of the new apo structure to that of LIV, the r.m.s.d. is 0.6 Å when the loop containing residues 291–298 is omitted. LBP has two inserted amino acids at this point that are not found in LIV. For domain 2 of the apo structures, r.m.s.d. values in the Cα position of ~0.5 Å are observed. The hinges of the two proteins are similar but not identical. In LIV, both W1 and W2 as described above are present in the model, but not W3; this is perhaps an artifact of the relatively low resolution of the LIV crystallographic data. Ser123 of LBP is replaced with an aspartic acid in LIV, which...
could form hydrogen bonds with W2 when the protein is closed. The carboxylate oxygens of the aspartate would then occupy the same space as W3 in the closed LBP structures.

At present, the only ligand-bound form of LIV is an open one for which coordinates are not available (10). In this 2.4-Å structure, leucine is bound only to domain 1, making hydrogen-bonding interactions that are highly analogous to those we observe for amino acids bound to LBP. The hydrophobic portion of the leucine bound in the LIV structure, however, is reported closely to domain 1, especially Leu77. The authors indicate that ligand and Leu77 are close enough to be stacked, which is not the case in our structures, where the average distance between the leucine ligand and Leu77 is 5 Å.

The closed ligand-bound structures of LBP provide a good basis for modeling the equivalent form of LIV. If domain 1 of LIV is superimposed on domain 1 of the LBP-leu structures, and domain 2 on its counterparts, the binding sites are seen to be extremely similar (Fig. 5). All of the hydrogen-bonding interactions that position the main chain atoms of the ligand are conserved (once the side chain of Glu226 has moved into place), and there are only two changes in residues that make direct contacts with the non-polar side chain of the ligand: Trp18 is replaced by a tyrosine and Tyr276 by phenylalanine.

Comparison with Other Binding Proteins—The structure of LBP is more distantly related to those of other periplasmic binding proteins, such as the ALBP, RBP, and GBP. All three show r.m.s.d. values of −2 Å when the Cos of the individual domains are compared with LBP, with approximately half of the backbone atoms matching, as expected from their 10-15% amino acid sequence identity. The domains in the LBP/LIV structures are also somewhat differently placed compared with the other binding proteins. Furthermore, the hinges of LBP and LIV are differently constructed than those of the other three proteins. In the RBP subfamily, all three connections provide direct links between the central β-sheets of the two domains. The first two are βα crossovers, while the third is a ββ crossover. Connections I and III in LBP are βα and ββ crossovers that link the β-sheets (although connection III is somewhat irregular); connection II is a βα crossover that is further away from the other two hinge segments and contains an inserted helix. In ALBP, RBP, and GBP, two aspartic acid residues that are Ramachandran outliers are essential to the construction of the hinge, one placed on each side of the binding cleft. No corresponding outliers are found in the LBP or LIV structures. In all of the proteins, water molecules are found in the hinge region and are observed to be important in conformational changes. However, their location and behavior during conformational changes varies. In our LBP structures no water molecules are visible near connection II, but three are present between connection I and III. ALBP has two water molecules, one between connection I and III and one between connection II and III. Similar waters are also present in the closed form of GBP. RBP binds water in a similar manner to ALBP but here the molecule between connection I and III is lost upon closing and new bonds between the main-chains are formed. All of these differences appear to be correlated with different motions of the domains with respect to each other.

The open conformation of LBP is probably stabilized only slightly by a network of hydrogen bonds linking Ser123, Thr127, and Asp330. In the case of ALBP, no new contacts between the two domains are observed in the open structures, but in RBP some new inter-domain contacts are formed, burying −200 Å² of the surface area. In the open LBP structure only −80 Å² is buried.

DISCUSSION

The LBP structures reported here, when combined with previous structural and biochemical data, provide an unusually complete picture of the motion and function of this protein. A number of open forms are expected to exist in solution, as witnessed by the three related conformers observed for LBP and its close relative LIV. LBP, like LIV (10), is expected to dock ligand first to the half-site in domain 1 of any of the open forms. The ligand will at this point be correctly positioned by hydrogen bonding of the α-amino and carboxylate groups of the ligand to Ser79, Gly100, and Thr102. The hydrophobic interactions needed for the side chain of the ligand will still be largely unsatisfied, although Sack et al. (10) noted earlier that the solvent-exposed area of the ligand is already decreased by 80% compared with free leucine. Upon ligand binding and closing of LBP, Trp18, Glu226, and Tyr276 must move to provide both space and correct interactions.

