Solvent-exposed Residues in the Tet repressor (TetR) Four-helix Bundle Contribute to Subunit Recognition and Dimer Stability*

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Dimerization specificity of Tet repressor (TetR) can be altered by changes in the core of the four-helix bundle that mediates protein-protein recognition. We demonstrate here that the affinity of subunit interaction depends also on the solvent-exposed residues at positions 128 and 179–184, which interact across the dimerization surface. TetR(B) and (D), two naturally occurring sequence variants, differ at position 128 with respect to the monomer-monomer distances in the crystal structures and the charge of the amino acids, being glutamate in TetR(B) and arginine in TetR(D). In vivo analysis of chimeric TetR(B/D) variants revealed that the single E128R exchange does not alter the dimerization specificity of TetR(B) to the one of TetR(D). When combined with specificity mutations in α10, it is, however, able to increase dimerization efficiency of the TetR(B/D) chimera with TetR(D). A loss of contact analysis revealed a positive interaction between Arg-128 and residues located at positions 179–184 of the second monomer. We constructed a hyperstable TetR(B) variant by replacing residues 128 and 179–184 by the respective TetR(D) sequence. These results establish that in addition to a region in the hydrophobic core residues at the solvent-exposed periphery of the dimerization surface participate in protein-protein recognition in the TetR four-helix bundle.

Protein-protein complexes play crucial roles in many biological processes such as gene expression, replication, DNA repair, signal transduction, enzyme regulation, and immune response (1–9). The rules determining specificity in protein-protein recognition are therefore of fundamental biological importance. Among the structural motifs mediating protein-protein recognition, assemblies of α-helices are probably most widespread (10). Oligomerization of transcription factors, e.g. is often accomplished by α-helical coiled coils (11), and four-helix bundles form dimerization surfaces in many proteins (12). The mechanistic details governing recognition specificity and binding affinity of four-helix bundle mediated protein oligomerization are only poorly understood. We investigated subunit recognition in the four-helix bundle of Tet repressor (TetR) dimers with the goal of determining generally applicable rules for this process. TetR sequence variants regulate tetracycline-induced expression of seven naturally occurring tetracycline-resistance determinants (classes A–E, G, and H; for a review, see Ref. 13). In the absence of tetracycline, dimeric TetR binds to tet operator (tetO) repressing transcription of the tet promoters. Nanomolar concentrations of tetracycline lead to dissociation of TetR from tetO to induce transcription. This transcriptional switch is exceptionally sensitive to low inducer concentrations, making it the system of choice for regulation of gene expression in many higher organisms, including plants, transgenic mice, and human cells (for two recent reviews, see Refs. 14 and 15).

Crystal structures of TetR(D) in complex with [ Mg-tetracycline] revealed a small N-terminal and a large C-terminal domain (16, 17). The latter mediates tetracycline binding and dimerization via a four-helix bundle formed by the helices α8 and α10 of each subunit. Disruption of the dimeric structure of TetR by urea concomitantly leads to denaturation of the subunits (18).

We have established that the sequence variants TetR(B) and TetR(D) do not form heterodimers because amino acids located in the core of the four-helix bundle in helix α10 of TetR(B) and TetR(D) lack structural complementarity (19). Adjusting all amino acids in α10 yielded a change in subunit recognition specificity but did not restore the full dimerization efficiency of the wild type. We demonstrate here that this requires only one additional exchange, E128R, located in helix α8 at the edge of the four-helix bundle. Furthermore, we construct a hyperstable TetR(B) variant by improving the interaction of Arg-128 with residues of the second monomer. Thus, interactions between partially solvent-exposed, hydrophilic residues can profoundly influence the specificity of subunit recognition in protein hetero-oligomers and improve the stability of homo-oligomers.

EXPERIMENTAL PROCEDURES

Materials and General Methods—Chemicals were obtained from Merck (Darmstadt), Serva (Heidelberg), Sigma (München), or Roth (Karlsruhe) and were of the highest purity available. Tetracycline was purchased from Fluka (Buchs). Enzymes for DNA restriction and modification were obtained from Boehringer Mannheim, Life Technologies, Inc. (Eggenstein), New England Biolabs (Schwalbach), or Pharmacia (Freiburg). Sequencing was carried out according to the protocol provided by Perkin Elmer for cycle sequencing.

