Electrostatic Forces Contribute to Interactions Between *trp* Repressor Dimers

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ABSTRACT The *trp* repressor of *Escherichia coli* (TR), although generally considered to be dimeric, has been shown by fluorescence anisotropy of extrinsically labeled protein to undergo oligomerization in solution at protein concentrations in the micromolar range (Fernando, T., and C. A. Royer 1992. *Biochemistry.* 31:3429–3441). Providing evidence that oligomerization is an intrinsic property of TR, the present studies using chemical cross-linking, analytical ultracentrifugation, and molecular sieve chromatography demonstrate that unmodified TR dimers form higher order aggregates. Tetramers and higher order species were observed in chemical cross-linking experiments at concentrations between 1 and 40 μM. Results from analytical ultracentrifugation and gel filtration chromatography were consistent with average molecular weight values between tetramer and dimer, although no plateaus in the association were evident over the concentration ranges studied, indicating that higher order species are populated. Analytical ultracentrifugation data in presence of corepressor imply that corepressor binding destabilizes the higher order aggregates, an observation that is consistent with the earlier fluorescence work. Through the investigation of the salt and pH dependence of oligomerization, the present studies have revealed an electrostatic component to the interactions between TR dimers.

INTRODUCTION

Many proteins that bind to specific sites on DNA and thereby modulate transcriptional activity have been shown to exist as oligomers (Miller and Reznikoff, 1980). More recently, protein-protein interactions have been implicated in both positive and negative regulation of a large number of eukaryotic transcription factors (Roeder, 1991; Lamb and McKnight, 1991; Miner and Yamamoto, 1991). The tryptophan repressor (TR) from *Escherichia coli* is a 107-amino acid polypeptide encoded by the trpR gene (Gunsalus and Yanofsky, 1980; Singleton et al., 1980). TR represses the transcription of the genes of a number of operons (trpEDCBA, aroH, aroL, mtr, and trpR) in response to increasing intracellular tryptophan (Rose et al., 1973; Zurawski et al., 1981; Heatwole and Somerville, 1991; Sarsero et al., 1991; Heatwole and Somerville, 1992). With the exception of the trpR gene, which is autogenously regulated, the gene products of the remaining operons are involved in the various pathways of aromatic amino acid biosynthesis.

The oligomeric state of TR has been generally considered to be dimeric (Joachimiak et al., 1983). The three-dimensional structures of apoTR, holoTR (the tryptophan-bound repressor) and the operator DNA-holoTR complex have been derived from crystallographic data (Schevitz et al., 1988; Joachimiak et al., 1987; Zhang et al., 1987; Lawson and Sigler, 1988; Otwinowski et al., 1988). The dimeric architecture is characterized by three interlocking helices (A–C) from each polypeptide chain forming the interface between monomers, and a helix-turn-helix DNA binding domain (D and E), with the final helix (F) folding back upon the core of the dimer. Comparison of the apo- and holoTR crystal structures shows that corepressor binding results in a change of the orientation of the D and E helices with respect to the core (Zhang et al., 1987). This tertiary conformational change has been proposed as the basis for the increase in specific DNA binding affinity of holoTR compared to apoTR (Zhang et al., 1987). More recently however, fluorescence studies on extrinsically labeled TR showed that it forms higher order oligomers in solution in the micromolar concentration range in absence of tryptophan, and that the binding of corepressor destabilizes these oligomers (Fernando and Royer, 1992). The specific destabilization of the TR oligomers by tryptophan suggests that oligomerization may play a role in modulating the DNA binding properties of TR. In the present study we have used analytical ultracentrifugation, molecular sieve chromatography, and chemical cross-linking to confirm the existence of higher order protein complexes between unlabeled TR dimers. In addition, we show that high salt and high pH destabilize these oligomers.

