An Src Homology 3-like Domain Is Responsible for Dimerization of the Repressor Protein KorB Encoded by the Promiscuous IncP Plasmid RP4

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KorB is a regulatory protein encoded by the conjugative plasmid RP4 and a member of the ParB family of bacterial partitioning proteins. The protein regulates the expression of plasmid genes whose products are involved in replication, transfer, and stable inheritance of RP4 by binding to palindromic 13-bp DNA sequences (5′-TTTAGC(G/C)GCTAAA-3′) present 12 times in the 60-kb plasmid. Here we report the crystal structure of KorB-C, the C-terminal domain of KorB comprising residues 297–358. The structure of KorB-C was solved in two crystal forms. Quite unexpectedly, we find that KorB-C shows a fold closely resembling the Src homology 3 (SH3) domain, a fold well known from proteins involved in eukaryotic signal transduction. From the arrangement of molecules in the asymmetric unit, it is concluded that two molecules form a functionally relevant dimer. The detailed analysis of the dimer interface and a chemical cross-linking study suggest that the C-terminal domain is responsible for stabilizing the dimeric form of KorB in solution to facilitate binding to the palindromic operator sequence. The KorB-C crystal structure extends the range of protein-protein interactions known to be promoted by SH3 and SH3-like domains.

KorB plays a direct role in the partitioning of the plasmid, acting together with another protein. KorB also functions as a transcriptional repressor of RP4 genes. It is a member of the ParB family of proteins that are involved in genome partitioning and encoded on plasmids and bacterial chromosomes (8–11).

Purified KorB exists as a dimer in solution (12), and even formation of a homo-oligomer was described (13). KorB has a size of 358 amino acids (39,011 Da) with an abundance of about 2000 molecules/cell. Despite its negative charge at neutral pH, KorB recognizes and binds specifically to the operator sequence, O9 (5′-TTTAGC(G/C)GCTAAA-3′), which occurs 12 times on the RP4 genome. The positions of these 12 operator sites (measuring from the middle of the O9 site) relative to RP4 promoters can be classified as follows. Class I sites are 39/40 bp upstream of a transcription start point, class II sites are further upstream or downstream of promoters but within 80–190 bp of a transcription start site, and class III sites are more than 1 kb away from any known promoter (14). KorB can repress the RP4 promoters carrying class I or class II O9 sites (4, 5, 14–16), but its role in the regions where class III operators occur has not been elucidated. However, the conservation of these sites on the IncP-1 relative of RP4, plasmid R751, whose complete sequence was compiled (17), suggests that even class III O9 sites play an important role in the expression of the genome. In conjunction with KorA, KorB represses the transcription of the kilA, trfA, and korAB operons. It is also involved in the negative control of kilB operons. KorA and KorB act cooperatively in transcriptional repression.

Detailed studies on the binding of KorB to the 12 operators present in RP4 showed that they fall into three groups according to the binding strength of KorB. The highest affinity site is O9,10, which occurs in the promoter transcribing genes for replication, trfAp. Purified IncC1 enhanced KorB binding to all O9 sites except O9,3, a site involved in partitioning (18). The 5-bp sequences flanking the 13-mer O9 site were found to affect KorB binding and IncC1 stimulatory activity. Flanking sequences on one side only were sufficient to specify the difference between O9,10 and O9,3. These differences also eliminated potentiation by IncC1. It was suggested that KorB contacts O9,10 flanking sequences and that IncC1 may alter the conformation of multimeric KorB so that it is better able to make these contacts, thus stabilizing the complexes once formed.

To elucidate the structural basis of KorB’s DNA-binding properties and the interaction with other proteins, a crystallographic analysis was initiated. Here we report the crystal structure of the C-terminal domain, KorB-C,1 consisting of 62

1 The abbreviations used are: KorB-C, C-terminal domain (amino acids 297–358) of KorB protein; KorB-N, N-terminal domain (amino acids 1–296).
KorB Dimerization through a C-terminal SH3-like Domain

EXPERIMENTAL PROCEDURES

Preparation and Crystallization—korB was overexpressed in E. coli, and the protein was purified as described by Balzer et al. (12). The highly soluble protein (>100 mg ml⁻¹) was subjected to crystallization experiments. After several months, a crystal with a hexagonal unit cell of a = 51.8 Å, c = 88.3 Å was obtained. Even under the most favorable assumptions of one KorB molecule per asymmetric unit and point symmetry 6 of the crystal, this unit cell is too small to accommodate the crystallographically independent copies. Based on the structure, we argue that the C-terminal domain is mainly responsible for stabilizing the dimeric form of KorB, thus enhancing specific operator binding by the repressor.