Bacterial Strains, Plasmids, and Phage—All bacterial strains are derived from Escherichia coli K12. Strain DH5α (hsdR17(r−m−)/recA1 endA1 gyrA96 thiI relA1 supE44 dcm lacZAM15 [λlac ZYA-argF1U169]) was used for general cloning procedures. Strain WH207 (lacZΔM15 galK2 rpsL1 recA1) was served as host strain for β-galactosidase assays. The plasmids pWH1200 (20), pWH520Δ26-53 (21), pWH506, and pWH53 (22), pWH620Δ26-53, pWH553/B/D/51-208, pWH553/B/D/51-178, pWH553/B/D/179-184, and pWH553/B/D/190-208 (19) as well as pWH1950 (23), which were used in the in vivo studies, for the construction of tetR(B/D) mutants and overexpression have been described.

Construction of tetR Variants—The mutation E128R was introduced into tetR(B) by polymerase chain reaction according to a three-primer method (24). The conditions of the polymerase chain reactions were as described (25). The products of the second polymerase chain reaction

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The abbreviations used are: TetR, Tet repressor; tetO, tet operator; WT, wild type.

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were purified, digested with MluI and BstEII, and cloned into likewise digested pHW553(B/D)179-208. The resulting pHW553 derivative was named pHW553(B/D)128. MluI/NcoI or MluI/BstEII fragments of tetR(B/D)179-208 and tetR(B/D)179–184 were cloned into likewise digested pHW553(B/D)128, yielding pHW553(B/D)128,179-208 and pHW553(B/D)128,179-184, respectively. tetR(B/D)51-127,129-178 and tetR(B/D)51-127,129-178,128A were constructed by introducing the R123E or R123A mutations in tetR(B/D)51-178 by polymerase chain reaction. pHW1950 derivatives were constructed for tetR(B/D)128, tetR(B/D)179-184, and tetR(B/D)128,179-184. The corresponding pHW553 derivatives were digested with XbaI and NcoI, and the TetR fragments were purified and cloned into likewise digested pHW1950. DNA of positive candidates was analyzed by digestion with restriction enzymes and sequencing of tetR.

β-Galactosidase Assays—Repression and induction efficiencies of the TetR variants as well as the negative transdominance efficiencies of tetR(B)Δ26-53 and tetR(D)Δ26-53 were assayed in E. coli WH207(tet50). The phage tet50 contains a tetA-lacZ transcriptional fusion integrated as single copy into the WH207 genome (22). Bacteria were grown in LB supplemented with the appropriate antibiotics. Quantification of induction efficiencies was done with 0.2 μg/ml tetracycline in overnight and log-phase cultures. β-Galactosidase activities were determined as described by Miller (26). Three independent cultures were assayed for each strain, and measurements were repeated at least twice.

Point Mutations in TetR—pWH1950 or pHW1950 derivatives were transformed into E. coli RB791. Cells were grown in 3 liters of LB at 28 °C in shaking flasks. Tet repressors were overexpressed by adding isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 1 mM at an A600 of 0.7–1.0. Cells were pelleted, resuspended in buffer A (50 mM NaCl, 2 mM dithiothreitol, and 20 mM Na3PO4, pH 6.8), and broken by sonication. TetR was purified by cation exchange chromatography and gel filtration as described (23).

Determination of the In Vitro Stability of TetR Variants—Circular dichroism (CD) measurements were performed on a Jasco 715 spectropolarimeter at protein concentrations of 5 μM in 0.5-cm cells. All measurements were carried out at a temperature of 22 °C. Equilibrium denaturation was performed by incubating protein samples overnight at the indicated urea concentration. We used F-buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol) for all spectroscopic measurements. Urea was obtained from ICN Biochemicals (Eschwege), and urea solutions were prepared every day. Renaturation was achieved by incubating the samples overnight at 8 M urea and then dialyzing them 200-fold with F-buffer. Thermodynamic calculations were done as described before (18).

