The transposon Tn5 carries a gene designated ble that confers resistance to bleomycin (Bm). In this study, we determined the x-ray crystal structures of the ble gene product, designated BLMT, uncomplexed and complexed with Bm at 1.7 and 2.5 Å resolution, respectively. The structure of BLMT is a dimer with two Bm-binding pockets composed of two large concavities and two long grooves. This crystal structure of BLMT complexed with Bm gives a precise mode for binding of the antibiotic to BLMT. The conformational change of BLMT generated by binding to Bm occurs at a β-turn composed of the residues from Gln97 to Thr102. Crystallographic analysis of Bm bound to BLMT shows that two thiazole rings of the bithiazole moiety are in the trans conformation. The axial ligand, which binds a metal ion, seems to be the primary amine in the β-aminoalanine moiety. This report, which is the first with regard to the x-ray crystal structure of Bm, shows that the bithiazole moiety of Bm is far from the metal-binding domain. That is, Bm complexed with BLMT takes a more extended form than the drug complexed with DNA.

Bleomycin (Bm), an antibiotic produced by Streptomyces verticillus, is widely employed in the treatment of several neoplastic diseases including non-Hodgkin's lymphoma, squamous cell carcinomas, and testicular tumors (1, 2). The Bm-Fe(II) complex, in conjunction with a reducing agent and oxygen, causes nucleotide sequence-specific DNA cleavage (3). It has been suggested that “activated Bm,” generated by the reductive activation of oxygen by a Bm-Fe(II) complex, cleaves DNA (3, 4). Recent studies have provided unequivocal evidence for Bm-mediated degradation of certain RNA substrates, notably transfer RNAs and tRNA precursor transcripts (5–7).

We have cloned and sequenced a gene, designated blmA, encoding a Bm resistance determinant from Bm-producing S. verticillus (8). The gene blmA encodes a Bm-binding protein, designated BLMA (8, 9). Tallysomycin-producing Streptallochus hindustanus produces a protein designated Shble protein, which binds to Bm like BLMA (10). When incubated with Bm-binding proteins, Bm loses both its antibacterial and DNA-cleaving activities (8, 10).

Although Bm has not been used as an antibacterial agent, almost all strains of methicillin-resistant Staphylococcus aureus, isolated in Hiroshima University Hospital, were resistant to the drug. A Bm resistance gene, designated blmS, has been cloned from chromosomal DNA isolated from methicillin-resistant S. aureus and sequenced (11). The gene product, designated BLMS, is also a Bm-binding protein (9).

The transposon Tn5, expressed in Escherichia coli, carries Bm resistance together with kanamycin and streptomycin resistances (12–14). The nucleotide sequence analysis of the Bm resistance gene, designated ble, has suggested that it encodes a protein consisting of 126 amino acids with a molecular mass of 14,058 daltons (14). In addition to its role as the Bm resistance gene product confers survival advantage to E. coli (15, 16). The amino acid sequence of the ble gene product, designated by us as BLMT (17), shares sequence homology with the BLMA protein (21%) and the Shble protein (25%). BLMT has been determined to be a Bm-binding protein (17, 18).

We have determined the crystal structure of BLMA at a high resolution of 1.5 Å by the single isomorphous replacement method including the anomalous scattering effect (19). Another group has determined the crystallographic analysis of the Shble protein at a 2.3 Å resolution (20). Both groups have independently provided a model that suggests that dimeric formation of the protein generates two pockets for binding to Bm. However, because the crystallization of BLMA and the Shble protein, which are complexed with Bm, has been unsuccessful until now, the precise binding mode between the protein and Bm has not been determined.