| Native | Derivative |
|--------|------------|
|        | PtCl₂ | Cis-platin(II) diamminchloride |
| Orthorhombic | P₂₁₂₁ | P₂₁₂₁ |
| Hexagonal | P₆₃ | P₆₃ |

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RESULTS

Structure Determination and Analysis—X-ray diffraction data were collected in-house and at beamline X31 of EMBL/DESY in Hamburg and processed with DENZO/HKL (20). The structure was solved in the orthorhombic space group using a MIRAS approach based on three diffraction data sets collected for two platinum derivatives (Table 1). One derivative data set was collected at the Pt L₃Ⅲ peak wavelength as established from a fluorescence scan to optimize the anomalous signal. Four heavy atom sites present in each derivative but differing in relative occupancy and anomalous contribution were identified by SOLVE (21). The phases were refined with MLPHARE, and the quality of the density was improved by solvent averaging using DM as implemented in the CCP4 package (22). A first inspection of the electron density revealed the noncrystallographic symmetry present in the asymmetric unit and permitted the subsequent phase improvement by noncrystallographic symmetry averaging in DM. This yielded an easily interpretable electron density map. An atomic model of KorB-C was built in O (23) and initially refined in XPLOR 3.7 (24). Finally, the structure was refined to convergence in REFMAC (25) using isotropic B values for all nonhydrogen atoms. Noncrystallographic symmetry restraints were gradually decreased and completely removed in the last refinement cycles. All solvent peaks were modeled as fully occupied water oxygens. Alternative conformations were modeled for a number of side chains and occupancies adjusted to yield similar B values for both conformers. The structure of KorB-C in the hexagonal space group was solved by molecular replacement with AMORE (26) using a protein dimer from the orthorhombic KorB-C structure as a model. The structure of hexagonal KorB-C was refined essentially as the orthorhombic structure.

Chemical Cross-linking and DNA Binding Assay—KorB-C protein (15 pmol) was treated with increasing concentrations of glutaraldehyde in a buffer consisting of 20 mM NaH₂PO₄ (pH 7.0), 100 mM NaCl, 1 mM dithiothreitol and 5% glycerol. After a 1-h incubation at 37 °C, the reaction was stopped by adding 1 μl Tris-HCl, pH 7.6, to a concentration of 100 mM. The products were reduced, heat-denatured, and electrophoresed on a 17.5% (w/v) polyacrylamide gel containing 0.1% (w/v) SDS.

Complex formation of KorB proteins with O₄ DNA was assayed by polyacrylamide gel electrophoresis. A DNA digest containing equimolar amounts of linear four-base 30 min at 37 °C with 3 or 9 pmol of KorB or with 2.5–45 pmol of either KorB-C or KorB-N and electrophoresed at 8 V cm⁻¹ on a nondenaturing 3% PAGE containing 40 mM Tris-acetic acid, pH 7.9, 20 mM sodium acetate, 2 mM EDTA according to Balzer et al. (12). DNA bands were visualized by staining with ethidium bromide.

RESULTS

Structure Analysis—The crystal structure of KorB-C, the C-terminal domain of the RP4-encoded repressor protein KorB,
was determined in two space groups. After refinement, all residues lay in the allowed or favored region in the Ramachandran plot for both structures. Stereochemical parameters were calculated by PROCHECK (27) and WHATCHECK (28) and were in the range expected for structures with similar resolution.

The orthorhombic structure was solved by multiple isomorphie replacement with contribution from the anomalous scattering of the platinum atoms. The asymmetric unit contains four copies of KorB-C. The final model consists of 1803 atoms and 250 solvent atoms. Of the expected 62 residues (297—358 of KorB), not all are visible in the electron density for each molecule. The N-terminal polypeptide region varies most, with molecule A starting from residue 305, molecules B and C from 302, and molecule D from 300. A number of side chains are not visible in the N-terminal region as well: the side chains of Pro 299, Lys 305, and Lys 306 in molecule B. The final structure is also reflected in high mean atomic displacement parameters assume values close to the B values (Table II). The diffraction component precision index (29) was determined in two space groups. After refinement, all independent copies, four times in the orthorhombic structure and twice in the hexagonal structure. All six copies share the general structural organization described above. This is evident from the root mean square (r.m.s.) deviations between equivalent atoms in the six molecules after least-squares superposition (Table III).