The new LBP structures provide a good basis for understanding the ligand specificity of this protein. The α-amino and carboxylate groups of an L-amino acid are held firmly in place by hydrogen bonds to protein atoms, predominantly those of domain 1. d-Amino acids cannot be accommodated in the same way, in agreement with the experimental observation that they do not bind (4). L-Leucine and L-phenylalanine, however, bind in a very similar manner with their side chains in the same hydrophobic pocket in the protein (Figs. 3 and 4). The larger side chain of phenylalanine forces a slight movement of Phe276, which results in more van der Waals contacts between ligand and protein. Although both ligands make hydrophobic contacts with Trp18, Tyr150, and Tyr276, phenylalanine has many more interactions with individual atoms in these residues. The additional contacts are probably part of the reason for the −2-fold tighter binding of phenylalanine to LBP (0.18 μM as compared with 0.40 μM for leucine (4, 5)). 4-Fluorophenylalanine has also been observed to bind tightly to LBP (Kᵢ 0.26 μM (4)), suggesting a similar effect. It seems possible that isoleucine could bind, as well, although this was not detected in previous tests. Valine should not bind as tightly, because its side chain will make fewer van der Waals contacts and leave a cavity at this position. The shape of methionine is also not as well suited to the
shape of the site, in agreement with observations that it does not bind at all (4).

The closed structures of LBP also allow a number of insights into the different specificity of LIV. The closed form of LIV was modeled in Fig. 5 by rotating the individual domains of the open structure onto their counterparts in LBP-Leu. All of the residues making hydrogen bonds to the main chain atoms of the ligand are conserved, and so leucine will be expected to bind to LIV in a very similar way. The only substantial differences between the two sites involve residues in the immediate vicinity of the ligand side chain, i.e., Trp\(^{18}\) of LBP is changed to tyrosine, Gly\(^{100}\) to alanine, and Tyr\(^{276}\) to phenylalanine. Trp\(^{18}\) of LIV is expected to make a hydrogen bond to His\(^{203}\), which will lock its conformation. Glu\(^{226}\) of LIV will be unable to hydrogen bond to residue 276 because of the tyrosine to phenylalanine replacement. The slightly larger Ala\(^{100}\) will also contribute to the shape of the binding site. Together, these changes create a different environment for the ligand side chain and so underlie the different specificity of LIV. The large side chain of phenylalanine is difficult to accommodate in LIV; is less likely to bind. The smaller side chains of isoleucine and valine have fewer steric problems and still bind LIV strongly (\(K_D\) values 0.40 and 0.7 \(\mu\text{M}\), respectively). However, LIV is unable to provide good hydrogen-bonding opportunities for threonine, which may account for its weaker binding compared with the isosteride side chain of valine. Serine and alanine can enter the site but will not be a good match for its size and character.

Comparisons with other binding proteins in the wider family indicate that more general similarities, and differences, exist. The hinges of LBP and LIV are similarly constructed, and the observed conformations suggest that their routes for opening/ closing will be similar. This is not the case for ALBP, RBP, and RBP, which belong to a different structural cluster (34) within the type I family of binding proteins, and have very different hinge construction. In LBP/LIV, movements of connections I and II are believed to dominate, while the changes of connection II are more passive. The known motions of the RBP/ALBP cluster are dominated by movements of connections I and II, while the movements of connection III seem to reflect attempts to adjust to motions in the other two. However, even RBP and ALBP, which have 35% amino acid sequence identity, have differences of 30–40° in the preferred directions of motion (23, 26). The available structures clearly show that some conformational routes are highly preferred for a particular protein, but changes in water and protein structure can allow many different outcomes in the various proteins. Water molecules appear to be a particularly good way of solving the non-trivial problems of moving three neighboring hinge segments without major complications.