In this study, we successfully crystallized BLMT uncomplexed and complexed with Bm; the former and latter structures were determined at 1.7 and 2.5 Å resolution, respectively. We describe the conformational differences of BLMT in the Bm-free and Bm-bound form. Although a structural model of Bm bound to an oligonucleotide, determined by two-dimensional NMR analysis, has been proposed (21, 22), we report a structural model based on x-ray crystallography.
EXPERIMENTAL PROCEDURES

Crystal Preparation—BLMT, overproduced using an E. coli host vec-
tor system, was purified according to the methods described previously
(17, 23). For crystallization, BLMT was adjusted to a final concentra-
tion of 21 mg/ml. Crystals were obtained by vapor diffusion at 25 °C
using the hanging-drop method (24) with the mother liquor of 25% PEG
6000 (polyethylene glycol), 0.1 M calcium acetate, and 0.1 M sodium
bicarbonate at pH 7.0. These crystals belong to the orthorhombic space
group C222, with unit cell dimensions \( a = 81.3 \text{ Å}, b = 85.0 \text{ Å}, \) and \( c = 78.8 \text{ Å} \). The crystal volume per unit of \( V_{\text{sym}} \), \( 25, \) is 2.42 \( \text{Å}^3/ \text{Da} \) with 2 BLMT monomers in the asymmetric unit, which corresponds to
a solvent content of 49%.

For crystallization of BLMT complexed with Bm, the protein was
incubated at a final concentration of 14 mg/ml for 1 h at room temper-
ature, 3-fifths excess of Bm, and then the excess of Bm was removed by
 centrifugal ultrafiltration (Fig. 1). The molar ratio of Blm to Bm was
suitable for complete binding to BLMT. The crystals of the BLMT-Bm
complex were obtained by vapor diffusion at 25 °C using the hanging-drop method with the mother liquor of 15% PEG
6000, 0.1 M ammonium sulfate, 0.02 M magnesium chloride, and 0.05 M MES-NaOH at pH 5.0. These crystals belong to the orthorhombic space
group \( P2_12_12 \), with unit cell dimensions \( a = 115.3 \text{ Å}, b = 117.9 \text{ Å}, \) and \( c = 79.9 \text{ Å} \). The \( V_{\text{sym}} \) is 2.14 \( \text{Å}^3/ \text{Da} \) with 2 complexes (2 \( = \) two nonmonomeric BLMT molecules complexed with two Bm molecules) in
an asymmetric unit. The value corresponds to a solvent content of 42.5%.

Data Collection and Processing—Diffraction data for the Bm-free
BLMT crystals were collected with synchrotron radiation (1.0 Å
wavelength) at beam 16B of the Photon Factory, the National Labora-
tory for High Energy Physics, Tsukuba, Japan. The data collection was
done with three sets of high resolution data up to 1.7 Å
resolution were recorded on Fuji imaging plates (200
\( 500 \text{ mm} \)) using a synchrotron radiation produced by a Rigaku RU-
300 rotating anode generator operated at 40 kV and 100 mA. The
crystal-to-detector distance was set to 95 mm. Each frame of the 1.5°
crystal oscillation was collected for 10 min. The diffraction spots in a
rotation range of 90° were recorded on a total of 60 frames. The data
processing was accomplished at 2.5 Å resolution with the R-AIXIS IIC
data processing software package. The number of reflections having
adequate intensities was small; this may be because of the noncrystal-
lographic translational symmetry causing the systematic weakness of
the intensities. Details of the data collection are summarized in Table I.

Structure Determination and Refinement of BLMT Complexed with Bm—The crystal structure of BLMT was solved by the molecular
replacement method using the programs in X-PLOR (29). The start
model was a dimeric BLMA structure previously determined at 1.5 Å
resolution (40) was used, reflections at low resolution together with weak reflec-
tions with \( h+k+l = \text{odd} \) (Table I). The atomic coordinates, obtained by the molecular replacement method,
were refined against the 18,250 reflections with \( F \text{gt} \) and \( F \text{lt} \) were 19.0 and 26.4%, respectively, for the reflections from 10.0 to 4.0 Å resolution. The number of reflections having ade-
quate intensities was small; this may be because of the noncrystal-
lographic translational symmetry causing the systematic weakness of
the intensities. Details of the data collection are summarized in Table I.

