The three-dimensional structure of the RNA-binding domain of ribosomal protein L2; a protein at the peptidyl transferase center of the ribosome

Atsushi Nakagawa, Takashi Nakashima¹, Masae Taniguchi, Harumi Hosaka, Makoto Kimura¹ and Isao Tanaka²

Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810 and ¹Laboratory of Biochemistry, Faculty of Agriculture, Kyushu University, Fukuoka 812-8512, Japan

Ribosomal protein L2 is the largest protein component in the ribosome. It is located at or near the peptidyl transferase center and has been a prime candidate for the peptidyl transferase activity. It binds directly to 23S rRNA and plays a crucial role in its assembly. The three-dimensional structure of the RNA-binding domain of L2 from Bacillus stearothermophilus has been determined at 2.3 Å resolution by X-ray crystallography using the selenomethionyl MAD method. The RNA-binding domain of L2 consists of two recurring motifs of ~70 residues each. The N-terminal domain (positions 60–130) is homologous to the OB-fold, and the C-terminal domain (positions 131–201) is homologous to the SH3-like barrel. Residues Arg86 and Arg155, which have been identified by mutation experiments to be involved in the 23S rRNA binding, are located at the gate of the interface region between the two domains. The molecular architecture suggests how this important protein has evolved from the ancient nucleic acid-binding proteins to create a 23S rRNA-binding domain in the very remote past.

Keywords: peptidyl transferase/ribosomal protein L2/ RNA-binding protein/X-ray structure

Introduction

In all living cells, protein synthesis is carried out in cellular organelles called ribosomes. These large ribonucleoprotein complexes are generally organized into two subunits of unequal size. In prokaryotic Escherichia coli ribosomes, the large (50S) subunit consists of 34 proteins and 5S and 23S rRNAs, while the small (30S) subunit is a complex of 21 proteins and 16S rRNA. All these proteins have been localized in the ribosome by immunoelectron microscopy (Gornicki et al., 1984) and neutron scattering (Capel et al., 1987). Furthermore, attempts to obtain high-resolution structures of individual ribosomal proteins by X-ray crystallography or NMR have elucidated the structures of approximately one-third of all ribosomal proteins (for a review see Yonath and Franceschi, 1997; Nikov et al., 1998; Ramakrishnan and White, 1998). These structural analyses have demonstrated that ribosomal proteins represent unique targets for structural study. They provide new insights into protein evolution as well as the protein-synthesizing machinery for the following reasons. (i) Ribosomal proteins have a variety of RNA-binding motifs and are possibly the origin of the more recently evolved families of RNA- and DNA-binding proteins. (ii) Ribosomal proteins have well-ordered tertiary structures with definite hydrophobic cores. In many cases, somewhat unstructured loops extend from these hydrophobic cores. These architectural features suggest a means by which these molecules bind to rRNA and create its tertiary structure. (iii) The high-resolution structure of each protein can be used as an atomic resolution probe for studying ribosome activity (Heilek and Noller, 1996) or to test the validity of the current model of rRNA (Tanaka et al., 1998). These structures should also facilitate the analysis of the whole particle by X-ray crystallography or by electron microscopy.

The fundamental activity of the ribosome is to decode the message of the mRNA and to form a peptide bond between peptidyl-tRNA and aminoacyl-tRNA (peptidyl transferase). Each subunit of the ribosome is responsible for one of these two activities, which have been localized by immunoelectron microscopy (Gornicki et al., 1984). While the decoding property resides in the small subunit, the peptidyl transferase activity is in the large subunit. A number of experiments have been carried out to elucidate the elements responsible for the ribosome activities, and particularly for the peptidyl transferase activity. Such experiments have shown that 23S rRNA plays a fundamental role in peptidyl transferase activity (Noller et al., 1992). This conclusion is strengthened further by the more recent finding that 23S rRNA fragments synthesized by in vitro transcription can catalyze peptide bond formation (Nitta et al., 1998). Nevertheless, it is obvious that some ribosomal proteins are implicated in peptidyl transferase activity, and of these, protein L2 is known to be the most important constituent of the peptidyl transferase center. Affinity labeling studies have localized protein L2 at or near the peptidyl transferase center of the 50S ribosomal subunit (Nierhaus, 1980). Chemical modifications (Fahnstock, 1975; Sumpter et al., 1991) and in vitro reconstitution (Hampel et al., 1981; Schulze and Nierhaus, 1982) have revealed that protein L2 is highly associated with the peptidyl transferase center of the large ribosomal subunit. A recent mutagenesis study has even identified His229 to be a key residue for enzymatic activity (Cooperman et al., 1995).