**Subunit Structure—**KorB-C is folded into a five-stranded antiparallel all-β structure (Fig. 1). Strands β1, β2, β3, and β4 are arranged as an antiparallel up-and-down β-sheet with intervening loops of different length. The curvature of the strands and the pronounced left-handed twist of the sheet permit strand β5 to complete the fold by an antiparallel interaction on the free side of β1. Strands β1 and β2 are connected by loop L1 comprised of a β-turn (His313—Arg316), whereas a 10-residue loop (L2) links β2 to β3. This loop contains two β-turns, from Ile321 to Arg324 and from Ala328 to Tyr331. The short loop L3 is stabilized by a network of hydrogen bonds linking Tyr336 oxygen with Gly340 nitrogen, Asp338 Oδ1 with Asp339 nitrogen, and Asp339 Oδ1 with Glu341 nitrogen. Finally, the connection L4 between strands β4 and β5 contains a 310-helical conformation of three residues (Leu347—Asp349).

The KorB-C monomer is observed in six crystallographically independent copies, four times in the orthorhombic structure and twice in the hexagonal structure. All six copies share the general structural organization described above. This is evident from the root mean square (r.m.s.) deviations between equivalent atoms in the six molecules after least-squares superposition (Table III). α-Carbon positions superimpose with r.m.s. distances of 0.5 ± 0.2 Å and all equivalent atoms with 0.95 ± 0.2 Å for all chains with the exception of chain A of orthorhombic KorB-C. This chain clearly differs in the conformation of its three C-terminal residues, but it is structurally similar to the other five elsewhere. The unique conformation of the C-terminal tripeptide of molecule A is not caused by un-
usual crystal contacts. Instead, it seems that the disorder of this molecule’s N-terminal part, for which electron density is observed only from residue Lys305 onward, is correlated to the altered C-terminal structure of molecule A of KorB-C. Otherwise, the most pronounced differences between KorB-C monomers are in the N-terminal peptide region, which, in the orthorhombic structure, adopts an extended conformation for chains C and D (see Fig. 3a), whereas it is less ordered in chains A and B. The extended N-terminal peptide of monomers C and D is stabilized by interactions with loop L2 from the other subunit of a dimer (see below) and interacts with the C-terminal strand β5.

Structural Similarity to Other Proteins—The DALI server (30) was used to identify structural similarities between KorB-C and other proteins. This search clearly indicated significant similarity of the KorB-C structure with the SH3 fold, predominantly known from protein domains involved in eukaryotic signal transduction (31) but occasionally also encountered in proteins from prokaryotes (32–35). Using chain D of the orthorhombic KorB-C structure, the four top hits are characterized by DALI Z scores of 4.8 for a mutant human tyrosine kinase (PDB entry 1LCK; Ref. 36), 4.5 for E. coli dihydrofolate reductase (1VIE; Ref. 37), 4.4 for HIV-1 integrase (1IHV; Ref. 38), and 4.4 for the N-terminal SH3 domain of the proto-oncogene product c-Crk (1CKA; Ref. 39).

Least-squares superpositions of KorB-C with these domains yield r.m.s. deviations between corresponding Cα atoms of 2.4 Å for the tyrosine kinase domain, 1.8 Å for the dihydrofolate reductase domain, 2.0 Å for HIV-1 integrase, and 1.8 Å for the SH3 domain from c-Crk.

All of these structures are characterized by the typical antiparallel up-and-down β-sheet arrangement, which, in some cases, appears split in two sheets (one three-stranded and the other two-stranded) crossing each other at right angles. In addition, a three-residue 310 helix is often present between strands β4 and β5. These features are perfectly conserved in the crystal structure of KorB-C (Fig. 2). Missing in the KorB-C structure is the long loop between strands β1 and β2 that is known to be responsible for the binding of eukaryotic SH3 domains to the proline-rich motifs of other proteins during signal transduction (31). A second difference is the elongated strand β5 of KorB-C. This strand is crucial for the dimerization of KorB-C (see below). In addition, there is an N-terminal elongation of the polypeptide chain of KorB-C, which will make the connection to the preceding KorB domain and is very flexible in the isolated C-terminal domain.