The refinement was started with data from 10 to 3.5 Å resolution and
finished at 2.0 Å resolution. Positional parameters and refinement for
individual isotropic \( B \)-factors were included in each refinement cycle. The

The current model contains two protein monomers in the asymmetric
unit, together with 111 water molecules, two tetraethylene glycol moe-
cities, and one calcium ion. Because Met\(^2\) and Leu\(^22\)--Ser\(^25\) were invis-
ible on the electron density due to their higher flexibility, they were
excluded from the current model. All side-chain atoms of the residues
Leu\(^24\), excised from the model, and the side-chain atoms of Gly\(^{89}\) were completely invisible and, above the Cβ, the atoms of the Gly\(^{22}\) side-chain are excluded from the model. A
Luzzati plot (36) revealed that the mean coordinate error was \( 0.2 \text{ Å} \). No residues except Trp\(^5\) are in the acceptable regions of the Ramancha-
dran plot (37, 38). A peptide bond between Tyr\(^{46}\) and Pro\(^{50}\) is in cis-
conformation from the dihedral angle of 61° in bond angle, 2.0° in improper angle (Table I). The average B-factors are 26.3 Å\(^2\) and 39.5 Å\(^2\) for 1892 all-protein atoms and for 138 non-protein atoms.

Structure Determination and Refinement of BLMT Complexed with
Bm—Pseudo-precession photographs indicate that the reflection inten-
sities \( (I(hkl)) \) were systematically weak if \( k \) was an odd number. When
calculated using the reflections from 10.0 to 4.0 Å resolution, the second
highest peak, which is 6% lower than the origin peak, was present at \( (x, y, z) = (0, 0.5, 0) \) on the Patterson map. This suggests that the noncrystal-
lographic translational symmetry parallel to the \( b \)-axis is present in the
crystal. To obtain the solutions of the rotation and the translation functions by decreasing the number of independent molecules per asymmetric unit, we made modifications as follows: when \( k \) is an odd number, the reflection
was ignored. Otherwise, if \( k = \text{even number} \), the Miller indices of \( F(hkl) \) were transformed as follows: when \( k \) is an odd number, the reflection

The crystal structure of BLMT complexed with Bm was selected
from each imaging plate were scaled to each other by the Fox and
Holmes method (28) and were then merged.

The diffraction data for a crystal of BLMT complexed with Bm was
collected with a R-AIXIS IIC image plate detector system using the
mirror monochromated CuK\(_\alpha\) radiation produced by a Rigaku RU-
300 rotating anode generator operated at 40 kV and 100 mA. The
crystal-to-detector distance was set to 95 mm. Each frame of the 1.5°
crystal oscillation was collected for 10 min. The diffraction spots in a
rotation range of 90° were recorded on a total of 60 frames. The data
processing was accomplished at 2.5 Å resolution with the R-AIXIS IIC
data processing software package. The number of reflections having
adequate intensities was small; this may be because of the noncrystal-
lographic translational symmetry causing the systematic weakness of
the intensities. Details of the data collection are summarized in Table I.

At the current stage of the X-PLOR refinement, \( R_{\text{free}} \) and the \( R \)-factor were increased to 32.5% and 20.8%, respectively, against the 18,250 reflections with \( F > 2 \sigma \) from 10 to 2.0 Å resolution. Further refinement was performed with the data set from
5.0 to 1.7 Å resolution using the program SHELXL-97 (35). The final
\( R \)-factor was 19.1% for 26,798 reflections between 5.0 and 1.7 Å
resolution.
The structure of Bm A2. The junctions between the molecular units comprising Bm A2 are indicated by wavy lines. The underlined N atoms are the putative equatorial ligands to the metal ion.

In the present study, we prepared the original topology and parameter files of the Bm molecule. The metal-binding domain in the Bm A2 molecule is composed of the β-aminoalanine, pyrimidinyl propionamide, and β-hydroxyhistidine moieties. The binding domain for DNA is composed of the γ-aminopropyl dimethylsulphonium and bithiazole moieties (Fig. 1). The parameters used for construction of a three-dimensional model of the former and latter moieties were obtained from the x-ray crystal structures of the P-3A-Cu(II) complex (40) and 3-2′′-phenyl-2,4′′-bithiazole-4-carboxamide)propyl dimethylsulphonium iodide (41), respectively. The parameters for threonine and methyl valerate in the BLMT molecule were prepared using the energy minimization structure, which is calculated by the program CAChe.