Apart from its functional interest, protein L2 is a primary 23S rRNA-binding protein (Fahnstock et al., 1973) and is known to play a crucial role in assembly of domain IV in 23S rRNA. Footprinting and cross-linking experiments have revealed that it binds to a highly conserved stem–loop structure encompassing nucleotides 1794 and 1825 (Gulle et al., 1988; Egebjerg et al., 1991).
in the *E. coli* 23S rRNA. This confinement of the L2-binding site to a short stretch of nucleotides in 23S rRNA, as well as this protein’s possible involvement in the peptidyl transferase center, makes ribosomal protein L2 a highly attractive target for structural analysis. In an attempt to elucidate the tertiary structure of L2 by X-ray crystallography, substantial efforts have been made to crystallize L2. While the intact protein (275 residues for *Bacillus stearothermophilus* L2) continues to resist crystallization, the recombinant protein corresponding to the central region of L2 (60–201), which has been shown to be a 23S rRNA-binding domain (Watanabe and Kimura, 1985), has now been crystallized (Nakashima et al., 1999). Here, we describe for the first time the tertiary structure of an RNA-binding domain of the protein which is involved in the peptidyl transferase center of the ribosome.

**Results and discussion**

**Structure description**

Figure 1 is a stereo view of the overall structure of the RNA-binding domain (RBD) of the ribosomal protein L2 from *B. stearothermophilus* (BstL2-RBD). Secondary structural elements of BstL2-RBD as defined by the DSSP program (Kabsch and Sander, 1983) are given in Figure 2. The molecule has an all β structure consisting of two domains of approximately the same size. The N-terminal domain (Arg62–Pro130) has a five-stranded β domains of approximately the same size. The N-terminal domain (colored cyan) has an OB-fold homologous to the S1 domain, and the C-terminal domain (colored pale green) has an SH3-like barrel motif. The fifth β-strand (colored orange) and a 3_10 helix (H1) connect two subdomains.

**RNA-binding site**

The 23S rRNA-binding site for protein L2 was defined as a short stem–loop structure encompassing the nucleotides from positions 1794 to 1825 in *E. coli* 23S rRNA. This confinement was derived from footprinting analysis of the L2–23S rRNA complex from *B. caldolyticus* (K.Watanabe, unpublished results); the *B. caldolyticus* protein L2 protects, from RNase digestion, nucleotides corresponding to positions 1794–1825 in the *E. coli* 23S rRNA. Furthermore, it has been reported that the nucleotides forming the target stem–loop structure in *E. coli* 23S rRNA are highly protected from chemical modifications when protein L2 is bound to 23S rRNA (Beauclerk and Cundliffe, 1988). These facts suggest that L2 interacts with the domain IV in the 23S rRNA covering the short stem–loop structure.

Structural analyses of protein–RNA complexes, such as the U1A spliceosomal protein complex with an RNA hairpin (Oubridge et al., 1994) and the aminoacyl-tRNA synthetase complexed with its cognate tRNAs (Cavarelli et al., 1993), have revealed that positively charged residues are likely to interact with negatively charged phosphate backbone atoms of an RNA, and aromatic residues are likely to undergo stacking interactions with base groups of an RNA (Oubridge et al., 1994; Bochkarev et al., 1997). Previously, we generated a number of BstL2 point mutations, in which all basic and aromatic residues within the BstL2-RBD were in turn replaced with Gln and Ala, respectively, and analyzed the binding activity to 23S rRNA fragment containing a target stem–loop structure (Harada et al., 1998). Figure 4 shows the (side chains of) amino acid residues that were identified by this experiment as essential for 23S rRNA binding. These essential amino acid residues for 23S rRNA binding will now be discussed in the context of the tertiary structure of the BstL2-RBD.
Structure of ribosomal protein L2

Fig. 2. A sequence alignment of the RNA-binding domain of ribosomal protein L2 from all phylogeny. Secondary structure elements as determined by the present work using the DSSP program (Kabsch and Sander, 1983) are also given. The numbering for residues refers to ribosomal protein L2 from *B. stearothermophilus*. The residues highlighted in red represent complete conservation and those in yellow conservative mutation. The residues that affect 23S RNA binding as identified by the mutagenesis work are marked by stars. Red stars represent the most important residues for 23S rRNA binding. Determinant residues (two glycine residues) for the OB-fold are also marked by circles.

