The Essential Transfer Protein TraM Binds to DNA as a Tetramer*

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The TraM proteins encoded by F-like plasmids are sequence specific DNA binding proteins that are essential for conjugative DNA transfer. We investigated the quaternary structure and the DNA binding properties of the TraM wild-type protein of the resistance plasmid R1 and two mutant forms thereof. Size-exclusion chromatography and differential scanning calorimetry showed that purified TraM protein (amino acids 2–127) forms stable tetramers in solution. A truncated version of the protein termed TraMM26 (amino acids 2–56) forms dimers. Thus, the dimerization and tetramerization domains can be assigned to the N-terminal and C-terminal domains of TraM, respectively. Further analyses using chemical cross-linking and light scattering corroborated the preferentially tetrameric nature of the protein but also suggest that TraM has a tendency to form higher aggregates. Band-shift and fluorescence spectroscopy investigations of TraM-DNA complexes revealed that the TraM protein is also tetrameric when bound to its minimal DNA binding site. The deduced binding constant in the range of $10^8 \text{M}^{-1}$ demonstrated a very strong binding of TraM to its preferred DNA sequence. Secondary structure analysis based on CD measurements showed that TraM is mainly $\alpha$-helical with a significant increase in $\alpha$-helicity (48 to 58%) upon DNA-binding, indicating an induced fit mechanism.

Bacterial conjugation can be regarded as a specialized macromolecular transport system by which DNA and protein or DNA alone is transferred from one bacterium to another (for reviews, see Refs. 1–4). The process of conjugal DNA transfer is cell-cell contact-dependent and resembles in this and other respects the type III and type IV toxin export systems of pathogenic bacteria (5). The transfer of the plasmid DNA occurs via an initial strand and site-specific cleavage at the origin of transfer (oriT) (6, 12) in a plasmid-specific way (7). Experimental evidence suggests that TraM has at least three functions in conjugation of F-like plasmids: i) it serves as a positive control element for transfer gene expression (8); ii) it participates in the formation of the R1 relaxosome where it stimulates TraI-catalyzed cleavage at the transfer origin in vivo (7); iii) TraM couples the relaxosome to the envelope-bound transport complex via its interaction with the inner membrane protein TraD (13).

The multifunctionality of the small 14-kDa TraM protein and its essential role in conjugation prompted us to further characterize the biophysical and biochemical properties of the TraM protein. We used the purified protein and two mutant variants of TraM which have been characterized previously in vitro (7, 8). In in vitro analyses, we mainly focused on the questions of which oligomerization state TraM acquires in solution or when bound to its DNA target site and which domains of the protein are responsible for dimer and tetramer formation. Since recent NMR data on the structure of a synthetic peptide consisting of 22 N-terminal amino acids of TraM (amino acids 1–22) showed a high degree of flexibility in aqueous solution (12), we also wished to determine a possible conformational change in TraM upon DNA binding.

EXPERIMENTAL PROCEDURES

Expression of TraM, TraMM13, and TraMM26—The TraM proteins were overexpressed from Escherichia coli cells harboring the plasmids pExTraM, pExTraMM13, or pExTraMM26. In these constructs the traM and traM mutant genes are under the control of the λ P8 promoter. The plasmids also contain the gene for the temperature-sensitive λ represor (cI857) which allows temperature-controlled protein expression.