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Abbreviations used: TR, trp repressor; apoTR, trp repressor in absence of its corepressor, L-tryptophan; holoTR, trp repressor bound by its corepressor, L-tryptophan; DNS-Cl, 5-(dimethylamino)naphthalene-1-sulfonyl chloride; TR-DNS, dansyl-labeled trp repressor; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

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MATERIALS AND METHODS

Preparation of TR

An *E. coli* strain, CY15071, transformed with the plasmid, pJPR2, which overproduces the trp repressor (Paluh and Yanofsky, 1986), was obtained from Dr. Charles Yanofsky (Stanford University). TR was purified according to Joachimiak and co-workers and Paluh and Yanofsky (Joachimiak

et al., 1983; Paluh and Yanofsky, 1986) with changes described by He and Matthews (1990). The purified repressor was >95% pure as estimated from silver staining of an sodium dodecyl sulfate (SDS)-polyacrylamide gel. Each fraction containing TR was stored at −70°C in the elution buffer (10 mM potassium phosphate, pH 7.6, with 0.5 M KCl). Protein concentrations were determined using an extinction coefficient of 1.45×10^4 $cm^{-1}M^{-1}$ per monomeric subunit (Joachimiak et al., 1983).

Cross-linking reaction

Protein for the cross-linking studies was stored at a concentration of 8 mg/ml as an ammonium sulfate slurry (10 mM sodium phosphate, pH 7.6, 0.3 M NaCl, 0.1 mM EDTA, 75% saturation ammonium sulfate) at −20°C. Before running the cross-linking reactions, the protein was dialyzed against 10 mM sodium phosphate, pH 7.6, 0.1 M NaCl, 0.1 mM EDTA. Grade I glutaraldehyde (25% aqueous solution) was purchased from Sigma in 1.0-ml aliquots and stored at −20°C. Glutaraldehyde was diluted freshly in deionized water and added to aliquots of apoTR at final concentrations of 0.01 to 5% glutaraldehyde. ApoTR concentrations ranged from 0.4 to 40 μM in dimer. Reactions were carried out at room temperature (ca. 25°C) for times ranging from 1 min to 24 h. Unless noted otherwise, the standard buffer for all cross-linking reactions reported here was 10 mM sodium phosphate, pH 7.6, 0.1 M NaCl, 0.1 mM EDTA. The cross-linking reactions were quenched by adding 100-fold molar excess (over glutaraldehyde) of $NaBH_4$, freshly prepared in 0.1 N NaOH, and incubating at room temperature for 20 min. The protein was precipitated by using ice cold trichloroacetic acid (10% w/v). The precipitated protein was collected by centrifugation and dried under vacuum.

Gel electrophoresis

Two different denaturing gel systems were used to analyze the cross-linked products: 15% acrylamide/SDS gels (SDS-polyacrylamide gel electrophoresis (PAGE) (Laemmli, 1970)) and 15% acrylamide/6 μM urea/0.1 M Na_2HPO_4/SDS gels, pH 6.2 (Carey, 1989) which provide higher resolution for low molecular weight proteins. Molecular weight markers were purchased from Sigma (St. Louis, MO) and stored as 1 mg/ml solutions at 4°C. After electrophoresis, gels were stained in 0.1% Coomassie Brilliant Blue R in methanol:acetic acid:water (4:1:5, v/v) and destained in acetic acid: methanol:water (1:1:8, v/v).

Analytical ultracentrifugation

Sedimentation velocity experiments were conducted with a Beckman Model E analytical ultracentrifuge equipped with an ultraviolet scanner, electronic speed control and RTIC temperature control; double-sector (12 mm) charcoal-filled Epon centerpieces and sapphire windows were used. The UV scanner was used to trace the reaction boundary during sedimentation routinely at 280 or 260 nm, depending upon the protein concentrations. Weight-average sedimentation coefficients, $\bar{s}$, were determined from the centroid. The observed values were normalized to standard conditions by correcting for solvent density and viscosity. For a system undergoing rapid equilibration, the weight average sedimentation coefficient is related to the stoichiometry and equilibrium constant since

$$\bar{s} = \frac{\sum_i S_i^\circ (1 - g_i C) K_i C_1^i}{\sum_i K_i C_1^i} \quad (1)$$

where S_i° is the sedimentation coefficient of the ith species at infinite dilution, g_i is the nonideality coefficient, C is the total protein concentration, K_i is the equilibrium association constant between the smallest associating species and any i-mer, and C_1 is the concentration of the smallest associating species. Repetition of sedimentation runs revealed an error of approximately ±0.15 s.