Fig. 3. A topological diagram showing the secondary structure of *Bst*L2-RBD. The molecule consists of 10 β-strands (indicated by arrows) and a short 3_{10} helix (indicated by a rectangle). Elements identified in the N-terminal OB-fold (S1 domain) are shown in blue, whereas the C-terminal elements in the SH3-like barrel are shown in yellow. Unlike in the ordinal OB-fold, the β-strands 4 and 5 are linked by a cross-over connection, leaving the fifth β-strand at the interface between the N- and C-terminal domains. The C-terminal β10 makes a hydrogen bond to the β5, and thus all the β-strands in the molecule are connected by the hydrogen bonds.

The site-directed mutagenesis of Arg86 or Arg155 significantly diminished RNA-binding affinity. In addition, Arg68 and Lys70 mutations caused a partial loss of RNA binding. The CD spectra of these four mutant proteins were indistinguishable from that of the wild-type *Bst*L2, suggesting that these basic residues make essential contacts with the target RNA. Arg86 is located on the loop between the first (β1) and second (β2) strand of the N-terminal domain, while Arg155 lies on the long loop between β6 and β7 in the C-terminal domain. These two residues are located in close proximity within the tertiary structure and locate at the gate of the cleft which is formed by the
N- and C-terminal domains of the BstL2-RBD. On the other hand, Arg68 and Lys70 are located on the N-terminal long extended structure preceding the first strand (β1), and the side chain of Arg68 protrudes into the cleft between the N- and C-terminal domains, while that of Lys70 protrudes into the solvent. There are six aromatic residues in the BstL2-RBD, and five of them were found in the current structure of BstL2-RBD; the missing Tyr61 should locate near the N-terminal domain. The mutants F66A, Y95A and Y102A show almost no binding affinity for the 23S rRNA fragment (Harada et al., 1998). The replacement of these aromatic residues seemed to cause more drastic effects on binding affinity than did replacement of basic residues, probably by perturbing the structure of BstL2-RBD. These three aromatic residues are located in the protruding loops of the N-terminal domain. Phe66 is at the interface between the two domains, with its side chain being close to Arg155. Tyr95 is located at the end of β2 and protrudes its side chain into the cleft. In contrast to Phe66 and Tyr95, Tyr102 extends its side chain towards the solvent along with the N-terminal extended structure.

Based on the distribution of these basic and aromatic residues, it can be anticipated that BstL2-RBD may have a large contact surface for the target RNA in the cleft formed by the interface region of the N- and C-terminal domains, where the RNA stem–loop structure might be wrapped by both domains, thus protecting it from RNase digestions and chemical modifications. In this putative interaction, Phe66 and Tyr95 probably participate in stabilizing the conformation of each domain, and Tyr102 may be involved in interaction with the N-terminal protruding loop of the BstL2-RBD. As described in our previous paper (Watanabe and Kimura, 1985), the BstL2-RBD was protected from proteinase digestion of the BstL2–23S rRNA complex, and it remained tightly bound to 23S rRNA. However, our recent study further demonstrated that C-terminal extension is also important for the BstL2 to bind to 23S rRNA (T.Nakashima and M.Kimura, unpublished result). It is likely that interaction of the C-terminal extension with the target RNA causes a conformational change in the RNA such that it binds to the BstL2-RBD. The C-terminus of the BstL2-RBD is located on the molecular surface between the N- and C-terminal domains. This finding further supports the idea that the target short stem–loop binds in the cleft between the N- and C-terminal domains of the BstL2-RBD.

**Similarity to other structures**

The tertiary structures of 16 ribosomal proteins have been determined thus far. Comparison of their structures with those of other known proteins in the Protein Data Bank (Abola et al., 1997) has revealed that many ribosomal proteins share structural motifs, such as ribonucleoprotein (RNP), double-stranded RNA-binding domains (dsRBDs), K homology (KH) and helix–turn–helix motifs, with RNA- or DNA-binding proteins (Ramakrishnan and White, 1998). Since the ribosome is an organelle of very ancient origin, these observations have led to the speculation that ribosomal proteins are evolutionary ancestors of the more recently evolved RNA- or DNA-binding proteins. It is well known that ribosomal protein L2 is the primary 23S rRNA-binding protein and one of the most important constituents in the peptidyl transferase center. It can thus rationally be expected that, among the 50 ribosomal proteins, the origin of protein L2 is particularly old. The importance of L2 has also been demonstrated by sequence comparison, i.e. both sequence (Wittmann-Liebold et al., 1990) (Figure 2) and immunological (Schmid et al., 1984) analyses have shown that L2 is highly evolutionarily conserved. It is also the largest protein constituent (with the exception of S1, which is important in Gram-negative bacteria for facilitating the initiation of translation, but is not considered to be a typical ribosomal protein) in the ribosome. For all these reasons, the molecular architecture of L2 has been of considerable interest.