The expression plasmids were constructed by cloning a EcoRI -PstI

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fragment with the λ repressor and traM genes from plasmid pKL5 (6) into the EcoRI-PstI cut pUC119 (14) vector. The resulting plasmid was designated pExtraM. For pExtraMM13 and pExtraMM26, the Xbal-PstI fragment from pExtraM containing only traM was exchanged against the corresponding mutated fragments from plasmids pBBR111 and pBBR112 to obtain the corresponding mutations. The correct size of the traM cassette in the resulting expression constructs was verified by DNA-sequencing. As an expression host strain, the L-protease-deficient E. coli strain UG10 (lon::Tn10, hsdR, hsdM1, tre, thi, lac, rpsL) was used. Best expression of TraM and TraM mutants was obtained by growing 5-ml precultures overnight at 30 °C, followed by inoculation in 250-ml cultures (TY medium (16 g of Tryptone, 10 g of yeast extract, 5 g of NaCl per liter). The cultures were then grown at 37.4–38.2 °C for 18 to 20 h. The given temperature range was essential for successful expression of the proteins. Selective antibiotics were present into the E. coli strain UG10 (lon::Tn10, hsdR, hsdM1, tre, thi, lac, rpsL) was used. Best expression of TraM and TraM mutants was obtained by growing 5-ml precultures overnight at 30 °C, followed by inoculation in 250-ml cultures (TY medium (16 g of Tryptone, 10 g of yeast extract, 5 g of NaCl per liter). The cultures were then grown at 37.4–38.2 °C for 18 to 20 h. The given temperature range was essential for successful expression of the proteins. Selective antibiotics were present at the following concentrations: 100 μg/ml epipenicid (dihydroamipenicillin); 15 μg/ml tetracycline. After incubation, the cells were harvested and broken using a French pressure cell. The cytoplasmic fractions were obtained by centrifugation and the TraM proteins further purified in consecutive chromatography steps.

**Purification of TraM, TraMM13, and TraMM26—**TraM wild-type (WT) protein and mutant TraMM13 were purified using a fast protein liquid chromatography system (Amersham Pharmacia Biotech) in three steps (6) with minor modifications. The cytoplasmic extract was loaded onto a 10-ml fast flow S-Sepharose cation-exchange column (Amersham Pharmacia Biotech) using 50 mM Trisphosphate, pH 6.8, as loading buffer. The fractions containing TraM or TraMM13 were loaded onto a Mono-Q HR 5/5 anion ion-exchanger column (Amersham Pharmacia Biotech) under the same conditions as in step 1. The proteins were eluted with a linear NaCl gradient and concentrated between each chromatography step by ultrafiltration (Amicon). TraM was eluted from the S-Sepharose column with 130–200 mM NaCl, from the Mono-Q with 260–450 mM and from the Mono-S with 100–200 mM NaCl. TraMM13 was eluted with similar NaCl concentrations.

TraMM26 was purified in a two-step purification scheme. TraMM26 was added to a 10-ml S-Sepharose fast flow cation-exchange column (Amersham Pharmacia Biotech) using 50 mM Trisphosphate, pH 6.8, as starting buffer. TraMM26 was eluted between 70 mM and 110 mM NaCl. The second step was a gel filtration step on a 125-ml Superose 12 prep grade column (Amersham Pharmacia Biotech) using 50 mM NaPO4/NaH2PO4 buffer, pH 4.0. Between the two steps, the fractions containing TraMM26 were concentrated using ultrafiltration high. Samples of the soluble fractions of the crude extracts and the purified proteins were analyzed on a 20% homogeneous Phast gel (Amersham Pharmacia Biotech).

TraM WT concentrations were determined by UV spectroscopy using a molar extinction coefficient of ε = 5180 M−1 cm−1 for the denatured protein and ε = 5355 M−1 cm−1 for the native protein, respectively (15). Alternatively, protein concentrations were determined using protein determination dye kits (Sigma Diagnostics; Bio-Rad) (16).

**Synthetic Oligonucleotides Used—**Oligonucleotides used in this study represent a part of sbmA, one of the binding regions for TraM in the oriT region of plasmid R161. Oligonucleotides were synthesized on a DNA synthesizer by the solid-phase phosphoramidite method. Dime-thoxytrityl-bearing fragments were purified by reversed-phase high performance liquid chromatography on a Sephasil peptide C8 column (Amersham Pharmacia Biotech) using the volatile buffer triethanolamine-acetate, pH 6.7, with an acetonitrile gradient. After evaporation, the oily residue was resolved in MilliQ water and the triethanolamine-acetate precipitated using EIOH/NaCl. The oligonucleotides were dissolved in annealing buffer (5 mM MgCl2, 10 mM Tris-HCl, pH 7.5), completely denatured (95 °C), and annealed by slowly cooling to room temperature. The concentrations were determined via UV-spectroscopy.

