The Conformation of the Glucocorticoid Receptor AF1/tau1 Domain Induced by Osmolyte Binds Co-regulatory Proteins*

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The activation domains of many transcription factors appear to exist naturally in an unfolded or only partially folded state. This seems to be the case for AF1/tau1, the major transactivation domain of the human glucocorticoid receptor. We show here that in buffers containing the natural osmolyte trimethylamine N-oxide (TMAO), recombinant AF1 folds into a more compact structure, as evidenced by altered fluorescence emission, circular dichroism spectra, and ultracentrifugal analysis. This conformational transition is cooperative, a characteristic of proteins folding to natural structures. The structure resulting from incubation in TMAO causes the peptide to resist proteolysis by trypsin, chymotrypsin, endoproteinase Arg-C and endoproteinase Gluc-C. Ultracentrifugation studies indicate that AF1/tau1 exists as a monomer in aqueous solution and that the presence of TMAO does not lead to oligomerization or aggregation. It has been suggested that recombinant AF1 binds both the ubiquitous coactivator CBP and the TATA box-binding protein, TBP. Interactions with both of these are greatly enhanced in the presence of TMAO. Co-immunoadsorption experiments indicate that in TMAO each of these and the coactivator SRC-1 are found complexed with AF1. These data indicate that TMAO induces a conformation in AF1/tau1 that is important for its interaction with certain co-regulatory proteins.

Transcriptional activation by the glucocorticoid receptor (GR) is mediated through the function of three regions in the protein, AF1/tau1, tau2, and AF2. Numerous mutational analyses have shown that of these, AF1/tau1 makes by far the largest quantitative contribution to transcriptional activation. Deletion or inactivating mutations of AF1/tau1 reduce the ability of the GR to activate transcription from test genes by at least 60–70% (1–3). As defined by molecular genetic analyses, tau2 and AF2 are located in the C-terminal ligand-binding domain (LBD) of the receptor, whereas AF1/tau1 lies N-terminal from the centrally located DNA-binding domain (DBD). In the human GR (hGR) AF1 is encompassed by amino acids 77–262 (3). Precisely how the AF1/tau1 transactivation region functions is unknown, in part due to lack of knowledge of its working structure; yet this structure must provide the basis for essential, specific interactions with other proteins. Studies undertaken in our laboratory and others (4, 5) have shown that when expressed independently in dilute aqueous solutions, AF1 appears to have little structure. Instead it appears to exist as a collection of conformers that overall appears as random coil. However, in the presence of the strong α-helix stabilizing agent trifluoroethanol, as many as three segments toward the C-terminal end of the AF1/tau1 region exhibit α-helical characteristics (4). Mutations in the GR tau1/AF1 transactivation region coupled with assays of function have suggested that this ability to form α-helical conformation in vitro correlates with the transactivation potential of the region (4). The subdomain of AF1/tau1 that subsumes the potential helices has been referred to as the AF1 core (AF1Δ), and when this core is expressed separately while connected to an exogenous DNA-binding domain, AF1 retains considerable transactivating activity. Mutations of acidic amino acids in AF1, including the core, had little effect on such activity; however, mutations altering several hydrophobic residues of AF1/tau1 significantly diminished its transcriptional activation potential (6).

In recent years, a number of proteins that modulate GR activity, such as GRIP1, RIP140, SRC-1, and CBP/p300, have been identified (7–9). These co-regulators bind the GR and presumably act as molecular bridges to the primary transcriptional machinery. Most co-regulators have been identified through their ligand-dependent interactions with the LBD, in particular, with the AF2 domain in members of the receptor family of which the GR is part (10–14). However, it is not known whether the co-regulators function exclusively through this part of the GR or whether they can also modulate the activity of AF1/tau1. Some may well do so. CBP and several other co-regulators have been shown to bind, if not strongly, to recombinant AF1/tau1 (15–17). A recent study using AF1, or the potential helices thereof connected to a GR DNA-binding domain could interact in a yeast system with a fragment of CBP to activate appropriate promoter-reporter constructs (17). AF1 has also been shown to bind TBP (16), the critical TATA box-binding protein that forms the basis for the multiprotein transcription initiation complex. This raises the possibility that the AF1 GR domain somehow directly influences the transcription machinery. In vitro transcription studies indicated that the holoGR acts to stabilize the preinitiation complex (18). One possibility is that a structured conformation of AF1/tau1 is induced or stabilized during its interaction with specific target proteins. This model in fact has been supported for the activation domains of some transcription factors (19, 20). Thus, data short of actual structural proof support the idea that conditional folding of the AF1/tau1 region is an important require-
ment for its interaction with target factors and subsequent role in gene regulation. In vivo, the particular conformer of AF1/tau1 required for transcriptional activation presumably is induced or stabilized by forces coming from intra- and intermolecular interactions. Knowledge of those factors and the conformation adopted by AF1/tau1 will lead to understanding of the role of this region in the transcription process, information essential to understanding how glucocorticoids affect gene regulation.