The current model contains 8 monomeric BLMT molecules, 8 Bm molecules, and 48 water molecules in the asymmetric unit. The monomeric BLMT molecule in the current model consists of the Thr5–Glu120 residues, because both terminal residues, Met1 and Glu121–Ser126, were invisible and poorly defined, respectively. The mean coordinate error was estimated from a Luzzati plot to be 0.32 Å. The Ramachandran plot indicates that the backbone torsion angles for 95.9% are in the acceptable region. Most of the remaining residues, because both terminal residues, Met1 and Glu121–Ser126, were invisible and poorly defined, respectively. The mean coordinate error was estimated from a Luzzati plot to be 0.32 Å. The Ramachandran plot indicates that the backbone torsion angles for 95.9% are in the acceptable region. Most of the remaining residues, because both terminal residues, Met1 and Glu121–Ser126, were invisible and poorly defined, respectively. The mean coordinate error was estimated from a Luzzati plot to be 0.32 Å. The Ramachandran plot indicates that the backbone torsion angles for 95.9% are in the acceptable region.

The overall structure of BLMT complexed with Bm is almost the same as that of the Bm-free BLMT (Fig. 4). The current model shows with accuracy that two Bm molecules bind to two Bm-binding pockets formed by the alternate arm exchange of two monomeric BLMT molecules, results in two large concavities and two long grooves.

Although a noncrystallographic symmetry restraint is not imposed in the final refinement, one monomeric BLMT molecule is almost the same as the partner’s monomer. The superposition of main-chain atoms on each BLMT monomer is shown in Fig. 3B. The r.m.s. positional differences between two monomeric BLMT molecules are 0.41 Å for main-chain atoms and 0.79 Å for all-protein atoms. Structural deviations occur at two turn regions, Ala52–Ser58 and Leu86–Thr102, and at a long loop composed of the Gln92–Gly97 residues. The conformational deviations may be generated because of the flexibility of these regions.

The electron density, which fall outside of the acceptable region, have poorly defined electron densities. The hydrogen bonding networks of the γ-aminopropyl dimethylsulphonium and bithiazole moieties (Fig. 1). The parameters used for construction of a three-dimensional model of the former and latter moieties were obtained from the x-ray crystal structures of the P-3A-Cu(II) complex (40) and 3-2′′-phenyl-2,4′′-bithiazole-4-carboxamide)propyl dimethylsulphonium iodide (41), respectively. The parameters for threonine and methyl valerate in the BLMT molecule were prepared using the energy minimization structure, which is calculated by the program CAChe.

RESULTS AND DISCUSSION

Overall Structure

The monomeric BLMT molecule consists of two α-helices and two β-sheets (β2–β5 and β6–β9), in addition to a short N-terminal β1-strand (Fig. 2A). The BLMT monomer exhibits two very similar domain structures connected to a long loop from Pro51 to Trp93. The first domain consists of the α1-helix and four β-strands (β2, β3, β4, and β5). The β2-strand is parallel to the β5-strand, and the other pairs of β-strands (β3 and β4, β4 and β5) are in an antiparallel configuration. The α1-helix plays a role as a linker connecting the β2- and β3-strands. Similar topology (α2βββ) is found in the second domain (α2-helix and β6–β9 strands). The hydrogen bonding networks of the β3- and β7-strands to each partner’s β-strand generate an atypical anti-parallel β-sheet because they are disordered (Fig. 2B).

Two monomeric BLMT molecules are related by a noncrystallographic 2-fold axis (Fig. 3A). The β3-strand interacts with the partner’s β6-strand, suggesting that the former strand plays a key role for the dimeric formation. The topology of BLMT is almost the same as BLMA (19) and the SbbL protein (20). The dimer formation of BLMT, generated by the alternate arm exchange of two monomeric BLMT molecules, results in two large concavities and two long grooves.

The bithiazole mode of Bm is inserted into the long groove running along the dimer interface (Fig. 5A). The first thiazolium ring, which is interacted by the hydrophobic effect with the Phe30B benzene, is also interacted by the polar effect with the Arg65A guanidino group. The second thiazolium ring is tightly stacked with two indole rings from Trp35B and Phe30A (Fig. 5B). The stacking effect contributes to stabilization of the Bm molecule.