**SBM1a, 5′-TGTGAATTCCATATGATTCCGACacgacGTTGAATCATAATCGGATTCACCA-3′; and SBM1aRev, 5′-GTTGAATTCGATATGATTCACGacacgacGTTGAAATCATATGATTCAC-3′. The annealed oligonucleotides consist of two pseudopalindromic binding motifs (half-sites; underlined) arranged as inverted repeats: The oligonucleotides represent one complete binding site (full-site) for TraM.**

SBM1loop, 5′-TGTGAATTCCATATGATTCCGCacgacGTTGAATTCCATATGATTCCGACacgacGTTGAATCATAATCGGATTCACCA-3′, is able to form a double strand (ds) hairpin structure (single strand loop sequence in lowercase letters) containing a complete binding site (full-site) for TraM in the orientation that the oligonucleotide SBM1loop was purified as described above except that extraction from the agarose gel was done by a freeze-squeeze method according to protocol of the manufacturer (FMC). The gel slice was frozen overnight at −70 °C and then thawed and subsequently centrifuged for 15 min. The supernatant was collected. To increase the yield, some microfilters of 0.1× Tris-EDTA were added to the slurry, and the procedure was repeated. Some supernatants containing the oligonucleo-
traM Binding to DNA

The oligonucleotide SBM1loop (340 nt) was incubated with various amounts of purified TraM in the same buffer as described for the EMSA with the labeled oligonucleotide. No competitor DNA was added to the reaction. After incubation for 15 min at 30 °C, 1 μl of 0.12% xylene cyanol was added, and the mixture was loaded onto a 3.5% agarose gel (MetaPhor, FMC). Subsequently, the gel was stained with ethidium bromide. In a similar experiment, TraM protein (1680 nm) was incubated with various amounts of SBM1loop.

Dynamic Light Scattering—A stock solution of TraM protein in 25 mM sodium phosphate, pH 7.0, was made at 20 °C with various amounts of SDS or with the oligonucleotide SBM1a. The solutions were centrifuged and filtered through a 0.2-μm Anatop™25Plus filter (Merck). The final protein concentration was 2 mg/ml (1.4 × 10^{-4} M) in buffer with 0.3% SDS, 0.05% SDS or with 3.5 × 10^{-3} M dsDNA, respectively. The laboratory-built goniometer was equipped with an Argon® laser (Spectra Physics, model 2060–5S, f_{0} = 5 watts, λ = 514.5 nm), single mode fiber detection and ALV-5000E correlator with fast expansion (ALV Germany). Measurements were carried out at a scattering angle of 90° at a temperature of 20 °C and a laser power of 200 milliwatts. The time dependence of the scattering intensity, represented by its correlation function, provided information on diffusional motion of the scatterers. Correlation functions from repeated experiments (typically 10 per sample over 5 min) were averaged. The diffusion coefficient D, determined from the correlation function, is related to the apparent hydrodynamic radius R_{H} by the Stokes-Einstein equation, i.e. an equivalent sphere with the radius R_{H} shows the same diffusion behavior as the particle under investigation and serves as a size parameter. A polydisperse system gives rise to a correlation function with a spectrum of different decay constants. Laplace inversion of the correlation function results in the intensity-weighted size distribution D(R_{H}) (17).