Naturally occurring solutes or organic osmolytes have been used to shift the thermodynamic balance so as to make unstructured proteins fold into native-like, functioning structures (21–26). One such osmolyte, trimethylamine N-oxide (TMAO), has been used very successfully to fold unstructured, inactive proteins into proteins with significant functional activity (27, 28). We recently demonstrated that TMAO induces secondary and tertiary structure in a GR fragment containing the entire N terminus plus the DBD (amino acids 1–500). The conformational transition of this GR fragment is cooperative in nature (5), a condition characteristic of proteins folding to their proper shape. Because most of the structural changes taking place in this GR fragment appear to be happening in the N-terminal domain, we hypothesize that TMAO induces an ordered conformation in the AF1/tau1 region, and this conformation is important for its interaction with target proteins. In this paper, we present studies of the TMAO-induced conformation in the AF1/tau1 region expressed alone. Our data suggest that TMAO induces secondary and tertiary structure in the AF1/tau1 region, and this induced conformation greatly enhances its interaction with certain co-regulatory proteins and TBP.

MATERIALS AND METHODS

Expression and Purification—Construction and expression of AF1/tau1 have been described (5). The bacteria containing the recombinant vector for GST-AF1/tau1 were induced with isopropyl-$\beta$-D-thiogalactopyranoside (0.5 mM) for 3 h, lysed, and extracted. The bacterial extracts were loaded onto a glutathione-Sepharose column at 4 °C. AF1/tau1 protein was eluted from the column by thrombin digestion, followed by a Superdex-75 exclusion column as described (5). For GST adsorption experiments, GST or GST-tagged protein was purified by eluting the bound protein using 50 mM Tris, 500 mM NaCl, 50 mM reduced glutathione, pH 8.3. Protein purity was analyzed by SDS-polyacrylamide gel electrophoresis stained with Coomassie Blue R-250 and was estimated to be greater than 95%. Fluorescence Emission Spectroscopy—Fluorescence emission spectra of purified AF1/tau1 protein in solution were recorded at various concentrations of TMAO. The spectra were monitored using a Spex Fluoromax spectrophotometer at excitation wavelengths of 278 or 295 nm as described (5). All measurements were made in 1 cm rectangular cuvettes thermostated at 22 °C, and all the data were corrected for the contribution of the solute concentrations. To prevent aggregation of protein we added proline at a constant molar ratio of TMAO:proline as 4:1 in all samples containing TMAO.

Circular Dichroism Spectroscopy—The CD spectra were recorded at 22 °C on an Aviv 62 spectropolarimeter using a 1.0-cm quartz cell, with the bandwidth of 1.0 nm and scan step of 0.5 nm. The CD spectra were recorded at a protein concentration of 1.0 mg/ml (in the presence and absence of 3 M TMAO) in 10 mM Tris, 10 mM NaCl, pH 7.9. The CD spectra were recorded at a protein concentration of 1.0 mg/ml (in the presence and absence of 3 M TMAO). The CD spectra were corrected for the contribution of solute concentrations. Each spectrum shown is a result of five spectra accumulated, averaged, and smoothed.