Although previous Protein Data Base searches using L2 sequences have not detected any homologous proteins,
the present structure analysis has revealed that the central region of L2 has two distinct domains which are related with known motifs: the N-terminal domain (positions 60–130 in BstL2) shows a topology identical to the oligonucleotide/oligosaccharide-binding (OB)-fold (Murzin, 1993), whereas the C-terminal domain (positions 131–201 in BstL2) shows a topology identical to the Src-homology 3 (SH3)-like barrel in the SCOP database (Murzin et al., 1995). Both OB-fold and SH3-like motifs are found in a variety of proteins having properties of DNA and RNA binding (Figure 5). Among the ribosomal proteins, the OB-fold motif was found in S17 (Golden et al., 1993), but no SH3-like motif has been found to date.

The most conserved features of the OB-fold are in strands 1 and 4 of the β barrel: (i) strand 1 is in the middle of the β-sheet and is long and strongly coiled; (ii) the coiling of strand 1 is promoted by a conserved small residue (glycine or sometimes alanine) in the first half of the strand and by a conserved β bulge in the second half; and (iii) the αC conformation (usually at glycine) at the beginning of strand 4 facilitates the turn of the protein β structure, as mentioned above. Because of this connection, the N-terminal domain of the E.coli ribosomal protein S1 (S1 domain) conserves the features of the OB-fold motif designated as SH3-like (Holm and Sander, 1993). Instead, the N-terminal domain of the BstL2-RBD structure analyzed here, all three of these features were conserved. These important residues are marked in Figure 2. In a typical OB-fold, however, β4 and β5 strands that are connected by a short loop form an antiparallel β-sheet, and the main chain hydrogen-bonds between β5 and β3 close the β-barrel. In the BstL2-RBD structure, β4 and β5 strands have a cross-over connection, thus forming a parallel β-sheet structure. Because of this connection, the β5 strand is not involved in closing the β-barrel structure, but is involved in the linker region between the N- and C-terminal domains. Furthermore, the N-terminal domain of BstL2-RBD lacks the α-helix, which caps the barrel end between β3 and β4 in the classical OB-fold (Holm and Sander, 1993). Instead, a short loop structure caps the β-barrel in the N-terminal domain of BstL2-RBD. In spite of these disagreements with a typical OB-fold, the overall topology of the N-terminal domain conserves the features of the OB-fold structure, as mentioned above.

The internal repeating unit of the E.coli ribosomal protein S1 (S1 domain) is also expected to have an OB-fold motif (Brycoat et al., 1997). The S1 domain was identified originally in E.coli ribosomal protein S1 as a six times repeating unit (Subramanian, 1983). Subsequently, a similar repeating unit was found in a large number of proteins, including E.coli polynucleotide phosphorylase (PNPase), eukaryotic translation initiation factor eIF2α, yeast RNA helicase-like protein PRP22, DNA-dependent RNA polymerase subunit from archaeon, plants and yeast, the Tex family of bacterial proteins, idiosyncratic domains of several aminoacyl-tRNA synthetases and the cold shock domain family. The three-dimensional structure of ribosomal protein S1 is not known, but PNPase that was demonstrated by sequence comparison to have the S1 domain has been analyzed recently by NMR (Brycoat et al., 1997). The results showed that the S1 domain has an entirely OB-fold motif (Figure 5).

When the sequence of the N-terminal domain of BstL2-RBD is aligned according to its structure with the sequences of the S1 domains in ribosomal protein S1, BstL2-RBD shares only a few residues with the corresponding domains. Similar observations that the structural resemblance is not reflected in the obvious homology at the sequence level have been noted in some cases for other ribosomal proteins, such as S5 and S6: their structures have a similar topology to the dsRBD and RNP domains, respectively (Ramakrishnan and White, 1998). Nevertheless, the consensus amino acids in the S1 domain, such as Gly and hydrophobic residues that are thought to play a structural role for antiparallel β-strands in the S1 domain, are well conserved in the BstL2-RBD. It is thus suggested that the N-terminal domain of the BstL2-RBD and the repeating units of ribosomal protein S1 have a similar structure.