Steady State Fluorescence—The extrinsic fluorescence probe 1,8-ANS was purchased from Sigma. Fluorescence spectroscopy was performed on a Hitachi F-2000 spectrophotometer, and measurements were obtained with both excitation and emission slit width set at 10 nm. A 1 × 1 cm quartz cuvette with a sample volume of 1 ml was used for all experiments. The sample was equilibrated at 20 °C and stirred during measurement to reduce photobleaching. Preliminary experiments proved that ANS binds to the TraM protein, giving an increase in intensity and a blue shift in the fluorescence spectrum. In further investigations, the displacement of ANS from the protein because of the addition of DNA could be shown. Preliminary experiments yielded optimized measurement conditions for the competition assay. The sample was chosen to be 1 mM (e = 5500 M^{-1} cm^{-1}) in 10 mM Tris-HCl, 100 mM NaCl, pH 7.5 (the effective concentration in the cell for the competition assay was 100 μM). The DNA concentrations were chosen to be 1 × 10^{-7} to 5 × 10^{-8} M, the protein concentration varied in the range from 10^{-8} to 10^{-6} M. The optimal excitation and emission wavelengths were determined to be 370 and 483 nm, respectively. DNA/ANS competition assays were conducted by titrating 5-μl aliquots of TraM stock solution to 1 ml of 0.12% xylene cyanol, pH 7.0, was incubated at 20 °C for 5 min) were averaged. The diffusion coefficient D, determined from the correlation function, is related to the apparent hydrodynamic radius R_{H} by the Stokes-Einstein equation, i.e. an equivalent sphere with the radius R_{H} shows the same diffusion behavior as the particle under investigation and serves as a size parameter. A polydisperse system gives rise to a correlation function with a spectrum of different decay constants. Laplace inversion of the correlation function results in the intensity-weighted size distribution D(R_{H}) (17).

Circular Dichroism Spectroscopy (CD)—CD measurements were performed in 10 mM KH_{2}PO_{4}/KOH, pH 7.5, buffer with a protein concentration of 3.5 × 10^{-5} M and a DNA concentration of 8.7 × 10^{-6} M SBM1loop in the complex solution. The investigations were carried out on a Jasco J-715 spectropolarimeter using a 0.05-cm pathlength cell equilibrated at 20 °C. Protein and complex spectra were base line-corrected by subtracting buffer and DNA spectra, respectively. For the heat-denaturation experiment, the sample cell was heated to 66 °C with a heat rate of 0.2 °C/min, and the thermal equilibration was awaited. Because of a temperature lag between the cell and the thermostating device, the actual sample temperatures were determined directly in the cell using a miniature thermocouple probe from Newport Electronics. Mean residue ellipticity [θ] was calculated as specified in the data analysis program provided by Jasco (mean residue weight 113.6 g/mol).

RESULTS

Rationale for the in Vitro Analysis of TraM, TraMM13, and TraMM26—For the in vitro analyses, three forms of the TraM protein were chosen: the TraM WT protein, TraMM13, and TraMM26. TraMM13 is a mutant TraM protein with amino acid exchanges SIST(37–40) to ATAS(37–40). The mutant protein neither affects the autoregulatory ability nor stimulation of transfer origin cleavage by TraI (7, 8). However, the mutant protein does affect transfer gene expression, which is dramatically reduced, as well as the conjugation frequency, which is reduced by three orders of magnitude (8). Therefore, only the function of TraM in controlling transfer gene expression but not the function of TraM as a relaxosomal component is impaired in the case of TraMM13. TraMM26 is a C-terminal deletion variant of TraM retaining only the first 56 amino acids. Despite the presence of the N-terminal DNA binding and recognition domain, TraMM26 is a loss-of-function mutant in all testable TraM features (7, 8).

Overexpression and Purification of TraM, TraMM13, and TraMM26—The TraM proteins described above were overexpressed from E. coli cells harboring the traM expression plasmids pExtraM, pExtraMM13, or pExtraMM26. In these constructs the traM and traM mutant genes are under the control of the bacteriophage λ P_{R} promoter. The cytoplasmic fractions were purified as described under “Experimental Procedures,” yielding >98% pure protein as estimated by SDS-PAGE (data not shown). The identities of the expressed and purified TraM proteins were verified by MALDI-TOF mass spectrometry. The analysis revealed that the first methionine is not present in the mature TraM WT protein. The same situation was encountered with the TraMM13 and TraMM26 proteins. Thus the mature TraM and TraMM13 mutant proteins overlap consists of 126 amino acids (amino acids 2–127), the TraMM26 mutant form is 55 amino acids in length (amino acids 2–56). The calculated molecular masses of the purified proteins correspond well to the values measured by the MALDI-TOF-MS analysis. The measured values were 14,314 Da for TraM (14,315 calculated), 14,253 Da for TraMM13 (14,257 calculated), and 6,280 Da for TraMM26 (6,280 calculated).