The Trp35B indole ring is interacted by a hydrogen bond with the Gln46B carboxylate. The Gln46B carboxylate, interacted by two salt bridges with the Arg96A guanidino group, is also stabilized by a hydrogen bond with the Thr102B hydroxyl oxygen (Fig. 5B). That is, a hydrogen bonding network is extended from Trp35B to Arg96A. In addition, the Arg96A and Trp35B are interacted with the first and second thiazolium rings, respectively.

The large concavity is formed mainly by the residues from one monomer. One side of the concavity has the hydrophobic residues. The linker, metal-binding, and sugar domains of Bm are buried in the large concavity (Fig. 5A) and stabilized by a large number of hydrogen bonds to the protein atoms.

In the metal-binding domain of Bm, an amino group attached
to the pyrimidine ring is interacted by a hydrogen bond with two backbone carbonyl oxygens of Phe^{60A} and Gly^{111A} (Fig. 6). The carbonyl oxygen of the pyrimidinyl propionamide moiety of Bm forms a hydrogen bond with the Arg^{90A} guanidino group (Fig. 6). The terminal amide group of the pyrimidinyl propionamide moiety forms three hydrogen bonds: its oxygen atom with the Arg^{115A} guanidino group, its nitrogen atom with the Ser^{61A} hydroxyl oxygen, and the Trp^{59A} carbonyl oxygen (Fig. 6). However, β-aminoalanine and β-hydroxyhistidine for the metal binding make no polar interactions with the protein atoms. Because the β-aminoalanine moiety is poorly defined in the electron density, the conformation may be unstable. However, its amide group orients parallel to the Trp^{59A} indole ring and seems to be stacked by the apolar interaction. In fact, the Trp^{59A} side-chain is flexible in the Bm-free form but rigid in the complexed form because the side-chain is stacked with the imidazole rings of His^{50B} and the amide group of the β-aminoalanine moiety of Bm.

Only mannose, but not gulose, in the sugar moiety of Bm is involved in the binding to the protein. The carbamoyl group, attached to the 3’ hydroxyl oxygen of mannose, makes two hydrogen bonds: its nitrogen atom with the carbonyl oxygens of Pro^{55A} and Ser^{56A} (Fig. 6). The 2’ and 4’ hydroxyl groups form a hydrogen bond with the Leu^{56B} carbonyl oxygen and the Arg^{50B} guanidino group, respectively. The 6’ hydroxyl group interacts by the hydrogen bond with the Arg^{50B} guanidino, Ser^{55B} carbonyl, and Gly^{87B} amide groups (Fig. 6).

The interaction between the protein and the linker domain of Bm is weak. Only one hydrogen bond is formed between the Arg^{115A} guanidino group and the carbonyl oxygen of the threonine moiety of Bm (Fig. 6). This occurrence is likely to be related with the flexibility of the domain.

The terminal γ-aminopropyl dimethylsulphonium moiety of Bm A_{γ}, having the positive charge, is not well defined in the electron density. The end of the long groove, which is the expected binding site for the γ-aminopropyl dimethylsulphonium moiety, has negatively charged residues Asp^{3}, Asp^{42}, Asp^{97}, and Glu^{120}. These residues are located around the positive charge of the γ-aminopropyl dimethylsulphonium moiety. However, no electrostatic interactions are observed between this moiety and the negatively charged residues, suggesting
that the negatively charged residues are necessary for the recognition of the ligand rather than for stabilization.