Limited Proteolytic Digestion—Sequential digestion of trypsin, chymotrypsin, endoproteinase Gluc-C, and endoproteinase Arg-C were used for proteolytic digests. Digestion was carried out with 5 μg of purified enzyme in 10 mM Tris, 10 mM NaCl, 2 mM dithiothreitol, pH 7.9. Except for Endo Arg-C, which was carried out at room temperature for 50 min, all other reactions were carried out at 4 °C for 20 min. Endo Gluc-C and Endo Arg-C were added at a protein:enzyme mass ratio of 50:1, whereas trypsin and chymotrypsin were added at 100:1. Reactions were terminated by adding SDS loading buffer and then placing the sample tubes in a boiling water bath. The samples were then run on SDS-PAGE gel and stained by Coomassie Blue R-250. For protein microsequencing, the proteolytic reactions were carried out in triplicate in the presence and absence of 3 M TMAO. After gel electrophoresis, the proteins were transferred onto PVDF membranes. The largest protected bands in the samples containing 3 M TMAO were pooled and sequenced by five cycles of Edman degradation (29).

RESULTS

TMAO Causes the hGR AF1/tau1 Transactivation Region to Fold into a Native-like Conformation—As with other related receptors, the GR contains several major functional domains. These are shown diagrammatically for the human GR in Fig. 1. The AF1/tau1 transactivation region, amino acids 77–262 is highlighted, with vertical lines above the bar indicating the location of the two Tyr and one Trp residues within AF1/tau1.

Fig. 1. Topological diagram of human GR showing its functional regions. The numbers indicate positions of amino acids. The vertical lines represent the positions of Trp (bold line), and Tyr (thin lines) residues in AF1/tau1.
Fluorescence emission spectra of AF1/tau1 in the absence and presence TMAO. The curves a, b, d and e show the spectra in the absence (---) and presence (-----) of 3.5 M TMAO. Experiments a-c were carried out in low salt; d-f were carried out in high salt. a and d, excitation at 278 nm; b and e, excitation at 295 nm. c and f show the reversible conformational transition of AF1/tau1 induced by TMAO, as monitored by change in emission maxima upon excitation at 278 nm with respect to increasing concentrations of TMAO. The spectra in low salt were recorded in buffer containing 10 mM Tris, 10 mM NaCl, 10 mM dithiothreitol, pH 7.9. Those of d-f were recorded in the same buffer except with 200 mM NaCl. The linear least squares best fit of experimental data to the two-state model of protein folding/denaturation using linear extrapolation methods (42) gives apparent thermodynamic parameters of TMAO-induced folding: \( \Delta G = -2.8 \pm 1.1 \text{ kcal/mol}, \) \( m = 1.1 \pm 0.6 \text{ in 10 mM NaCl, and } \Delta G = -3.9 \pm 0.7 \text{ kcal/mol, } m = 1.6 \pm 0.3 \text{ in 200 mM NaCl.}\)

Because the ultracentrifugation studies (discussed below) required a buffer containing 200 mM NaCl, we repeated the fluorescence emission spectra in this buffer to find out whether the structural changes we observed in AF1/tau1 following TMAO exposure occurred at this salt concentration. In Fig. 2, (d and e) the quantum yield of the fluorescence is increased significantly following TMAO exposure, as was seen in Fig. 2 (a and b). Interestingly, the blue shift in emission maxima at 295 nm is significantly increased compared with that at low salt (Fig. 2, b versus e), indicating that higher salt concentration favors the conformational transition of folding of AF1/tau1 by TMAO. The TMAO-induced conformational transition in AF1/tau1 in 200 mM salt also is cooperative (Fig. 2f) and reaches saturation at 3 M compared with that of 3.5 M in 10 mM salt (Fig. 2c). These fluorescence emission studies thus indicate that the TMAO-induced cooperative conformational transition in AF1/tau1 is more favored at 200 mM.

The fluorescence emission spectra of AF1/tau1 in the presence and absence of TMAO suggest that TMAO causes tertiary structure to form in this protein. To acquire further evidence for tertiary structure occurring in AF1/tau1, we recorded the near-UV CD spectra of this protein in the presence and absence of TMAO (Fig. 3). Comparison of the spectra shows that in the presence of TMAO, the maximum at around 290 nm is significantly increased, reflecting perturbation of the Trp residue. These data support the conclusion that TMAO causes three-dimensional structure to occur in the domain.