TraM Forms Stable Tetramers in Solution—The quaternary structures of the purified TraM proteins were investigated in a...
first approach by size-exclusion chromatography. The resulting chromatogram and a calibration curve, which was used to determine the approximate molecular masses for TraM, TraMM13, and TraMM26, are shown in Fig. 1. The molecular mass determined by this method for TraM and TraMM13 is 54 kDa, which is a value that correlates well with the calculated molecular weights of TraM and TraMM13 tetramers (57 kDa). For TraMM26, the C-terminal truncated variant of TraM, a molecular mass of 12.6 kDa was obtained corresponding exactly to the value expected for TraMM26 dimers. To test for a possible dissociation of the TraM tetramer, lower concentrations of TraM were tested. Within the detection limit of the size-exclusion chromatography, the elution time remained unchanged.

To further confirm the aggregation state of TraM WT in solution, heat denaturation experiments were conducted on a DSC. In these experiments, TraM showed a thermal unfolding transition with a maximum at 42.2 °C and 38.3 °C for scan rates of 1 °C/min and 0.5 °C/min, respectively (Table I). An irreversible unfolding transition occurred at approximately 60 °C, resulting in the precipitation of the protein. Because the thermal unfolding of TraM is irreversible, the transition temperature showed a strong scan rate dependence (20). The data representing the first transition were base line corrected and normalized. The resulting curves were used for fitting a non-two-state unfolding model (Fig. 2), leading to the thermodynamic parameters shown in Table I. The ratio of $\Delta H/\Delta H_F$ was 0.24 for the 0.5 °C/min scans, indicative of a single coupled transition of four identical subunits (20).

TraM Tends to Form Higher Aggregates in Solution—In another approach, chemical cross-linking of purified TraM and TraMM13 with glutaraldehyde was used to determine the ability of TraM to form multimeric structures. As can be seen in Fig. 3A, defined multimers of TraM are formed with increasing glutaraldehyde concentration. At the lowest concentration of 0.001% glutaraldehyde (lane 3 in Fig. 3A), only bands corresponding to monomers, dimers, trimers, and tetramers of the TraM protein are visible. In lanes 4 and 5, the dominant band is shifted to a position corresponding to the tetrameric form of TraM. However, higher complexes appear as well, migrating slower in the SDS gel. Fig. 3B shows that cross-linking of TraMM13 to a higher multimeric complex (Mc in lanes 4, 6, and 8) is favored in comparison with the formation of defined multimers of the TraM protein. These higher multimeric complexes are trapped at the interface of the stacking gel and the running gel. This difference with respect to the formation of multimers between TraM and TraMM13 was not detectable in the size-exclusion chromatography experiment in which both proteins were present as tetramers.

The aggregation behavior of TraM was further surveyed with dynamic light scattering, a method suitable for obtaining the hydrodynamic radius of the protein in its native state. The results are presented in Fig. 4. TraM at a concentration of 2 mg/ml generates a peak with a maximum of 5.6 nm in the intensity distributions, $D[R]$, corresponding to an aggregate of the size of 12 to 16 monomeric subunits. A second peak occurs above 30 nm, which is probably because of the presence of small amounts of impurities or protein aggregates. It should be emphasized that the number of these large particles is small taking into account their contribution to the apparent size distribution scales with radius $R^6$ for globular aggregates. Measurements at varying concentrations (1 to 10 mg/ml) showed a concentration dependence of the oligomerization behavior (data not shown).

Searching for conditions to resolve the aggregates, we found that SDS at concentrations of 0.05 and 0.30% lead to deaggregation of TraM to the dimeric and monomeric forms, respectively. These results were initially obtained by cross-linking studies (data not shown) and correspond to results from the light scattering experiments. There a significant decrease of the $R_g$ with increasing concentrations of SDS was observed yielding particle sizes of 2.7 (0.05% SDS) and 2.2 nm (0.30% SDS). Adding DNA oligonucleotides with the specific binding site for TraM also resulted in a decrease in particle size ($R_g = 3.9$ nm) and a strong broadening of the protein peak, which

![Figure 1](image1.png)