The DALI-server (Holm and Sander, 1993) detected that the C-terminal domain of BstL2-RBD has a similarity with proteins having an SH3-like barrel motif in the SCOP classification (Murzin et al., 1995). Figure 5 shows two of the proteins that have an SH3-like barrel. These are N-terminal domain of the proto-oncogene product c-Crk (Wu et al., 1995) and the DNA-binding domain of HIV-1 integrase (Lodi et al., 1995). The five-stranded β-barrel motif designated as SH3-like was first reported for an R67 plasmid-encoded dihydrofolate reductase from trimethoprim-resistant bacteria (Narayana et al., 1995) and subsequently has been found in SH3 domains. The SH3-like barrel is often described as a β-sandwich comprising two sets of three-stranded antiparallel β-sheets. The SH3-like barrel motif is also found in BirA, the repressor of the E.coli biotin biosynthetic operon (Wilson et al., 1992).

Recently, the structure of an eukaryotic or archaebacterial translation initiation factor (IF-5A) (Kim et al., 1998; Peat et al., 1998) was determined, and revealed that this protein has, like BstL2-RBD, OB-fold and SH3-like barrel domains joined with a flexible hinge. Interestingly, the N-terminal domain of IF-5A is an SH3-like barrel and the C-terminal domain is an OB-fold, the reverse of the situation in L2. Although there is no evidence that these molecules are genetically related, this finding supports the idea that the proteins that are involved in translation processes have evolved from the pool of the relatively small number of repertoires of the ancient nucleic acid-binding proteins by certain genetic events, such as gene duplication and gene shuffling. The structural comparison of the BstL2-RBD and IF-5A shows, however, a significant difference in domain constructions. While the OB-fold and SH3-like barrel domains in IF-5A are linked by a long linker and seem to act as two individual functional domains, these two domains in L2 are related more intimately, as described above. The genetic event required for the advent of ribosomal protein L2 (or its RBD) is not merely gene fusion but rather a more complex process; it requires the insertion between β4 and β5 to make the cross-over connection possible, which in turn allows the formation of inter-domain hydrogen bonds between β5 and β10 such that the two domains are nearly fused into one and function as an RBD. In this context, it may be worthwhile to note that the longest insertion of amino residues is observed between β4 and β5 in the case of mitochondrial L2 of Saccharomyces cerevisiae (Figure 2), which further supports this idea.
**Fig. 5.** Comparison of the fold of N- and C-terminal subdomains of BstL2-RBD with other proteins having similar motifs. (Left) (A) The OB-fold domain of the N-terminal half of BstL2-RBD. The fifth strand (β5) shown in orange is at the linker region between the OB-fold and the SH3-like barrel. (B) The S1 RNA-binding domain of *E.coli* polynucleotide phosphorylase (Bycroft et al., 1997) and (C) ribosomal protein S17 (Golden et al., 1993). (Right) (A) The SH3-like barrel domain of the C-terminal half of BstL2-RBD, (B) the N-terminal domain of the proto-oncogene product c-Crk (Wu et al., 1995) and (C) the DNA-binding domain of HIV-1 integrase (Lodi et al., 1995).

**Conclusions**

The present structural analysis demonstrates that even the largest protein component in the ribosome consists of domains of only ~70 amino residues. This domain construction is commonly observed for all larger ribosomal proteins (L1, L6, L9, S5 and S8), and possibly reflects the early genetic events of protein evolution. The N- and C-terminal domains of BstL2-RBD are OB-fold and SH3-like barrels, respectively. Both structural motifs are often found in RNA- or DNA-binding proteins, and their RNA-binding sites have been predicted to be regions constructed by the loops protruding outward from the barrel axis. In the BstL2-RBD structure, the corresponding regions are formed by L_{12} (the loop between β1 and β2), L_{34} and L_{67}. Since the residues Arg86 and Arg155 that are essential for 23S rRNA binding are located on L_{12}
and $L_{67}$, respectively, it is likely that the BstL2-RBD interacts with its target RNA in a way similar to those of other proteins containing either an OB-fold or an SH3-like barrel domain. However, these essential Arg residues in BstL2-RBD are not conserved in other OB-fold and SH3-like barrel proteins and, in addition, BstL2-RBD appears to bind the target RNA on the cleft formed by the N- and C-terminal domains, which probably confers on protein L2 a specific recognition for the target stem–loop structure.