The protein was dialyzed overnight into appropriate buffer. The experiments were conducted at either 4 or 20°C. Within experimental uncertainties, there is no apparent temperature effect on the measured values. Samples in buffer for more than 48 h at 4°C were excluded from further analysis, as values for those samples continued to decrease with time, although prior to 48-h storage, no change was observed. The concentration range of TR examined was 8×10^{-6} to 5×10^{-4} μM in dimer.

Analytical gel chromatography

Solutions of apoTR or holoTR were dialyzed against the column running buffer at 4°C overnight, and the final concentration determined by absorbance. Protein was either used immediately or stored for a maximum of 48 h. Errors in both broad and narrow zone chromatography apparent molecular weight values is estimated at ± 2000 M_r.

Broad zone chromatography

Broad zone analytical gel chromatography was employed to determine the molecular weights of apoTR or holoTR as described previously by Beckett et al. (1991) and Heyduk et al. (1992). Experiments were performed on a water-jacketed Sephacryl 200 HR column (1 × 28 cm) in buffer containing 0.1 M potassium phosphate, pH 7.5. The eluate was monitored by fluorescence. Generally, 10 ml of protein solution was applied to the column to ensure a symmetric broad zone profile. The column was calibrated with ribonuclease A (13.7 kDa), chymotrypsinogen (25 kDa), ovalbumin (43 kDa), bovine serum albumin (67 kDa), and blue dextran 2000 (average 2000 kDa). The flow rate, measured by weighing the liquid eluted in a specific time period, was typically kept at 17 ml/min with a peristaltic pump.

Narrow zone chromatography

A similar Sephacryl 200 HR column (1 × 36 cm) was employed for small zone chromatography at 20°C. The column was calibrated with the same mixture as the broad zone column. All samples were applied in and eluted with 0.1 M potassium phosphate, pH 7.5, or in the same buffer with 0.5 M KCl. The flow rate, measured by weighing the liquid eluted in a specific time period, was kept at 16.7 ml/min with a peristaltic pump.

Fluorescence

Dansylation

ApoTR was labeled with DNS-Cl as described by Fernando and Royer (1992). Chymotryptic digestion of the labeled repressor followed by gel filtration and SDS-PAGE analysis (Carey, 1989) indicated that the DNS labeling occurred at the α-amino group. The average labeling ratio for 25 preparations was determined as described by Fernando and Royer (1992) to be 1.0 ± 0.2 DNS/dimer.

Fluorescence data acquisition and analysis

Steady state measurements were performed on an ISS KOALA fluorometer (Urbana, IL) in analog acquisition mode using a 300-watt xenon arc lamp from ILC Technologies (Sunnyvale, CA). Steady-state polarization measurements were measured in L-format exciting at 340 nm with 8-nm bandwidth and using a Y460 cuton filter (Hoya Optics, Freemont, CA) in emission. Buffer background was subtracted for all samples. L-Tryptophan was ultrapure (ICN Biomedicals, Cleveland, OH). Lifetime measurements were performed using the multifrequency phase/modulation acquisition electronics from ISS, Inc (Urbana, IL) with the KOALA sample compartment as described by Fernando and Royer (1992). Multifrequency phase and modulation data were analyzed using the program Globals Unlimited (LFD, Urbana, IL). Multiple data sets for the magic angle lifetime experiments at differing protein, KCl, or tryptophan concentration and pH were analyzed simultaneously, linking the lifetime values across data sets and allowing the fractional intensities to vary. A full description of the current generation of fluorescence global analysis software can be found in Beechem et al. (1992).

Anisotropy (A) was calculated from the parallel and perpendicular emission component intensities as follows:

$$A = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}. \quad (2)$$

The observed steady-state anisotropy values were used in conjunction with the average lifetime calculated from the fits of the frequency response data as a linear fractional-intensity-weighted sum of the recovered lifetimes (τ) to calculate an average rotational correlation time for the DNS probe (τ_c) using the following relation (Perrin, 1926), where A_o is the limiting anisotropy of the probe (0.31 for DNS (Weber, 1952)).

$$\frac{A_o}{A} - 1 = \frac{\tau}{\tau_c} \quad (3)$$

Dilution correlation time curves were the average of three to four experiments on different TR-DNS preparations. The error for the correlation time within each preparation was near ±3 ns, although this increased slightly with decreasing concentration. The error on the averaged correlation times was similar, except in absence of added KCl. For those preparations, there was a larger variation in anisotropy values between different preparations (±8 ns) although the quality of the data within each preparation was the same for all profiles.