**Fig. 1.** Determination of the molecular weight of native TraM, TraMM13, and TraMM26. TraM WT protein, TraMM13, or TraMM26 were separated using a Superose 12 matrix and 50 mM Na-phosphate, pH 7.2, 150 mM NaCl buffer. BSA, β-lactoglobulin, cytochrome c, aprotinin, and vitamin B12 were used as calibration proteins. A, a chromatogram for the TraM proteins is shown. B, determination of the molecular masses of TraM and TraMM13 tetramers (57 kDa). For TraMM26, the C-terminal truncated variant of TraM, a molecular weight of 12.6 kDa was obtained corresponding exactly to the value expected for TraMM26 dimers. To test for a possible dissociation of the TraM tetramer, lower concentrations of TraM were tested. Within the detection limit of the size-exclusion chromatography, the elution time remained unchanged.

**Table I**

| Sample   | Sample I          | Sample II         |
|----------|-------------------|-------------------|
| Scan rate [°C/min] | 1.0               | 0.5               |
| $T_m$ [°C]       | 42.12 ± 0.06      | 38.32 ± 0.12      |
| $\Delta H$ [kcal/mol] | 24.6 ± 0.2      | 28.2 ± 1.9        |
| $\Delta H_F$ [kcal/mol] | 128 ± 0.7        | 115 ± 0.4         |

$^a$ Values are averages from two independent scans with refill. Standard deviations are derived from the least squares fitting procedure.
could be because of unspecific DNA-binding of TraM and heterogeneity of the complex.

**TraM Binds to the DNA as a Tetramer**—Because TraM binds to an approximately 160-bp DNA region near the transfer origin of plasmid R1, we needed to define a minimal TraM binding site for subsequent experiments in which we wished to determine how TraM binds to the DNA. A minimal binding site for TraM was experimentally determined. According to this information, the synthetic oligonucleotide SBM1loop was synthesized (for details, see “Experimental Procedures”). Binding of TraM, TraMM13, and TraMM26 to the ³²P-labeled ds DNA oligonucleotides carrying the cognate binding site with high affinity. To further characterize stoichiometry and binding affinity of TraM to different oligonucleotides, a fluorescence competition assay using 1,8-ANS as a fluorescent probe was devised (Fig. 7A) (21). Conducted with saturating concentration of TraM and DNA oligonucleotide, this assay yielded the stoichiometry of binding to be 4.4 ± 0.61 (protein monomers to dsDNA). In limiting concentrations (concentrations close to the dissociation constant), the assay yielded the binding constant of TraM to its cognate dsDNA carrying a complete binding site to be in the range of 1–5 × 10⁴ M⁻¹ for various oligonucleotides (Fig. 7B) (19). Both the binding constant and the determined stoichiometry for the TraM-DNA complexes are in good agreement with the results of the EMSA described above.

**Refolding upon DNA Binding**—To detect a possible conformational change of TraM upon DNA binding, we performed secondary structure analysis of TraM and its DNA complex with CD spectroscopy. At 20 °C, TraM alone showed a mainly α-helical secondary structure with an α-helix content of 48%, calculated with the fitting programs provided by the manufacturer according to Yang, et al. (22). At the same temperature in the DNA complex, the α-helical content increases to 58% (Fig. 8). Upon thermal unfolding, the CD signals reduce significantly.

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² P. Verdinò, W. Keller, H. Strohmaier, K. Bischof, H. Lindner, and G. Koraimann, unpublished results.
but retain some secondary structure, which can be fitted mainly by random coil and β-sheet, whereas the α-helix content reduces to 24% (Fig. 8). At 65.8 °C, no significant difference in secondary structure between the free protein and the protein-DNA complex can be observed.