Conformational Change of BLMT by Binding of Bm

Fig. 7A shows the superposition of the BLMT-Bm structure on the Bm-free structure. The positional r.m.s. differences between two structures are 0.53 and 0.77 Å for the main-chain atoms and all-protein atoms, respectively. The marked positional deviation occurs at the residues located on the loop and turn regions, such as His$^{50}$–Ala$^{57}$, Glu$^{83}$–Gly$^{87}$, and Gln$^{97}$–Thr$^{102}$. In particular, the Gln$^{97}$–Thr$^{102}$ residues, composed of a β-turn between the β7- and β8-strands, move about 2 Å toward the partner’s monomer. The conformational change enables the Trp$^{99}$ indole ring of one monomer to approach the Trp$^{35}$ indole ring of the partner’s monomer. The conformational approach results in the formation of stable structure of the long groove and strong intercalating interaction with the bithiazole moiety of Bm. However, the corresponding region of the Bm-free BLMT appears to be highly flexible with large B-factors (Fig. 7B).

Comparison with Bm-binding Proteins from Other Microbial Sources

Overall Structure—The amino acid sequence homology between BLMA and the Shble protein is ~60%, whereas that between BLMT and BLMA, or between BLMT and the Shble protein is only 21–25%. Nevertheless, the superposition of the main-chain atoms of BLMT on that of BLMA or the Shble protein shows that the overall structure of BLMT is almost the same as those of BLMA and the Shble protein (Fig. 8A). The r.m.s. positional differences for 114 residues of the core region composed of β-sheets are 1.25 Å between BLMT and BLMA and 1.35 Å between BLMT and the Shble protein. The marked structural deviation found among three Bm-binding proteins occurs away from the gravity center of dimeric molecules.

The conformation of a long loop connecting α2-helix and the β7-strand in BLMT is more stable than that in BLMA or the Shble protein. The stability is likely to be generated by the deletion of one amino acid in this region (Fig. 8B). Otherwise, the turn region between the β7- and β8-strands of BLMT is highly flexible. The flexibility may be generated by the insertion of an amino acid into the long loop (Fig. 8B).

Substrate Specificity—Table II lists the dissociation constants ($K_d$) to Bm and phleomycin (Phm) for BLMT and other Bm-binding proteins. Phm is an analog of Bm and has a thiazolinylthiazole instead of a bithiazole moiety in Bm; that is, the second thiazolium ring in Phm is reduced and nonplanar. We have observed that the affinity between BLMT and Phm is stronger than between BLMA and Phm. Although the $K_d$ value to Phm for the Shble protein has not been determined yet, there is a report that the minimum inhibitory concentration of Phm to E. coli expressing the Shble protein is much lower than that of Bm (20). This suggests that the binding affinity of the Shble protein to Phm is lower than to Bm. The difference of the dissociation constant between Bm and Phm can be explained as follows. The turn region of BLMT composed of the Gln$^{97}$–Thr$^{102}$ residues, which is the Bm-binding domain, is more flexible than those of BLMA and the Shble protein. The flexibility of the binding domain in BLMT is generated by the insertion of an
additional amino acid into the turn region (Fig. 8B). The flexibility might decrease the substrate specificity for binding.

**Bm-binding Sites**—Aromatic residues corresponding to Phe30 and Trp35 of BLMT, which interact with the bithiazole moiety of Bm, are present in BLMA, BLMS, and the Shble protein (Fig. 8B), suggesting that the aromatic rings are interacted by the hydrophobic effect with the bithiazole moiety. An amino acid corresponding to Trp99 of BLMT, which interacts with the bithiazole moiety of Bm, is also present in BLMS and the Shble protein. The Trp residue of these proteins is likely to interact with the bithiazole moiety. BLMA is replaced by Ala instead of Trp99. A crystallographic analysis of BLMA complexed with Bm showed that Pro101 interacts with the bithiazole moiety.2

The negatively charged residues of the BLMT-Bm complex are located around the positive charge of the γ-aminopropyl dimethylsulphonium moiety of Bm A2, but no electrostatic interactions are found between the protein and Bm. The distribution of the negatively charged residues in BLMT (Fig. 8B) resembles those in other Bm-binding proteins (19, 20). These negatively charged residues might be helpful in the recognition of the ligand.