The Structure Induced in AF1/tau1 by TMAO Resists Proteolysis—As another way of evaluating the changes in the tertiary structure of the AF1/tau1 region brought about by TMAO, we carried out limited proteolytic digestions of the protein with four different proteases in the presence and absence of the osmolyte. To be sure that the compound was not interfering with enzyme activity, we first tested trypsin activity on the AF1 core. As another way of evaluating the changes in the tertiary structure of the AF1/tau1 region brought about by TMAO, we carried out limited proteolytic digestions of the protein with four different proteases in the presence and absence of the osmolyte. To be sure that the compound was not interfering with enzyme activity, we first tested trypsin activity on the AF1 core.


At 2 M or higher TMAO concentrations, AF1/tau1 appears to be mostly protected, suggesting that it has folded into a tertiary structure that moves the residues attacked by these enzymes to positions not easily reached by them. Similar results were obtained when AF1/tau1 was digested with the proteases Endo Gluc-C and Endo Arg-C (Fig. 4b), although in the case of Endo Gluc-C, the protection was not as complete. In the case of Endo Arg-C, full protection was seen only at 3 M TMAO.

We compared the peptide patterns resulting from digestion of heat-denatured and non-denatured AF1/tau1, to see whether the non-denatured form contained folded regions resistant to peptidases. The data (not shown) indicated no difference in patterns, consistent with lack of significant structure in native recombinant AF1/tau1. We then compared the peptide patterns of the folded forms induced by TMAO. Upon exposure to each of the four proteases, both non-denatured and denatured AF1/tau1 show closely similar protected digestion patterns after incubation in the presence of 3 M TMAO for 15 min up to 16 h (data not shown). The similar patterns of protected peptides at every time point in the presence of 3 M TMAO demonstrate that TMAO can induce similar structures in AF1/tau1 whether it is initially non-denatured or denatured. Acute (15 min) TMAO exposure is enough to fold the recombinant protein domain into a tertiary structure not distinguishable, in this test, from that produced by the longest exposure. These observations suggest that once in a sufficient concentration of TMAO, AF1/tau1 folds rapidly to a protease-resistant shape and that it remains in this conformation, irrespective of the duration of TMAO exposure.

Four of the most prominent protected bands seen after partial digestion of AF1 in the presence of 3 M TMAO were identified by sequencing their N termini. In Fig. 5 the positions of these bands in the AF1/tau1 domain are shown diagrammatically. A long region starting at the N terminus of AF1/tau1 was protected from digestion by trypsin, chymotrypsin, and Endo Gluc-C. Many potential substrate sites for these enzymes exist within that region. All three enzymes cut in a relatively short region lying approximately beyond amino acid 217. Although we did not identify them by sequencing as yet, the sizes of smaller peptides protected against these three enzymes suggest that in TMAO a relatively short segment of AF1/tau1 beyond amino acid 217 is open to attack. Peptide 4 was produced by Endo Arg-C, which has only two potential substrate sites in AF1/tau1. The protected site is Arg-214. This result is consistent with the interpretation that in the TMAO-folded portion, a short region beyond amino acid 217 is open to proteolysis. It is evident from these results that TMAO-induced structure in AF1/tau1 is not confined to only one part of the molecule.

**AF1/tau1 Exists as a Monomer Both in the Absence and Presence of TMAO**—It is evident from our data that TMAO exposure apparently leads to the formation of a significant amount of secondary and tertiary structure in the AF1/tau1. The observed structural changes in the presence of 3 M TMAO might originate from an oligomerization of the protein induced by the presence of the co-solvent. Analytical sedimentation studies were conducted to monitor the effects of TMAO on the quaternary structure of the protein. Data from sedimentation velocity studies of AF1/tau1 in the absence and presence of 3 M TMAO were found to fit a single species model, as shown in Fig. 6. No additional species were detected, and the total amount of protein loaded in the cell was accounted for in that single species. The s² (g, w) value for AF1/tau1 obtained in the presence of 3 M TMAO is ~2.4, which corresponds to the expected value for a globular protein of ~20–25 kDa (37). However, the s² value for AF1/tau1 in buffer without TMAO is ~1.6, consistent with that of a protein of the same molecular weight but one that assumes either an asymmetric shape or adopts an unstructured conformation. Sedimentation equilibrium experiments were also run under similar conditions at three different speeds (20,000, 25,000, and 30,000 rpm). The results (not shown) indicated the presence of a protein of a molecular mass of 21 kDa, which corresponds to the monomeric molecular weight for AF1/tau1. Thus these ultracentrifugation studies clearly indicate that up to 0.5 mg/ml AF1/tau1 exists as a monomer in the aqueous solutions employed in this study and
that exposure to 3 M TMAO does not induce protein aggregation.