DISCUSSION

TraM actively participates in several steps of conjugation: it is involved in relaxosome formation (6, 7), regulation of transfer gene expression (8), and also in physically connecting the relaxosome via its interaction with the inner membrane protein TraD to the cell envelope-bound DNA transport complex (13). In all these processes, DNA binding plays an essential role. In previous studies, it has been shown that the N-terminal sequence of TraM is responsible for DNA binding (6, 8) and also confers specificity to the DNA binding reaction (7). The results of the experiments performed with purified TraM protein and two mutant forms thereof presented here show that TraM forms a stable tetramer in solution and that tetramerization is necessary for high affinity DNA binding. In gel filtration experiments, TraM WT and the SIST(37–40) to ATAS(37–40) mutant TraMM13 elute as a tetramer. In contrast, the C-terminal deletion mutant TraMM26 forms dimers in solution accompanied by a loss of high affinity DNA binding (Fig. 5A), despite the DNA recognition region being in the N-terminal part of TraM. The loss of the DNA binding activity in the case of TraMM26 corroborates the in vivo data in which TraMM26 was found to be a loss of function mutant, which negatively affected conjugation, autoregulation, transfer gene expression (8), and strand and site-specific nicking at the oriT (7). On the other hand, mutant TraMM13 affected transfer gene expression but no other functions in conjugation (7, 8). The DNA binding ability of TraMM13 was equivalent to the binding of TraM WT to DNA with the notable exception that slightly more DNA was shifted in the EMSA (Fig. 5). This effect could be because of an enhanced stability of TraMM13 in the DNA-protein complex as compared with TraM WT. The enhanced stability would also explain the negative effect of TraMM13 on transfer gene expression. TraMM13 presumably down-regulates its own expression to a level which does not permit sufficient co-expression of the positive regulator protein TraJ (8).

Secondary structure analysis based on CD measurements indicates that TraM forms a mainly α-helical protein whose α-helicity increases upon DNA-binding. The prediction of secondary structure with various prediction methods (23) yields four prominent α-helices for the TraM sequence comprising approximately 60% of the total TraM structure. This corresponds well to the value of 58% α-helix content experimentally determined from the CD spectra of the TraM-DNA complex.
The formation of the N-terminal helix is most likely responsible for the increase in α-helicity upon DNA-binding. It has been shown that a peptide sequence comprising the first 22 amino acids of TraM is flexible in aqueous solution and that α-helix formation is induced in a mixture of trifluoroethanol/water (12). This newly formed α-helix matches in size the observed increase in α-helical signal of the TraM WT upon DNA binding. The increase in α-helicity suggests an induced fit of the TraM protein to its cognate binding site, an effect that has been described for several DNA binding proteins, e.g. bZIP transcription factors, where the basic domain is unfolded in the free state of the protein and only acquires its α-helical content upon binding of DNA. At 65.8 °C no difference in secondary structure between the protein and the DNA complex can be observed.

DNA complex (28) and the tetramerization domain (29, 30) have been solved as separate structures. Because of the sequential arrangement of binding sites, a tetrameric protein has to bind to linear dsDNA as a dimer of dimers (C2 symmetry instead of the higher D2 or C4 symmetry usually found in tetrameric proteins). However, in both cases mentioned—p53 and LacI—the tetramerization domain turns out to be of the higher D2-symmetry, which necessitates a flexible linker connecting the tetramerization domain with the lower symmetric DNA-binding core. This linker is 30 residues long in the case of p53 (29) and approximately 8 residues in the case of LacI (26). For TraM, two helices are predicted (23) for the C-terminal part connecting by a loop of 19 residues, which appears to be sufficient for the proposed linker function.

Hydroxyl radical footprinting analysis of the DNA TraM complexes, using the oriT region of resistance plasmid R1, yielded a protection pattern with a spacing of 11 base pairs (11). The regular spacing of the protected sites suggests that TraM binding of consecutive particles occurs on the same side of the DNA. In light scattering experiments, we could show that TraM has a tendency to form higher aggregates at high concentrations (>35 μM) with an average hydrodynamic radius $R_H$ of 5.8 nm. Binding to DNA reduces the apparent particle size of TraM in solution to 3.9 nm $R_H$. Thus the diameter of the tetramer bound to an oligonucleotide representing one complete binding site from sbmA appears to be approximately 8 nm, which corresponds to 23 base pairs in linear B-DNA. Therefore, the size of the complex observed in the light scattering experiment almost exactly fits the protection pattern of the hydroxyl radical footprint, provided that one TraM tetramer occupies two adjacent recognition half-sites (NNTGA(ATT)/TCANN).

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