RESULTS

Tet(B/D) Repressors—Using chimeric TetR(B/D) repressors, we demonstrated previously that residues in helix α10 are critical for the different dimerization specificities of TetR(B) and TetR(D); however, further changes are required to achieve WT affinity of subunit binding (19). The monomer-monomer distances in the TetR(D)/[Mg-tetracycline]3− crystal structure and a structural model of TetR(B) are different at residues in α10 and at position 128 in α8 (19). Thus, we constructed five new Tet(B/D) repressor dimers to analyze the influence of mutations at position 128 on dimer formation. The sequences of these five dimeric TetR(B/D) repressors and those from the previous study used here again are depicted in Fig. 1. We introduced the mutation E128R into TetR(B) and TetR(B/D)179-208. The resulting repressors were named TetR(B/D)128 and TetR(B/D)128,179-208, respectively. TetR(B/D)51-127,129-178 and TetR(B/D)51-127,129-178,128A were made to analyze whether the residue at position 128 influences dimerization of TetR(B/D) repressors by attractive interactions with TetR(D) or repulsive interactions with TetR(B). These TetR(B/D)51-127,129-178,128A derivatives contain either the Arg→Glu or the Arg→Ala mutation at position 128. To evaluate if the interaction of the TetR(D) residues at positions 128 and 179−184 can be used to construct a hyperstable protein, we also introduced the mutation E128R in TetR(B/D)179-184 leading to TetR(B/D)128,179-184.

In Vivo Repression and Induction Efficiencies—The TetR variants were transformed into E. coli WH207(tet50) to test in vivo operator binding activity and inducibility. pHW553 derivatives, which constitutively produce low levels of TetR (22), were used as expression plasmids. Repression of the tetA-lacZ transcriptional fusion and inducibility by tetracycline were quantified at 37 °C. The results are shown in Table I. The presence of WT TetR(B) results in repression of β-galactosidase activity to 2.4% of that in the absence of TetR. All TetR(B/D) variants show slightly increased repression efficiencies. Repressor variants with different amino acids only at position 128 show similar repression efficiencies. All chimeric TetR(B/D) repressors were to the same extent inducible by tetracycline as WT TetR. Thus, the E128R exchange does not impair in vivo repression or inducibility of TetR.

The Mutation E128R Increases the Dimerization Efficiency of TetR(B/D) Chimera with TetR(D) but Does Not Affect Dimerization of TetR(B) with TetR(D) (Ref. 26-53) and TetR(D)Δ26-53 variants indicated the amount of dimer formation of the TetR(B/D) variant with TetR(B) and TetR(D), respectively (19). The results are also shown in Table I. Efficient dimerization of TetR(B/D)Δ26-53 is only observed for Tet(R)B and TetR(B/D)128. No dimerization is detected between TetR(B/D)Δ26-53 and TetR(B/D)51-208, TetR(B/D)179-208, or TetR(B/D)128,179-208. TetR(D)Δ26-53 dimerizes only with TetR variants containing at least the TetR(D) amino acids 179–208. This confirms that the main determinants of the dimerization specificities of TetR(B) and TetR(D) are located in helix α10 and that the dimerization efficiency of TetR(B/D)Δ26-53 with TetR(B) is not affected when E128R is present as a single exchange. The dimerization efficiency of TetR(D)Δ26-53 is about 4-fold higher for TetR(B/D)128,179-208 compared with TetR(B/D)179-208. In fact, TetR(B/D)128,179-208 exhibits the same dimerization efficiency with TetR(D)Δ26-53 than TetR(B/D)51-208, which contains the complete TetR(D) protein core. These data demonstrate that only the E128R exchange is needed to restore WT dimerization efficiency of TetR(B/D)179-208 with TetR(D)Δ26-53.

Loss of Contact Analysis—The increased dimerization efficiency of TetR(B/D)128,179-208 with TetR(D) might be caused by an attractive interaction of Arg-128 or a repulsive interaction of Glu-128 with the TetR(D) monomer. To distinguish between these two possibilities, we investigated the effect of different residues at position 128 on dimerization of TetR(B/D)51-178 with TetR(D). TetR(B/D)51-178 was chosen because it forms dimers with TetR(B) and TetR(D) (Ref. 19; Table I). We formed a mutant of contact analysis by mutating Arg-128 of TetR(B/D)51-178 to A128 in TetR(B/D)51-127,129-178, A128 (Fig. 1). As a control, we also constructed TetR(B/D)51-127,129-178, which contains the TetR(B) amino acid Glu-128. Dimerization with TetR(D)Δ26-53 is neither detectable for TetR(B/D)51-127,129-178, A128 nor for TetR(B/D)51-127,179-208. In contrast, the dimerization efficiency of TetR(B/D)Δ26-53 with these TetR variants is not affected (see Table I). Thus, the Arg-128 residue increases the dimerization efficiencies of TetR(B/D) repressors with TetR(D) by forming an attractive interaction with the other TetR(D) monomer.