**TMAO Facilitates the Interaction of AF1/tau1 with Important Target Proteins**—It is presumed that AF1/tau1 makes physical interactions with other factors in order to transactivate gene(s) (16) and that conditional folding is important for these interactions (6). We therefore evaluated whether the conformation induced in AF1/tau1 by TMAO is important for specific protein-protein interactions. By using a GST “pull-down” assay with GST-AF1/tau1 attached to solid beads as the adsorptive reagent, we observed that in the presence of TMAO, several proteins from HeLa cell nuclear extracts were adsorbed. Fig. 7 shows an example of the results. It is evident from the Coomassie-stained gel that when sufficient TMAO is present, GST-tau1 binds only certain proteins from HeLa nuclear extracts. Several higher molecular weight bands that are not prominent in the extract become so in the adsorbed proteins, and one low molecular weight band that is prominent in the crude extract is strongly retained on the GST-AF1/tau1 column. GST alone or GST-AF1 in low concentrations of TMAO did not retain these proteins. Several similar experiments using both unlabeled and metabolically labeled proteins confirmed the fact that in TMAO certain proteins have high affinity for AF1/tau1. In the experiment shown, after binding the proteins to the GST-AF1 on beads in the presence of 3 M TMAO, the column with bound proteins was washed extensively with a buffer containing no TMAO. Subsequent elution and electrophoresis of the retained proteins showed that removal of TMAO from the washes had not completely released these proteins from the GST-AF1, suggesting that once the AF1/tau1 complexes were formed in the presence of TMAO, they were relatively stable (Fig. 7, compare lanes 5 and 6).

To begin to identify specific AF1/tau1 target factors, we screened the bound proteins by immunoreacting these with antisera against a selected set of known co-regulators. RIP140, CBP, NCoR, TBP, GRIP, p/Cip, SRC-1 have been shown previously to interact with nuclear receptors and/or to affect receptor-specific transcriptional activation (7–14). No immunoreactions were observed with the sera we employed for N-CoR, GRIP1, RIP140, or p/Cip, either in the presence or absence of 3 M TMAO. (One such experiment is shown in Fig. 8.) Consistent with previous reports (6), in the absence of TMAO we detected a weak interaction with TBP and CBP. This was increased dramatically in the presence of 3 M TMAO (Fig. 8). In the absence of TMAO the antisera did not detect a p250/300 band in the adsorbed HeLa nuclear proteins but did show a slight reaction with a protein of 160 kDa. But in 3 M TMAO, a reaction at 250/300 kDa and a very strong reaction at 160 kDa were seen. To determine whether TMAO truly enhanced an interac-

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**Fig. 6.** Sedimentation coefficient distribution of AF1/tau1 as determined from sedimentation velocity. The peak \( s^* \) corresponds to the apparent sedimentation coefficient. a, in buffer; b, in 3 M TMAO.

**Fig. 7.** TMAO enhances GST-AF1/tau1 interactions with several proteins from HeLa cell nuclear extract in a GST pull-down assay. Proteins were identified by SDS-PAGE and Coomassie Blue staining. Lane 1, molecular weight markers; lane 2, nuclear extract; lane 3, GST-AF1/tau1 with no nuclear extract; lane 4, GST-AF1/tau1 + nuclear extract; lanes 5 and 6, GST-AF1/tau1 + nuclear extract + 3.0 M TMAO. The beads used in lane 5 were washed with a buffer with 3 M TMAO, whereas in lane 6, beads were washed with buffer only. Horizontal lines to the right indicate two of the 5 or 6 proteins retained in TMAO.