Dimer Structure of KorB-C—In the structure of KorB-C determined from orthorhombic crystals, the molecules in the asymmetric unit are related by noncrystallographic dyad axes. The KorB-C molecules in pairs A/C and B/D each are related by a 2-fold axis. A third 2-fold axis is found between the molecules A and C and, respectively, B and D. This axis is nearly, but not perfectly, parallel to the other axes and does not, therefore, establish 222-point symmetry. Instead, there are two similar pairs of KorB-C molecules, A/C and B/D (Fig. 3a).

In the hexagonal crystal form, two KorB-C molecules comprise the asymmetric unit. These two molecules (A and B) share the same arrangement with respect to each other with the molecule pairs A/C and B/D from the orthorhombic structure. The arrangement of six independent KorB-C molecules in two different crystal forms (P212121 and P63) thus follows one common pattern: a dimer formation involving molecule pairs A/C and B/D of orthorhombic KorB-C and A/B of hexagonal KorB-C, which is promoted mainly by interactions of residues from the C-terminal strand, β5.

Dimer Interface—To examine which intermolecular interactions stabilize the observed dimer-like arrangement of KorB-C molecules (Fig. 3b), the subunit interface was analyzed by the approach of Jones and Thornton (40, 41) using their Protein-Protein Interactions server (available on the Internet at www.biochem.ucl.ac.uk/bsm/PP/). According to this analysis, 990 ± 90 Å² of accessible surface are buried in the subunit interface for each of the six independent KorB-C subunits, corresponding to 23 ± 1% of the total accessible surface. The subunit interface area has a length of 31 ± 3 Å and a breadth of 25 ± 1 Å, and 63 ± 5% of the interface is contributed by nonpolar atoms. By having an extended buried surface area of nearly 1000 Å² per subunit, which represents nearly one-fourth of the total accessible surface, nearly two-thirds of which are hydrophobic, the KorB-C pairs observed in our crystal structures thus have characteristics of true functional dimers.

Three segments of polypeptide chain are involved in inter-subunit interactions. The largest part of the interface is contributed by residues anchored in strand β5, namely Leu352, Leu355, and Glu357. The hydrophobic interactions promoted by a zipper-like arrangement of leucine residues are perfectly conserved in all three independent dimers (Fig. 4a), and the residues involved are perfectly ordered as is evident from the surrounding electron density (Fig. 4b). These interactions also involve the side chain of Leu347, which is part of the segment of

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**TABLE III**

| P63 | A  | 0.790 | B  | 1.436 | 0.975 | 1.125 | 0.971 |
|-----|----|-------|----|-------|-------|-------|-------|
| P212121 | A  | 0.301 | B  | 1.532 | 1.172 | 1.108 | 1.015 |

**FIG. 2. Structural similarity of KorB-C and the SH3 domain of c-Crk.** The two domains were superimposed with an r.m.s. deviation of 1.8 Å for 41 matching Ca atoms. Note the truncation of loop L1 of KorB-C relative to c-Crk.
310 helix within loop L4. Stabilizing polar interactions include at least four hydrogen bonds per subunit (not shown) and a conserved salt bridge between Glu 357 and Arg 325, the latter being located in the long loop L2. This salt bridge is formed only once in the orthorhombic A/C dimer because of the unusual conformation of the C-terminal tripeptide in subunit A (green chain in Fig. 4a), which places Glu 357 in a position that is not suited for direct interaction with the Glu 357 side chain.

KorB-C Dimer Formation in Solution—To prove that the dimeric state of KorB-C is not induced by crystallization, KorB-C was cross-linked in solution with glutardialdehyde. Analysis of the cross-link products by SDS-PAGE (Fig. 5a) reveals that with increasing concentration of reagent increasing amounts of KorB-C are sequestered into the dimeric form. At 0.25 mM glutardialdehyde, half of the protein in the reaction mix is cross-linked, and at 1 mM reagent much of the KorB-C is dimeric. Even at this highest reagent concentration, no significant amounts of higher multimers are obtained. These results agree well with earlier cross-linking studies using intact KorB protein (12) that showed dimer formation, but no higher oligomers, at similar protein and glutardialdehyde concentrations.