**Structure of Bleomycin**

Despite the powerful investigation of the x-ray crystal structure of Bm for the last two decades, the structure has not yet been determined. Therefore, the controversial and elusive points on the structure of the Bm molecule must be resolved; for example, it is not yet clear whether the two thiazolium rings in the bithiazole moiety of the Bm molecule are in cis- or trans-conformation. The crystallographic study of bithiazole derivatives has suggested that the two thiazolium rings may be in trans-conformation (41, 42). Some groups have determined that the solution structure of bithiazole moiety bound to DNA and suggested that the cis-conformation is favored to account for the upfield shift of the chemical shift of the bithiazolium ring protons (43, 44). In the present study, we tried to model both cis- and trans-type bithiazole moiety. From the electron density map obtained in this study, we conclude that the two thiazolium rings are in trans-conformation.

The ligands of the Bm molecule for the metal ion and its chirality also remain ambiguous. Most investigators agree on the fact that the equatorial ligands are the secondary amine of the β-aminoalanine moiety, the amide nitrogen of the β-hydroxyhistidine moiety, and the nitrogens from the pyrimidine and the imidazole rings. However, three possibilities are emphasized for the axial ligand: (i) the primary amine of the β-aminoalanine moiety (45–47); (ii) the carbamoyl nitrogen of the mannose moiety; and (iii) the primary amine of the β-aminoaalonine moiety and the carbamoyl nitrogen of the mannose moiety (48–50). Although our model of BLMT complexed with Bm does not contain the metal ion, we suggest that the primary amine of the β-aminoaalonine moiety is suitable as an axial ligand for the metal ion. In the Bm molecule, while the car-

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2 M. Sugiyama, M. Hayashida, Y. Matoba, M. Maruyama, and T. Kumagai, unpublished data.
FIG. 6. Hydrogen bonding network of Bm to BLMT in the large concavity. The residues from Bm and BLMT are colored in black and gray, respectively. The oxygen and nitrogen atoms are colored in red and blue, respectively. The dotted lines indicate hydrogen bonds.

FIG. 7. Conformational change of BLMT by binding to Bm. A, superposition of BLMT uncomplexed with Bm onto that complexed with Bm. Ca backbones of BLMT uncomplexed and complexed with Bm are colored in red and blue, respectively. B, B-factor of BLMT uncomplexed and complexed with Bm. The black and red lines indicate the average B-factor for main-chain atoms of BLMT uncomplexed and complexed with Bm, respectively.

FIG. 8. Comparison of BLMT with other Bm-binding proteins. A, superposition of three Bm-binding proteins. Ca backbones of BLMT, BLMA, and Shble proteins are colored in green, blue, and red, respectively. B, alignment of Bm-binding proteins from different microbial sources. Red and blue indicate acidic and basic residues, respectively. Purple and green arrowheads indicate an amino acid deletion and insertion in BLMT, respectively.
The same chirality as the propositions (45–47). Judging from the model, Bm A2 complexed with DNA have a more extended form, that is, the bithiazole moiety folds back into a large concavity of BLMT and stabilized by a number of hydrogen bonds including several hydrogen bonds with DNA as well as with BLMT. The linker and metal-binding domains of Bm, located in the minor groove of DNA, are stabilized by several hydrogen bonds with DNA including base pair-specific ones.

The binding mode of Bm complexed with BLMT may be similar to that with DNA for the following reasons. First, the bithiazole moiety is intercalated with the aromatic rings in both cases of DNA and BLMT. Second, the linker and metal-binding domains are buried in the minor groove of DNA or the large concavity of BLMT and stabilized by a number of hydrogen bonds. Finally, the positive charge of the terminal amine of Bm may be necessary for the electrostatic interaction with DNA or BLMT.

A model of the DNA-Bm complex shows that Bm exhibits a more compact form, that is, the bithiazole moiety folds back toward the metal-binding domain (21, 22). However, x-ray analysis of Bm complexed with BLMT shows that the Bm molecule has a more extended form, that is, the bithiazole moiety is far from the metal-binding domain.

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TABLE II
Ligand specification of Bm-binding proteins

| Ligand | BLMT | BLMA | Shible complex |
|--------|------|------|---------------|
| $K_d$ to Bm | 32 | 280 | 55<sup>a</sup> |
| $K_d$ to Phm | 87 | 1,800 | ND<sup>b</sup> |

<sup>a</sup> Dumas et al. (20).
<sup>b</sup> Not determined.