**Fig. 8.** TMAO enhances the association of TBP, CBP, and SRC-1, but not all known steroid receptor cofactors, with AF1/tau1. In the upper three panels, GST-AF1/tau1 linked to glutathione-Sepharose beads were used to adsorb proteins from HeLa cell nuclear extracts (see “Materials and Methods”). Lanes 1, nuclear extract without adsorption to beads. Lanes 2, adsorption without TMAO. Lanes 3, adsorption in the presence of 3 M TMAO. The data shown are immunoreactions of the proteins resolved by denaturing gel electrophoresis, using antibodies to GRIP1, TBP, or CBP. Lower panel shows the immunoreaction to antibodies against AF1 (resolved by gel electrophoresis), after immunoprecipitation with the indicated antibodies. Lanes 1, 3, and 5, immunoadsorption in the absence of TMAO. Lanes 2, 4, and 6, immunoadsorption in 3 M TMAO.
tion between CBP and AF1/tau1, we transfected COS-1 cells with a CBP plasmid designed to express CBP constitutively. In extracts from these cells we found that in the presence of 3 M TMAO a protein of 250/300 kDa, reactive to the anti-CBP antiserum, was trapped by the GST-AF1 column (Fig. 8). Among the other HeLa proteins trapped in the presence of 3 M TMAO on the GST-AF1 column was TBP. Only a weak reaction was seen unless TMAO was present.

To confirm further these AF1/tau1 interactions, we carried out immunoadsorption experiments, using CBP and TBP primary antibodies in HeLa nuclear extracts that had been supplemented with recombinant AF1/tau1. We also probed for adsorbed proteins with an antibody to the 160-kDa protein SRC-1. After allowing the extracts to react with each antiserum, the protein complexes were trapped by adding secondary antibodies linked to inert beads. The beads were washed extensively, and the bound proteins were released and resolved by electrophoresis in denaturing conditions on polyacrylamide gels. An antiserum to amino acids 150–175 of the hGR was used to identify AF1/tau1. In the absence of TMAO, a small amount of AF1 was found to have been retained on the beads precipitated with anti-CBP and anti-TBP. No SRC-1/AF1 interaction was seen without TMAO. In the presence of 3 M TMAO, a very strong interaction of AF1 with each of the other proteins has been shown to have occurred. Taken together, these protein-protein interaction data indicate that TMAO-induced folding in AF1/tau1 is important for its interaction with target factors. Among these may be CBP, TBP, and SRC-1.

DISCUSSION

Deletion studies have indicated that hGR lacking the AF1/tau1 transactivation region retains only a small portion of its transactivation activity (1, 2); hence, it has been concluded that this region plays a major role in gene regulation by GR. It has been reported that AF1/tau1 makes physical contact with certain other proteins to activate genes (6, 15–17). It has also been shown that a C-terminal portion of AF1/tau1 is indispensable for its transactivation activity. This “core” region has a propensity to form a-helix, and in the presence of trifluoroethanol, it can form three helical segments (4). These potential helices seem important for functional interactions with a fragment of CBP in a yeast system (17). The induced fit model of folding hypothesizes that the AF1/tau1 region is unstructured in vivo until it adopts a more ordered conformation (38). In this paper we have studied the conditional folding of recombinant AF1/tau1 in the presence of a naturally occurring osmolyte, TMAO, which has been shown to fold proteins into native-like structures (27). Our results clearly demonstrate that TMAO causes a significant amount of secondary/tertiary structure to form in the AF1/tau1 region. The fluorescence emission data indicate a shift of hydrophobic amino acids into a more non-polar environment, just the type of change seen as natural hydrophobic forces drive protein folding. In both low salt and near physiological salt concentrations, the conformational transition is cooperative, a hallmark of naturally folding proteins (35, 36), and the free energy shift is similar to that seen during spontaneous folding of globular proteins. Osmolyte-driven stabilization of protein folding is in fact a process used often in nature (23). Due to its solvophobic effect on the peptide backbone (25), TMAO forces thermodynamically unstable proteins to fold to active conformations without altering the rules for folding (25). Osmolytes, including TMAO, serve this purpose in a wide range of organisms. Based on these facts and on our observations, it is highly likely that TMAO enhances folding to a native-like structure in AF1/tau1. The near-UV CD and the fluorescence emission data both point to this conclusion. These data monitor signals indicating that the 2 Tyr and 1 Trp residues move to more hydrophobic locations, e.g., to the interior of the protein. The TMAO-induced increased sH value without a change in molecular weight suggests that the protein is more tightly packed and assumes a globular structure in the presence of this co-solvent. Since the breadth of the sedimentation profiles shown in Fig. 6 is proportional to the diffusion coefficient of the protein, the larger breadth in the profile for AF1/tau1 in the presence of 3 M TMAO indicates a higher diffusion coefficient. A higher diffusion coefficient for the same molecular weight protein implies that the hydrodynamic shape of the protein is more compact or symmetric. Thus, the hydrodynamic data are in complete agreement with the conclusion that the presence of 3 M TMAO induces AF1/tau1 to assume a more structured state without inducing formation of large aggre-
Conformational changes from other factors such as cross-domain interactions, binding to glucocorticoid response elements, or even steroid binding could also play a significant role.