The finding of dimers in cross-linking studies is also in line with the crystal structure of KorB-C that shows a close, dyad-symmetric interaction between subunits. The N terminus of KorB-C extends out of the compact dimer structure, its terminal residues being disordered to various degrees in all copies of the domain seen in the crystals. It thus appears possible that the 296 missing N-terminal residues can be added to form the intact KorB protein without disturbing the dimerization mode of KorB-C described above. In other words, the dimerization of KorB’s C-terminal domain found in the crystal structures of KorB-C may be expected to occur in the same way in the intact KorB protein and, possibly, its DNA complexes.

DNA Binding Studies—To probe the possibility that KorB-C is directly involved in specific operator binding, the DNA binding of KorB, KorB-C, and KorB-N, the N-terminal protein fragment (amino acids 1–294), was assayed by PAGE (Fig. 5b). Even at the highest KorB-C concentration used, corresponding to 45 pmol of protein, no binding of KorB-C to a fragment containing an O8 site could be detected. In contrast, both KorB and KorB-N bound specifically to the O8-containing DNA fragment at much lower concentrations, albeit not with the same affinity: whereas the band corresponding to the O8 fragment is completely shifted at 3 pmol of KorB (O8”), no shift is detect-
KorB Dimerization through a C-terminal SH3-like Domain

The RP4-encoded KorB protein is a transcriptional repressor that negatively controls the expression of RP4 genes by binding to pseudosymmetric 13-bp operator sequences (OB) present 12 times on the plasmid. Here we have determined the structure of KorB's C-terminal domain, KorB-C, from two different crystal forms. KorB-C comprises the 62 C-terminal amino acid residues of KorB. It forms a globular domain shaped as a five-stranded antiparallel β-barrel with a topology and three-dimensional structure reminiscent of SH3 domains as often present in proteins involved in eukaryotic signal transduction. The six crystallographically independent copies of KorB-C observed in the two crystal forms are arranged as three homodimers with internal pseudodyad symmetry and an extensive, predominantly hydrophobic subunit interface. By chemical cross-linking experiments, it was demonstrated that KorB-C forms dimers in solution as well.

In conjunction with the available biochemical evidence, the crystal structure suggests that KorB-C functions as the dimerization domain of KorB. Chemical cross-linking has earlier shown that intact KorB is a dimer in solution (12), and the formation of KorB tetramers has been reported as well (13). The extensive subunit interface present in KorB-C dimers (see Figs. 3 and 4) may very well suffice to stabilize the dimeric state of intact KorB. Our KorB-C crystal structure provides no evidence for tetramerization, since there are no tight and recurring interaction motifs between dimers, but we cannot exclude the possibility that under certain conditions N-terminal regions of KorB might support further oligomerization yielding, for example, tetramers. In a recent experiment (14), the deletion of the 17 C-terminal amino acids reduced the multimerization of KorB in vitro and, at the same time, reduced KorB's binding specificity for DNA fragments containing OB sequences. In particular, the KorB deletion mutant lost the ability to repress through class II operators but not through class I operators. These results agree with our comparative DNA-binding experiments (Fig. 5b), where we detected specific binding to OB sites by a KorB construct lacking the complete C-terminal domain. Thus, there is strong evidence not only that the C-terminal domain is responsible for KorB dimerization but also that the formation of multimers is linked to KorB's repressor function. Based on the crystal structure, we predict that a KorB deletion mutant Δ7 lacking just seven C-terminal residues should have similar properties as the Δ17 mutant, since this protein would lack the residues Leu352, Leu355, and Glu357 that provide a large part of the dimer interface. A KorB deletion mutant Δ2 would reveal the contribution of the Arg325–Glu357 salt bridge to dimer sta-
bility. Further evidence for a functionally relevant repressor dimerization promoted by the C-terminal domain comes from a study involving the KorB homolog ParB, where the deletion of the C-terminal part and the site-directed mutagenesis of several C-terminal residues, including several leucines, was shown to prevent protein dimerization and binding to parS sites on the P1 plasmid (8).

A dimeric structure of KorB, mainly promoted by its C-terminal domain, should permit specific binding to the O_{5} sites of RP4, which have internal pseudosymmetry. KorB-C has no detectable affinity to O_{5}-containing DNA (see Fig. 5a); thus, it is unlikely that the C-terminal domain of intact KorB contributes directly to specific DNA binding. This is in line with the detection of sequences resembling the canonical helix-turn-helix motif common to many bacterial repressors in the N-terminal part of KorB (12). The functional role of the C-terminal domain in gene regulation by KorB thus appears to rest in its ability to dimerize the intact protein, which in turn is important for specific high affinity operator DNA binding by KorB. Further functions of the C-terminal domain are quite possible, for example in interacting with RP4-encoded proteins KorA (42), TrbA (42, 43), or IncC (18, 44, 45), which cooperate with KorB in RP4 gene repression.