Introduction of the TetR(D) Residues at Positions 128 and 179−184 Results in a Hyperstable TetR(B) Variant—To test whether the TetR(B) homodimer is stabilized by interaction of Arg-128 with residues of the second monomer, we constructed TetR(B/D)128,179-184. This variant contains Arg-128 and...
128 and those TetR(D) residues which contact Arg-128 across the dimerization surface. TetR(B/D)179-184 and TetR(B/D)128,179-184 show identical dimerization efficiencies with TetR(B) and TetR(D)26-53 and TetR(D)26-53 (Table I). Thus, the E128R exchange does not alter the dimerization specificity of TetR(B/D)179-184.

### Urea-dependent Stability of TetR(B/D) Variants—

The in vitro stabilities of TetR(B), TetR(B/D)128, TetR(B/D)128,179-184, and TetR(B/D)128,179-184,128A were determined by urea-induced unfolding. After unfolding in 8 M urea and refolding by a subsequent 200-fold dilution in urea-free buffer, the CD spectrum of the respective TetR was identical to that of the native protein (data not shown) and more than 92% of the tetracycline-binding activity was recovered after the unfolding/refolding cycle.

Figure 1. **Amino acid composition of Tet(B/D) repressors.** The TetR(D) amino acid sequence is given in the one-letter code. Residues that build the dimerization surface in the TetR(D)/[(Mg-tetracycline)2] crystal are marked by triangles. Amino acids of TetR(B) that are identical to the corresponding TetR(D) residue are indicated by dashes. The lines below the sequence alignment indicate the parts of the TetR(B/D) chimera, which are encoded by tetR(D). α-Helices of the TetR(D)/[(Mg-tetracycline)2] crystal structure are shown as rectangles above the sequence alignment.

### Table I

| TetR variant | Induction<sup>a</sup> | Repression | Transdominance of deletion mutants<sup>b</sup> |
|--------------|------------------------|------------|-------------------------------------------------|
|              | % β-Galactosidase      | % β-Galactosidase | % β-Galactosidase | Derepression factor | % β-Galactosidase | Derepression factor |
| TetR(D)      |                        |            | TetR(D)Δ26-53                                          |                      | TetR(B)Δ26-53                                      |                      |
| TetR(B)      |                        |            | TetR(B)Δ26-53                                          |                      | TetR(B)Δ26-53                                      |                      |
| (B)          | 92 ± 5.3                | 2.4 ± 0.0  | 1.6 ± 0.0                                            | 1.1                  | 21 ± 0.5                                             | 14                   |
| (B/D)128     | 105 ± 2.1               | 1.1 ± 0.1  | 1.7 ± 0.1                                            | 1.5                  | 21 ± 0.1                                             | 19                   |
| (B/D)128,179-208 | 97 ± 2.6             | 0.9 ± 0.1  | 16 ± 0.5                                            | 18                   | 1.0 ± 0.0                                             | 1.1                  |
| (B/D)128,179-208 | 101 ± 1.3             | 1.0 ± 0.1  | 16 ± 1.3                                            | 20                   | 1.1 ± 0.0                                             | 1.1                  |
| (B/D)179-184 | 101 ± 1.3               | 1.0 ± 0.1  | 16 ± 1.3                                            | 20                   | 1.1 ± 0.0                                             | 1.1                  |
| (B/D)179-184 | 99 ± 3.3                | 1.5 ± 0.0  | 12 ± 0.0                                            | 1.0                   | 11 ± 2.2                                             | 9                    |
| (B/D)128,179-184 | 101 ± 1.3             | 1.2 ± 0.0  | 13 ± 0.3                                            | 1.3                   | 7 ± 0.9                                              | 10                   |
| (B/D)128,179-184 | 104 ± 2.2             | 1.1 ± 0.0  | 12 ± 3.7                                            | 7.9                   | 3.7 ± 0.4                                             | 4.1                  |
| (B/D)128,179-184,128A | 87 ± 1.7           | 1.0 ± 0.1  | 1.1 ± 0.0                                           | 1.1                   | 5.1 ± 0.2                                             | 5.1                  |
| (B/D)128,179-184,128A | 96 ± 3.6           | 1.0 ± 0.0  | 1.1 ± 1.1                                           | 1.1                   | 4.1 ± 0.1                                             | 4.1                  |

<sup>a</sup> Induction was determined at 0.2 μg/ml tetracycline. The 100% expression level of β-galactosidase corresponds to 4502 ± 181 units.

<sup>b</sup> Transdominance measurements were done according to Ref. 22.