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REFERENCES

1. Dahlman-Wright, K., Almlof, T., McEwan, I. J., Gustafsson, J. A., and Wright, A. P. H. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1619–1923
2. Giguere, V., Hollenberg, S. M., Rosenfeld, M. G., and Evans, R. (1986) Cell 46, 645–652
3. Hollenberg, S. M., and Evans, R. M. (1986) Cell 55, 899–906
4. Dahlman-Wright, K., Baumann, H., McEwan, I. J., Almlof, T., Wright, A. P. H., Gustafsson, J. A., and Hard, T. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1699–1703
5. Baskakov, I. V., Kumar, R., Srinivasan, G., Ji, Y., Bolen, D. W., and Thompson, E. B. (1999) J. Biol. Chem. 274, 10693–10696
6. Almlof, T., Gustafsson, J. A., and Wright, A. P. H. (1998) Biochemistry 37, 9586–9594
7. Mccarroll, J., Lanz, B., and O'Malley, B. W. (1999) Endocr. Rev. 20, 321–344
8. Glass, C. K., Rose, D. W., and Rosenfeld, M. G. (1997)Curr. Opin. Cell Biol. 9, 222–232
9. Horwitz, K. B., Jackson, T. A., Bain, D. L., Richer, J. K., Takimoto, G. S., and Tung, L. (1996) Mol. Endocrinol. 10, 1677–1177
10. McEwan, I. J., Wright, A. P. H., and Gustafsson, J. A. (1997) BioEssays 19, 153–160
11. Hong, H., Kohli, K., TrivediA., Johnson, D. L., and Stallcup, M. R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4948–4952
12. Cavailles, V., Dauvois, S. S., L'Horset, F., Lopez, G., Hoare, S., Kushner, P. J., and Parker, M. G. (1996) EMBO J. 15, 3741–3751
13. Onate, S. A., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1995) Science 270, 1534–1537
14. Chagov, J. C., Kwoh, R. P., Lamb, N., Nagwire, M., Montminy, M. R., and Goodman, R. H. (1993) Nature 365, 855–859
15. Henriksen, A., Almlof, T., Ford, J., McEwan, I. J., Gustafsson, J. A., and Wright, A. P. H. (1997) Mol. Cell. Biol. 17, 3065–3073
16. Ford, J., McEwan, I. J., Wright, A. P., and Gustafsson, J. A. (1997) Mol. Endocrinol. 11, 1467–1175
17. Warnmark, A., Gustafsson, J. A., and Wright, A. P. H. (2000) J. Biol. Chem. 275, 15014–15018
18. Tsai, S. Y., Srinivasan, G., Allan, G. F., Thompson, E. B., and O'Malley, B. W. (1990) J. Biol. Chem. 265, 17055–17061
19. McEwan, I. J., Dahlman-Wright, K., Ford, J., and Wright, A. P. H. (1996) Biochemistry 35, 8584–8593
20. Shen, F., Triezenberg, S. J., Hensley, P., Porter, D., and Knutson, J. R. (1996) J. Biol. Chem. 271, 4872–4877
21. Matsudaria, P. (1987) J. Biol. Chem. 262, 10035–10038
22. Stafford, W. F. (1992) Anal. Biochem. 203, 295–301
23. Van Holde, K. E. (1971) Biophysics, pp. 98–121, Prentice-Hall, Englewood Cliffs, NJ