RESULTS

Oligomerization of unmodified TR

Prior fluorescence studies using extrinsically labeled TR (Fernando and Royer, 1992) provided evidence for oligomerization of apoTR dimers and their destabilization by corepressor. As a first step in understanding any eventual physiological role for the interactions between TR dimers, it was important to unequivocally establish their existence using unmodified protein and alternative biophysical techniques.

Chemical cross-linking

Intersubunit cross-linking has been a useful chemical technique for quaternary structural analysis (Wold, 1972; Peter and Richards, 1977; Jaenicke and Rudolph, 1986). ApoTR (40 μM) was cross-linked in absence of corepressor at glutaraldehyde concentrations ranging from 0.01 to 2.5% for 20 min. The best resolution of protein bands on analytical gels was found at a glutaraldehyde concentration of 0.01%. A time course for reaction conducted at 0.01% glutaraldehyde (40 μM apoTR) was analyzed using both phosphate and Laemmli gels (Fig. 1, *a* and *b*, respectively) to resolve separately the low- and high-molecular weight ranges. The disappearance of monomer and the increasing intensity of higher order bands with time are evident in both gels. The molecular weights of higher order multimers were estimated from a calibration curve obtained from the molecular weight standards in Fig. 1 *b* (Laemmli gel). In addition to dimers, multimers of molecular weights corresponding to trimers, tetramers, and higher order complexes are detected. While cross-linking is clearly not an equilibrium methodology, the appearance of tetramer and higher order species provides evidence that the unlabeled apo-protein dimers interact in solution. For each multimer type a multiplet of closely spaced bands is observed, presumably reflecting different

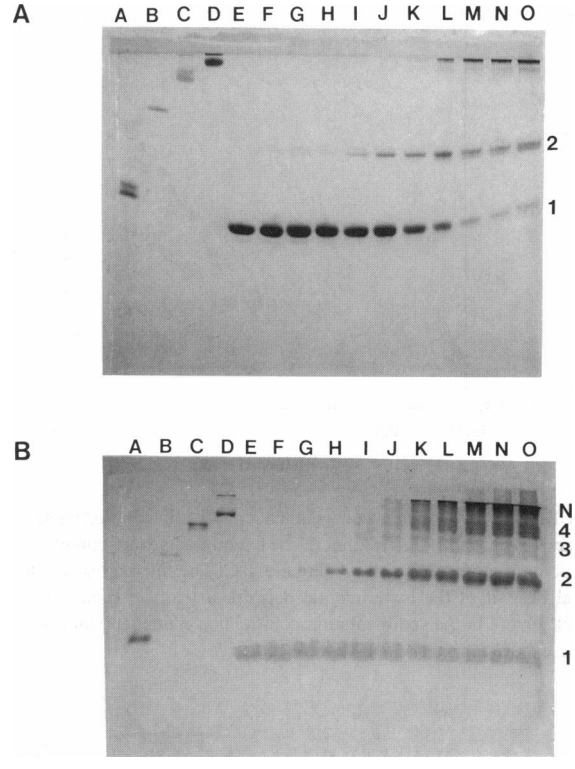

FIGURE 1 Time course for glutaraldehyde cross-linking of 40 μM repressor dimer. (*A*) Analysis was carried out by urea SDS-PAGE. Lanes A–D, standards in order of increasing molecular weight: α-lactalbumin, carbonic anhydrase, ovalbumin, bovine serum albumin. Lanes E–O each contain 8 μg of apoTR. Lane E, untreated apoTR; Lane F, apoTR treated identically to the protein in Lanes G–O with the exception of glutaraldehyde exposure. Lanes G–O, apoTR reacted with 0.01% glutaraldehyde for 0, 1, 5, 10, 20, 50, 120, 240 min, and 15 h, respectively. (*B*) Analysis was carried out by Laemmli SDS-PAGE. Lanes A–F, identical to *A*. Lanes G–O, apoTR reacted with 0.01% glutaraldehyde for 0, 5, 10, 20, 50 min and 2, 5.5, 8.5, 30 h, respectively. The total amount of apoTR loaded in each lane was 8 μg for lanes G–I, and 12, 14, 16, 24, 32, and 40 μg for J–O, respectively. The numbers 1, 2, 3, 4, and N in the margin mark the locations of monomer, dimer, trimer, tetramer and higher order bands, respectively.