A consistent feature within both structural clusters of this family of binding proteins is the occurrence of multiple, but related, open forms. This is apparently correlated with the fact that none of these proteins makes many inter-domain contacts that could stabilize a particular conformer. This trend is different from that found in the other major structural class of binding proteins, represented by the maltose-binding protein and lactoferrin. In these type 2 binding proteins, many contacts stabilize one particular type of open structure (35, 36). In both classes of binding protein, it is believed that the open forms will be in conformational equilibrium with closed ones, even in the absence of bound ligand, i.e., that the hinge motions require very little energy. Apparently, a valid open form need not be unique, but it must: (a) allow the ligand to enter a binding site that is completely inaccessible in the closed form and (b) be sufficiently different from the closed form that competition for the membrane-bound permease is not a serious issue. Evaluations of the kinetic data for transport systems suggest that non-productive interactions of the ligand-free proteins with the permease are indeed of significance (37, 38). In both classes of binding proteins, the closed forms show much less variation in the available structures. It appears that, provided the primary criteria are met, the conformational changes that link the open and closed forms of a particular binding protein can follow virtually any pattern, as long as each conformation is compatible with its sequence and structure.

REFERENCES

1. Boos, W., and Lucht, J. M. (1996) in Escherichia coli and Salmonella typhi-

murium: Cellular and Molecular Biology (Niedhart, F., Ingraham, R. C., Lin, K., Low, K., Magasanik, B., Reznikoff, W. C., Riles, M., Schaechter, M., and Unharger, H., eds) pp. 1175–1209, American Society for Microbiology, Washington, D. C.

2. Tatusov, R. L., Mushegan, A. R., Bork, P., Brown, N. P., Hayes, W. S., Borodovsky, M., Rudd, K. E., and Koonin, E. V. (1996) Curr. Biol. 6, 279–291

3. Gerstein, M., Laskowski, R. A., and Chothia, C. (1994) Biochemistry 33, 6739–6749

4. Luck, L. A., and Johnson, C. (2000) Protein Sci. 9, 2573–2576

5. Adams, M. D., Maguire, D. J., and Oxender, D. L. (1991) J. Biol. Chem. 266, 6209–6214

6. Salepek-Sondhi, B., and Luck, L. A. (2002) Protein Eng. 15, 855–859

7. Salepek-Sondhi, B., Swarts, D., Adams, P. S., and Luck, L. A. (2002) J. Biomol. Struct. Dyn. 20, 381–390

8. Rahmanian, M., Claus, D. R., and Oxender, D. L. (1973) J. Biological 116, 1258–1266

9. Sack, J. S., Trakanov, S. D., Tsagkoulis, I. H., and Quicho, F. A. (1989) J. Mol. Biol. 206, 193–207

10. Sack, J. S., Saper, M. A., and Quicho, F. A. (1989) J. Mol. Biol. 206, 171–191

11. Oh, G. A., Trakanov, S., Trevirolle, J., and Quicho, F. A. (1993) J. Biol. Chem. 268, 16241–16247

12. McPherson, A. J. (1982) Preparation and Analysis of Protein Crystals, John Wiley and Sons, New York

13. Ozonowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326

14. Collaborative Computing Project Number 4 (1994) Acta Crystallogr. Sect. D Biol. Crystallogr. 50, 760–763

15. Bernstein, F. C., Koetzle, T. F., Williams, G. J. B., Meyer, E. T., Jr., Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T., and Tasumi, M. (1977) J. Mol. Biol. 112, 535–542

16. Berman, H. M., Westbrook, J., Feng, Z., Gislikland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., and Bourne, P. E. (2000) Nature 404, 255–262

17. Navaza, J., and Saludjian, P. (1997) Methods Enzymol. 276, 581–594

18. Jones, T. A., Zou, J.-Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–115

19. Murshudov, G. N., Vagin, A., and Dodson, E. J. (1997) Acta Crystallogr. Sect. D Biol. Crystallogr. 53, 240–255

20. Kleywegt, G. J., and Jones, T. A. (1996) Acta Crystallogr. Sect. D Biol. Crystallogr. 52, 842–857

21. Harris, M., and Jones, T. A. (1996) Acta Crystallogr. Sect. D Biol. Crystallogr. 52, 1201–1203

22. Deisenhofer, J., and Michel, H. (1985) Nature 317, 618–624

23. Kleywegt, G. J., and Jones, T. A. (1996) Structure 4, 1395–1400