24. Johnson, M. L., and Fraiser, S. C. (1985) Methods Enzymol. 10693–10696
25. Johnson, M. L., and Fraiser, S. C. (1985) Methods Enzymol. 117, 301–342
26. Johnson, M. L., Correa, J. J., Yphantis, D. A., and Holvorson, H. R. (1981) Biochem. 36, 575–588
27. Chervenka, C. H. (1969) A Manual of Methods for the Analytical Ultracentrifuge, p. 45, Spinc Division, Beckman Instruments, Palo Alto
28. Tjian, R., and Maniatis, T. (1994) Cell 77, 5–8
29. Kawai, R., Baskakov, I. V., Srinivasan, G., Bolen, D. W., Lee, J. C., and Thompson, E. B. (1999) J. Biol. Chem. 274, 24737–24741
30. Balasubramanian, J., and Maniatis, T. (1994) Cell 77, 5–8
31. McEwan, I. J., Dai, J., McManaman, J. L., Takimoto, G. S., and Horwitz, K. B. (2000) J. Biol. Chem. 275, 7133–7132
32. McEwan, I. J., Wright, A. P., and Gustafsson, J. A. (1997) Mol. Endocrinol. 11, 1467–1175
33. Warnmark, A., Gustafsson, J. A., and Wright, A. P. H. (2000) J. Biol. Chem. 275, 15014–15018
34. Tsai, S. Y., Srinivasan, G., Allan, G. F., Thompson, E. B., and O'Malley, B. W. (1990) J. Biol. Chem. 265, 17055–17061
35. McEwan, I. J., Dahlman-Wright, K., Ford, J., and Wright, A. P. H. (1996) Biochemistry 35, 8584–8593
36. Shen, F., Triezenberg, S. J., Hensley, P., Porter, D., and Knutson, J. R. (1996) J. Biol. Chem. 271, 4872–4877
37. Matsudaria, P. (1987) J. Biol. Chem. 262, 10035–10038
38. Stafford, W. F. (1992) Anal. Biochem. 203, 295–301
39. Van Holde, K. E. (1971) Physical Biochemistry, pp. 98–121, Prentice-Hall, Englewood Cliffs, NJ
40. Johnson, M. L., and Fraiser, S. C. (1985) Methods Enzymol. 117, 301–342
41. Johnson, M. L., Correa, J. J., Yphantis, D. A., and Holvorson, H. R. (1981) Biochem. 36, 575–588
42. Chen, H., Srinivasan, G., and Thompson, E. B. (1997) J. Biol. Chem. 272, 25873–25880
43. Balasubramanian, J., and Maniatis, T. (1994) Cell 77, 5–8
44. Kawai, R., Baskakov, I. V., Srinivasan, G., Bolen, D. W., Lee, J. C., and Thompson, E. B. (1999) J. Biol. Chem. 274, 24737–24741
45. Balasubramanian, J., and Maniatis, T. (1994) Cell 77, 5–8
46. Kawai, R., Baskakov, I. V., Srinivasan, G., Bolen, D. W., Lee, J. C., and Thompson, E. B. (1999) J. Biol. Chem. 274, 24737–24741
47. Balasubramanian, J., and Maniatis, T. (1994) Cell 77, 5–8
48. Kawai, R., Baskakov, I. V., Srinivasan, G., Bolen, D. W., Lee, J. C., and Thompson, E. B. (1999) J. Biol. Chem. 274, 24737–24741
49. McEwan, I. J., Dai, J., McManaman, J. L., Takimoto, G. S., and Horwitz, K. B. (2000) J. Biol. Chem. 275, 7133–7132
50. McEwan, I. J., Dai, J., McManaman, J. L., Takimoto, G. S., and Horwitz, K. B. (2000) J. Biol. Chem. 275, 7133–7132