Stokes radii from different extents of cross-linking, consistent with the presence of four lysines per monomer. The time dependence of the appearance of the multiplet bands suggests that as cross-links accumulate, the multimer becomes more compact and thus migrates more rapidly.

Analytical ultracentrifugation

Sedimentation velocity experiments were carried out as another means of examining the interactions between apoTR dimers. The observed sedimentation coefficient of apoTR varies as a function of protein concentration as shown in Fig. 2. Throughout the concentration range of apoTR studied (4–300 μM) the protein sedimented as a single peak, although the value for $\bar{s}$ increased with increasing protein concentration. Sedimentation behavior of this type is consistent with an equilibrium that involves dimerization (i.e., dimer and dimer of dimer) (Gilbert, 1955, 1959). The sedimentation data were analyzed accordingly, as described under Materials and Methods. Based on the results shown in Fig. 2,

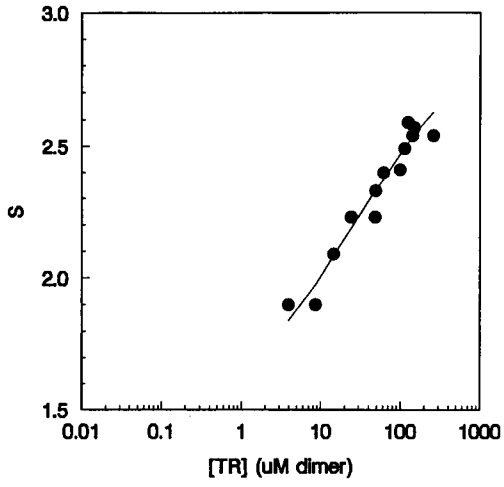

FIGURE 2 Relation between weight average sedimentation coefficient, $\bar{s}$, and apoTR concentration as dimer. The experiments were conducted in 0.1 μM potassium phosphate at pH 7.5 and 4°C. The line represents the theoretical best fit of the experimental data for a tetramer-dimer association model. Errors in the sedimentation coefficients estimated from repetitions of the experiment near 15 μM were ± 0.15 $\bar{s}$.

S_1° assumes a value of 1.9 S. Using the relationship of $S_i/S_1 = (i)^{2/3}$ (Schachman, 1959), the value of S_2° can be estimated at 3.0 S. Using these estimated parameters, theoretical values of as a function of C can be fitted to the observed values. The best fit curve to a dimer-tetramer equilibrium yielded a K_d of 55 μM and $S_2^\circ = 3.1$ S. Values of 1.9 and 3.1 S are consistent with globular protein structures of 26 and 52 kD, respectively, indicating that apoTR can undergo a rapid dimer-tetramer interaction on the timescale of this separation (minutes). However, the lack of a high concentration plateau suggests the formation of higher order species as well. Since the dimer-tetramer model is only an approximation itself, the actual value of the tetramer-dimer dissociation constant is approximate at best. Nonetheless, these results demonstrate that apoTR dimers can oligomerize, in agreement with the cross-linking studies and the earlier fluorescence work of Fernando and Royer (1992). Also confirming the previous fluorescence observation, it can be seen in Table 1 that the presence of tryptophan decreased the observed sedimentation coefficient, implying corepressor-induced oligomer destablization.

TABLE 1 Effect of solution parameters on sedimentation coefficient and apparent molecular weight

Technique*	Condition	[TR] (μM)	MW_{app}‡ or $\bar{s}$
Gel Filtration	pH 7.5	20	34,500
	pH 7.5, 500 mM KCl	20	25,000
Sedimentation	pH 7.5	15	2.3 S
	pH 7.5, 500 mM KCl	28	1.8 S
	pH 7.5, 81.7 μM L-tryptophan	16	1.9 S

* The buffer employed was 0.1 M potassium phosphate at 20°C.
‡ Determined by small zone gel filtration chromatography.