128 and those TetR(D) residues which contact Arg-128 across the dimerization surface. TetR(B/D)179-184 and TetR(B/D)-128,179-184 show identical dimerization efficiencies with TetR(B)Δ26-53 and TetR(D)Δ26-53 (Table I). Thus, the E128R exchange does not alter the dimerization specificity of TetR(B/D)179-184.

**Urea-dependent Stability of TetR(B/D) Variants**—The in...
TABLE II
Reversible denaturation and thermodynamic data of TetR variants

| TetR variant | Reversibility a | From CD b | ΔG [kJ mol⁻¹] | urea [M] |
|--------------|----------------|-----------|---------------|---------|
| (B)          | 96             | 74.6 ± 4  | 4.7           |         |
| (B/D)128     | 92             | 77.2 ± 3  | 4.9           |         |
| (B/D)179–184 | 93             | 77.9 ± 1  | 4.8           |         |
| (B/D)128,179–184 | 95         | 86.7 ± 3  | 5.6           |         |

TetR variant: (B) TetR(B) WT, (B/D) TetR(B/D) chimera containing the TetR(D) amino acids.

Reversibility: activity [%].

From CD: determined by the change in the CD signal at 220 nm. The protein concentration was 5 μM monomer, and the measurement was carried out at 22 °C in 0.5-cm cells. The respective TetR variants are: _ _, TetR(B) WT; ∞, TetR(B/D)128; _ _, TetR(B/D)179–184; and _ ..., TetR(B/D)128,179–184.

In contrast to the dimerization with TetR(D), dimerization of TetR(B/D) variants with TetR(B) is not affected by mutations at position 128. The negative transdominance efficiencies of TetR(B)Δ26-53 over TetR variants differing only in the residue position 128—like TetR(B) versus TetR(B/D)128, TetR(D)/B/D)179–184 versus TetR(B/D)128,179–184, and TetR(B/D)51-178 versus TetR(B/D)51-127,129-178 or TetR(B/D)51-127,129-178,128A—are almost identical. Thus, amino acids at position 128 are not important for dimer formation with TetR(B), and no attractive interaction between the amino acid at position 128 and the second monomer exists in the TetR(B) dimer.

The tetO-binding activities and inducibilities of the TetR variants are only marginally affected by the mutations E128R, R128E, or R128A. The mutants showing improved heterodimerization efficiency and in vitro stability are functional. On the other hand, all TetR(B/D) mutants with a reduced dimerization efficiency show a loss of function. This verifies that no major changes are introduced in the structure of TetR.

The properties of the mutations can, therefore, be interpreted using the TetR(D) crystal structure. Arg-128 is located at the N terminus of α8 in this structure and forms a hydrogen bond to Gln-184′ in α10, the two helices of the four-helix bundle dimerization surface (Ref. 17; Figs. 3 and 4). Because positions 128 and 184 bear Glu and Pro in TetR(D), respectively, the hydrogen bond is almost identical. Thus, amino acids at position 128 are not important for dimer formation with TetR(B), and no attractive interaction between the amino acid at position 128 and the second monomer exists in the TetR(B) dimer.

In agreement with the conclusions discussed so far, the stability of TetR(B) is profoundly increased by introducing Arg-128 and the TetR(D) residues contacting Arg-128 across the dimerization surface. The stability of TetR(B) is not changed by replacing either residue at 128 or 179′–184′ by the corresponding TetR(D) amino acids, but combining the mutation in TetR(B/D)Δ128,179–184 increases ΔG by 12 kJ mol⁻¹. Hydrogen bond formation between Arg-128 and Gln-184′ most likely is

DISCUSSION

Negative transdominance reflects the amount of dimer formed in vivo by the TetR deletion mutants used as probes and the TetR variant of interest (19). A comparison of the negative transdominance efficiencies of TetR(D)Δ326-53 over TetR(B/D)-51-127,129-178, TetR(B/D)51-127,129-178,128A, and TetR(B/D)-51-178 as well as TetR(B/D)128,179-208 and TetR(B/D)128,179-208 shows that mutations at position 128 influence the dimerization potential of TetR(B/D) repressors with TetR(D). Dimerization of TetR(B/D)51-178 with TetR(D) is abolished after mutating Arg-128 to Gln-184. Despite that, TetR(B/D)179-208 is increased after introducing additional E128R exchange. The dimerization efficiency of TetR(B/D)128,179-208 is almost identical to that of TetR(B/D)51-208, which contains the complete TetR(D) protein core. The single amino acid Arg-128 is essential for dimerization of TetR(B/D)51-178 with TetR(D) and sufficient to restore WT dimerization efficiency in TetR(B/D)179-208. Thus, no other TetR(D) residues than Arg-128 and those in the segment 179–208 influence the dimerization between TetR(B/D) repressors and TetR(D).