**Materials and methods**

**Crystallization and data collection**

Selenomethionyl recombinant BstL2-RBD (60–201) was prepared from an overexpressing clone of E. coli as described elsewhere (Nakashima et al., 1999). Crystals of the selenomethionyl BstL2-RBD grown by hanging drop vapor diffusion at 18°C, by equilibrating against a reservoir solution containing 10–15% (w/w) PEG20000 in 0.1 M MES buffer at pH 6.5. A 10 μl drop consisting of equal volumes of the reservoir solution and of a solution of 10–15 mg/ml protein in 10 mM Tris–HCl buffer at pH 7.5 with 1 mM dithiothreitol (DTT) was kept at room temperature. Crystals were grown within a few days to a size of up to 0.2×0.2×0.1 mm³. Crystals with the same space group but slightly different cell dimensions were grown under the same buffer at pH 6.5. A 10 μl drop consisting of equal volumes of the reservoir solution and of a solution of 10–15 mg/ml protein in 10 mM Tris–HCl buffer at pH 7.5 with 1 mM dithiothreitol (DTT) was kept at room temperature. Crystals were grown within a few days to a size of up to 0.2×0.2×0.1 mm³. Crystals with the same space group but slightly different cell dimensions were grown under the same crystallization conditions. In all cases, the space group of the crystals was triclinic, P1. The cell dimensions of the crystal used for structure determination were $a = 28.05$ Å, $b = 36.19$ Å, $c = 69.71$ Å, $\alpha = 99.6°$, $\beta = 95.9°$ and $\gamma = 102.6°$. The crystal contains two molecules in the asymmetric unit, with a solvent content of 43% and a $V_M$ value of 2.18 Å³/Da, which is in the range of most common protein crystals (Matthews, 1968). The crystals were suspended on a loop in a thin liquid film of stabilizing solution (Teng, 1990) and frozen at 100 K directly in a cold nitrogen gas stream with a Cryostream Cooler (Oxford Cryosystems, Oxford, UK) (Cosier and Glazer, 1986). MAD data of recombinant selenomethionyl BstL2-RBD were collected from a single crystal, pre-reacted by soaking in 25–30% sucrose in the reservoir solution for a few minutes, on station BL18B at the Photon Factory, KEK, Tsukuba, Japan (Watanabe et al., 1995). The recorded image on an imaging plate was read by a drum-type IP reader, IPR4080 (Sakabe et al., 1995). Data were integrated using the HKL suite (Otwinowski and Minor, 1997). Scaling and processing were performed using the CCP4 program suite (Collaborative Computational Project, 1994). Table I summarizes the results of the data collection.

**Structure determination and refinement**

The Bijvoet and dispersive anomalous difference Patterson maps were solved by the Patterson superposition method using the SHELXS-97 program (Sheldrick, 1997). Heavy-atom parameter refinement and phase calculations were carried out using the program SHARP (de la Fortelle and Bricogne, 1997). Minor sites of heavy atoms subsequently were found in residual maps after the refinement of heavy atom parameters by SHARP (Table II). The initial electron density map subsequently was calculated by Cromer and Liberman’s method (Sasaki, 1989).
improved by real-space solvent flipping with SOLOMON (Abrahams and Leslie, 1996; Abrahams, 1997) using the procedure in SHARP. The atomic model was built using the graphics program ‘O’ (Jones et al., 1991). The model was refined against the ‘Edge’ data of selenomethionine BrL2-RBD with the program CNS (Brünger et al., 1998) using positional and temperature factor refinement followed by a simulated annealing refinement. A non-crystallographic symmetry restraint was applied to keep the two molecules in a similar conformation except some loop region. At the current stage of refinement, the model has an R-factor of 23.2% for 91% of the data between 20 and 2.3 Å, including 62–194 and 60–196 residues for crystallographically independent molecules, respectively, and 22 water molecules, for a total of 2063 atoms. The free R-factor (Brünger, 1992) for the remaining 9% of the data within this resolution range is 27.4%. The r.m.s. deviation from standard values (Eng and Huber, 1991) of bond lengths and bond angles are 0.01 Å and 1.50°, respectively. Eighty-eight percent of non-glycine and non-proline residues are in the most favored regions, and 12% of non-glycine and non-proline residues are in the additional allowed regions as defined in PROCHECK (Laskowski et al., 1993). The coordinates will be deposited in the Protein Data Bank (Abola et al., 1997).

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