Analytical gel filtration chromatography

Examination of apoTR by broad zone and narrow zone analytical gel chromatography demonstrated a protein concentration dependent elution profile (Fig. 3) over a wide concentration range. A single peak (narrow zone) or transition (broad zone) was observed at all concentrations for these samples, implying rapid equilibration on the separation timescale. The apparent molecular weight derived from gel filtration by both methods was calculated by comparison of the elution volume of samples and standards. With both methods, the range of apparent molecular weight values is consistent with rapid equilibration between dimer (26 kD) and tetramer (52 kD) between 0.04 and 100 μM TR, similar to the results obtained by analytical ultracentrifugation. The absence of a plateau over 3 log units of TR concentration, however, indicates a more complex equilibrium, since a simple tetramer-dimer dissociation should go from 10 to 90% completion in 2.3 log units of protein concentration. The

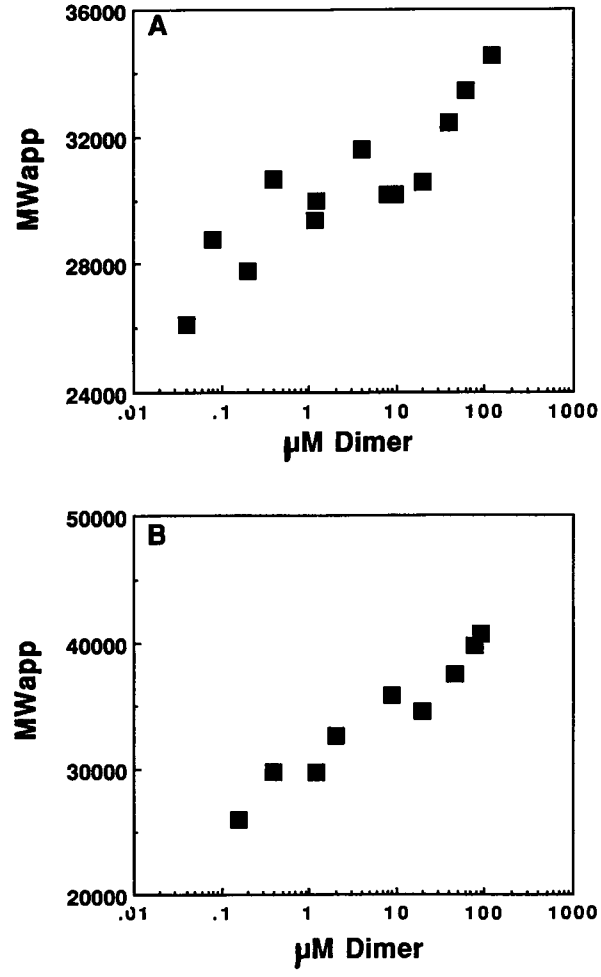

FIGURE 3 Relation between apparent molecular weight and apoTR concentration determined by gel filtration chromatography. Columns were run in in 0.1 M potassium phosphate, pH 7.5, 20°C. (*A*) Broad zone gel filtration data; (*B*) Narrow zone gel filtration data. Errors in apparent molecular weight values estimated from repetition of the broad and narrow zone experiments in the micromolar range were near ± 2000 MW.

continued decrease in the apparent molecular weight at concentrations below 1 μM suggests the onset of dimer dissociation. The lack of a plateau at the high concentration end of the profile suggests that a population of oligomers of higher order than tetramer cannot be ruled out. A numerical analysis of these data in terms of equilibrium constants between the species would require plateau values for the average molecular weight as well as a more thorough knowledge of the number and stoichiometry of the species involved. Nevertheless, these experiments using a complementary biophysical technique on unlabeled protein confirm the earlier fluorescence anisotropy work of Fernando and Royer (1992) using extrinsically labeled TR, that showed a propensity for TR dimers to oligomerize.