The mechanism of action by which Arg-128 influences dimerization with TetR(D) is revealed by the mutations R128E and R128A in TetR(B/D)51-178. Both mutations abolish dimerization with TetR(D). Because R128A is a loss of contact mutation, the increased dimerization efficiency of TetR(B/D) repressors containing Arg-128 with TetR(D) is caused by a positive interaction and not a repulsive interaction of the TetR(B) amino acid Glu-128 with the TetR(D) monomer.

Fig. 2. Urea-induced denaturation curves obtained from the change of the CD signal at 220 nm. The protein concentration was 5 μM monomer, and the measurement was carried out at 22 °C in 0.5-cm cells. The respective TetR variants are: _ _, TetR(B) WT; ∞, TetR(B/D)128; _ _, TetR(B/D)179–184; and _ ..., TetR(B/D)128,179–184.

The identical results were obtained by following fluorescence.
Dimerization by Four-helix Bundles

Fig. 3. Stereo view of TetR(D)–[(Mg-tetracycline)]4 structure. Tetracycline is not shown. The α helices are depicted as ribbons. The two monomers are colored yellow and gray, respectively, and the four-helix bundle is highlighted by thicker lines. The side chain of the solvent-exposed residue Arg-128 is shown in blue, and the respective residues at 179–184′ in the other monomer are shown in red.

Fig. 4. Proposed interaction of the TetR(D) residues Arg-128 (blue) with Q184′ (red). The respective residues of TetR(B) are overlaid in orange (Glu-128) and purple (Pro-184). Helices belonging to different monomers are shown in yellow and gray as in Fig. 3.

... responsible for the increased stability of TetR(B/D)128,179–184, but the nature of that interaction cannot be concluded from the current data. The negative transdominance presently cannot be quantitatively related to the dissociation constant or ΔΔG. Because the negative transdominance correlates with the amount of heterodimer formed in vitro (19) and the thermodynamic stability in vitro, it provides a valid qualitative measure of dimerization efficiencies.

Recognition between polypeptides has been investigated in several proteins. One of the best analyzed examples is the complex formed between the human growth hormone and its receptor. A saturating alanine-scanning mutagenesis of the hormone-receptor interface supported the so-called “hot spot concept” of protein-protein recognition (27), where only a few amino acids of a larger interface account for the affinity in protein complexes. These residues are mostly hydrophobic and are located in the center of the interaction surface, whereas the primarily hydrophilic residues at the periphery of the interaction surface are unimportant (7, 27). The main determinants of the TetR(B/D) dimerization specificity are four residues of helix α10 that are located in the center of the four-helix bundle (19). Therefore, the hot spot concept seems also to be valid for protein recognition during TetR dimerization. However, an additional mechanism must influence protein-protein recognition in TetR because even the exchange of the complete α10 is not sufficient to restore WT dimer formation with TetR(D) in a Tet(B/D) repressor. The results presented here establish that the additional recognition present in TetR only depends on the partially solvent-exposed residues at position 128 and at 179–184. The arginine residue at position 128 is 95% solvent-exposed (calculated using GRASP (31)) compared with a surface accessibility of 89, 83, and 79% for the arginine residues at positions 28, 62, and 87, respectively, which are not involved in dimerization. The leucine residue at position 193 in the center of the four-helix bundle, where it contributes to dimerization specificity (19), shows 0% solvent accessibility. Thus, the specificity of protein-protein recognition in the TetR four-helix bundle is mediated by a dual mechanism that is determined by the buried residues in the center of the dimerization surface and also by partially solvent-exposed residues located at the edge of the dimerization surface. Dimerization of leucine zipper peptides is profoundly influenced by the interaction of hydrophilic residues at the Glu and Gly positions, and mutations at these positions can be sufficient to change dimerization specificity (28, 29). However, recent results demonstrated that the patterns of charged residues at the Glu and Gly positions are not sufficient to predict dimerization specificities of many leucine zipper mutants (30). These results support the view that the dual mechanism of protein recognition established for the TetR four-helix bundle might as well apply for other protein complexes.

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