The KorB-C monomer is folded into a five-stranded antiparallel β-barrel with an approximately perpendicular arrangement of the paired strands β3 and β4 over a three-stranded sheet formed by the shared strand β2, by β1 and β5. This fold is radically different from the O_{5} fold, another five-stranded antiparallel β-barrel domain of similar size with Greek key topology as present, for example, in the bacterial cold shock proteins (46). The KorB-C fold instead strongly resembles the SH3 domain fold as seen in the tyrosine kinase Src (47), α-spectrin (31), c-Crk (39), and many other proteins. The β-sheet core of KorB-C superimposes quite well with these proteins, and the structural similarity extends to the presence of a short 3_{10}-helical segment in loop L4 of KorB-C, which is characteristic for the eukaryotic SH3 domains (48, 49). Whereas the loops L2, L3, and L4 of KorB-C are quite similar to their counterparts in eukaryotic SH3 domains, usually denoted as n-Src loop, distal loop, and 3_{10} loop, the RT loop of these is radically truncated to a β-turn that comprises loop L1 of KorB-C. A further difference between KorB-C and the canonical SH3 domain concerns the elongated strands β1 and β5 of the former (see Fig. 2). Nevertheless, the structural similarity between KorB-C and eukaryotic SH3 domains justifies denoting the KorB-C fold as SH3-like. Even with a knowledge of the three-dimensional structure, sequence similarity between KorB-C and eukaryotic SH3 domains is hardly detectable.

Besides KorB-C, only a few proteins from prokaryotes are known that display a SH3-like fold. It was noted early on (50) that the C-terminal domain from the E. coli biotin holoenzyme synthetase/biorepressor (51) structurally resembles SH3 domains. Other SH3-like domains are present in dihydrololate reductase (37), the photosystem I accessory proteins from Synechococcus sp. (33) and Nostoc sp. (52), Thermus thermophilus phenylalanine tRNA synthetase (34), Corynebacterium diphtheriae diptheria toxin repressor, DtxR (53), E. coli exonuclease I (54), and the minor coat protein g3p of fd phage (55). The DNA-binding protein Sso7d from Sulfolobus solfataricus (32) is an example for a SH3-like archaeal protein. Finally, we may point out that HIV-1 integrase, although not a bacterial protein, contains a DNA-binding domain that displays an SH3-like fold (38).

Eukaryotic SH3 domains are primarily involved in promoting protein-protein interactions and participate in biological processes such as signal transduction or membrane-cytoskeleton interactions. The most common mode of protein binding is the association of the SH3 domain with a proline-rich region of the cognate protein usually adopting a polyproline type II helical conformation (49). These interactions depend on the RT loop of the SH3 domain. In KorB-C, a similar mode of binding to proline-rich protein motifs is precluded, since the corresponding loop 1 is truncated (see Fig. 2). A number of further functions have been suggested for SH3-like domains from a variety of sources. These include DNA binding for the archaeal Sso7d protein (32) and the N-terminal domain of HIV-1 integrase (38), which depends on the dimeric state of the latter and ion binding for the SH3-like domain from DtxR (53). In some proteins, no clear function could be assigned to their SH3-like domains (34, 51).

We have established that the C-terminal KorB-C domain promotes the dimerization of the transcriptional repressor KorB through a symmetric interaction of its SH3-like fold. The formation of homodimers has earlier been reported for the SH3-like domain from HIV-1 integrase (see above) and for the epidermal growth factor receptor pathway substrate 8 (Eps8), where intertwined dimers coexist with monomers (56, 57). Heterodimeric interactions between SH3 domains have been described for the two SH3 domains of the mammalian growth factor receptor-binding protein Grb2 (58) and for the Grb2-Vav interaction (59). All of these SH3-SH3 interactions are different from the KorB-C homodimers described in this paper, where a close symmetric association is predominantly supported by residues located in an extended C-terminal polypeptide region. The crystal structure of KorB-C thus extends the modes of protein-protein interactions known for SH3 domains and SH3-like domains. In promoting the dimerization of KorB and thereby supporting DNA binding by this bacterial repressor, the novel SH3-SH3 interactions seen in KorB-C serve a clear biological function.

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