Effects of solution conditions on TR oligomers

In an attempt to understand the chemical basis for the interactions between apoTR dimers, analytical utracentrifugation, gel filtration chromatography and fluorescence anisotropy experiments were carried out under various solution conditions. As shown in Table 1, both the sedimentation coefficient and the apparent molecular weight by gel filtration decreased significantly upon addition of 500 mM KCl. The TR concentration dependence of the fluorescence correlation time of dansyl labeled TR was derived from anisotropy and lifetime measurements carried out at as a function of pH and salt concentration both in absence and presence of tryptophan as described under Materials and Methods, and is presented in Fig. 4, *a* and *b*. Regardless of conditions, a protein concentration-dependent increase in the correlation time is observed between 1 and 100 μM (Fig. 4) similar to the profiles reported earlier by Fernando and Royer (1992). All curves converge at low concentration to a plateau of near 10 ns, corresponding to the dimer. With no added salt or tryptophan, the maximum correlation time at pH 6.0 is near 60 ns (Fig. 4 *a*), clearly indicating that at this pH, near the pI of 5.9, oligomers of apoTR of higher order than tetramer are formed. Increasing pH from 6.0 to 7.6 and then 8.5 results in a destabilization of these oligomers as evidenced by a decrease in the correlation times at concentrations above those corresponding to the dimer plateau value. At pH 7.6, the addition of 200 mM KCl leads to a significant decrease in the average correlation times even at the highest TR-DNS concentration consistent with a shift in the equilibrium toward the dimer. NMR structural studies of TR were carried out at 500 mM NaCl in order to avoid aggregation phenomena at the millimolar concentrations used in such studies (Hyde et al., 1989; Arrowsmith et al., 1991). Comparing Fig. 4, *a* and *b*, it can be seen that the oligomer destabilizing effects of high salt and high pH are cumulative with the previously demonstrated destablizing effect of tryptophan (Fernando and Royer, 1992).

DISCUSSION

Results from chemical cross-linking, analytical ultracentrifugation, and gel filtration chromatography on unmodified TR confirm earlier fluorescence results on extrinsically labeled protein (Fernando and Royer, 1992) which demonstrated that dimers of TR interact to form higher order oligomers and that the binding of the corepressor, tryptophan, destabilizes these oligomeric interactions. No high concentration plateau was evident in any of the experiments, implicating oligomeric species of higher order than tetramer.

Examination of the effects of solution conditions on the interactions between TR dimers revealed that both high salt concentration and high pH lead to destabilization of these multimers, indicating that complexes between TR dimers are stabilized by electrostatic interactions. Destabilization of multimers by pH in the range of 6.0 to 8.5 suggests that titratable groups such as histidine or the amino terminus may

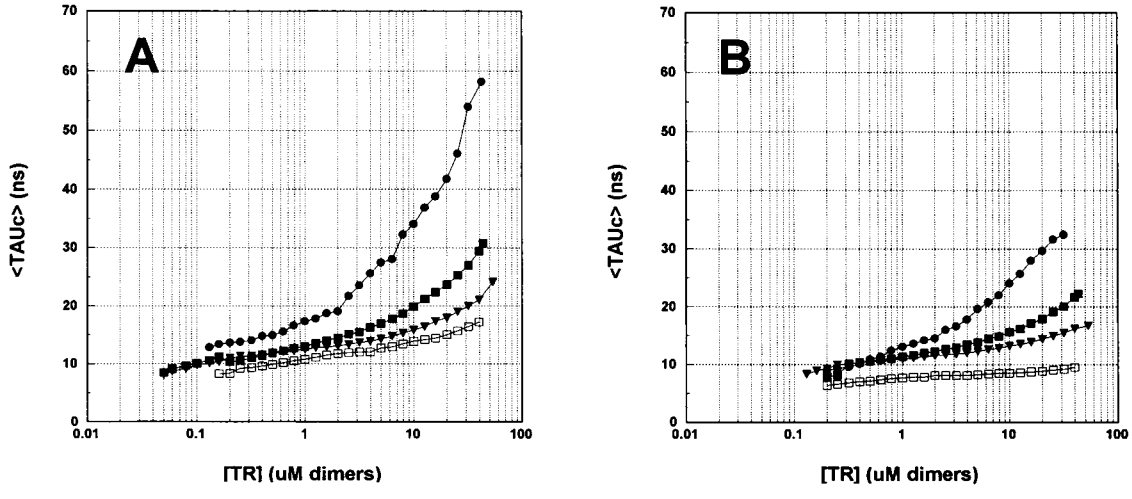

FIGURE 4 The influence of salt and pH on TR correlation times. (*A*) Results of experiments in absence of L-tryptophan in 10 mM potassium phosphate, in 0 KCl, pH 7.6 (■); 200 mM KCl, pH 7.6 (□); 0 KCl, pH 6.0 (●); and 0 KCl, pH 8.5 (▼). (*B*) Results in the presence of 0.4 mM L-tryptophan in 10 mM potassium phosphate, in 0 KCl, pH 7.6 (■); 200 mM KCl, pH 7.6 (□); 0 KCl, pH 6.0 (●); and 0 KCl, pH 8.5 (▼). The results shown are the mean at each TR-DNS concentration for three experiments.

be involved in oligomerization. A recent structural determination of TR dimers tandemly bound to DNA implicates the amino terminus in a protein-protein interaction (Lawson and Carey, 1993). Because the site of DNS labeling in these studies is on the amino terminus, the sensitivity of the DNS fluorescence lifetime and average emission energy to TR concentration (data not shown) also implicates this part of TR in multimer interactions, even in the absence of DNA. Labeling on the amino terminus may influence the extent of oligomerization. However, qualitatively, the results of cross-linking, analytical ultracentrifugation, and broad zone chromatography on the unlabeled repressor were quite similar to those obtained using fluorescence of the dansyl labeled protein. Oligomerization was observed in the same concentration ranges and was destabilized by high salt and tryptophan. Since the complexity of the oligomerization reaction precludes a true numerical analysis of the data from any of these techniques, any small effects of labeling on the oligomerization properties of the protein cannot be revealed. The danslyated protein binds to operator DNA with the same affinity as wild type as assessed by gel retardation experiments. Likewise, dynamic light scattering experiments using both labeled and unlabeled repressor show no significant differences in the average apparent molecular weights and polydispersities (data not shown).

The influence of corepressor on protein-protein interactions in this system suggests a physiological role for these oligomeric interactions in regulating *trp* gene expression. The in vivo concentration of the apo-repressor (low tryptophan levels) is relatively high, nearing 1 μM (Gunsalus et al., 1986). At comparable in vitro concentrations in 200 mM salt and at pH 7.6, the apoprotein is primarily dimeric. However, when comparing in vitro and in vivo conditions one must bear in mind that macromolecular crowding effects in vivo can result in large contributions of the activity coefficients to the chemical potentials of macromolecules effectively increasing their concentrations by as much as two to three orders of magnitude (Cayley et al., 1992; Jarvis et al., 1990). Local concentration gradients and any effect of non-specific DNA on oligomerization could also influence the extent of oligomerization of the apo-repressor in vivo.

It has become increasingly clear that DNA influences the interactions between holoTR dimers. Chemical protection (Kumamoto et al., 1987) suggested that two or more tandem holoTR dimers can bind to the various operator DNA sequences which are targeted by TR. The number of dimers bound was proposed to correlate with the differential regulation of these operons in vivo. It has been shown that these complexes of higher stoichiometry are dependent upon the target DNA sequence, length and symmetry (Liu, 1992; Carey et al., 1991; Haran et al., 1992; Liu and Matthews, 1993a), and that the complexes formed are specific in that they also depend upon corepressor (LeTilly and Royer, 1993; Liu and Matthews, 1993a). The three-dimensional structure of a tandem 2:1 TR-operator DNA complex has recently been derived from crystallographic data (Lawson and Carey, 1993). Moreover, stoichiometry and cooperativity studies on DNA binding by super-repressor mutants of TR revealed that some of these proteins, known to bind with higher affinity than wild type (Hurlburt and Yanofsky, 1990), also bind cooperatively with higher stoichiometries (Liu, 1992; Liu and Matthews, 1993b). Thus, DNA binding by super-repressor oligomers may help to explain their phenotype, which is the ability to repress tryptophan biosynthesis at extremely low concentrations of tryptophan. The fact that these super-repressors are charge change mutants implicates electrostatic interactions in oligomerization, in agreement with the salt and pH effects reported here. Destablization of wild type TR multimers by the corepressor, tryptophan, taken together with the changes in operator affinity, stoichiometry, and cooperativity of the super-repressor mutants, argues strongly that protein-protein interactions play a role in regulating gene expression by TR. Protein multimer-dependent modulation of operator binding would provide another level of complexity to the mechanism of action of this autogenously regulated repressor.

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