Induced α-Helix Structure in AF1 of the Androgen Receptor upon Binding Transcription Factor TFIIF†

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ABSTRACT: In recent years, it has become clear that in many proteins, significant regions are encoded by amino acid sequences that do not automatically fold into their fully condensed, functional structures. Characterization of the conformational propensities and function of the nonglobular protein sequences represents a major challenge. Striking among proteins with unfolded regions are numbers of transcription factors, including steroid receptors. In many cases, the unfolded or partially folded regions of such proteins take shape when the protein interacts with its proper binding partner(s), that is, the molecules to which it must bind to carry out its function. The AF1 domain of the androgen receptor (AR) shows little structure, when expressed as a recombinant peptide. It has been shown previously that AF1 interacts with transcription factor TFIIF *in vitro*. Using Fourier transform infrared (FTIR), we tested whether this interaction can induce structure in the AR AF1. Our results demonstrate that the recombinant AR AF1 can acquire significantly higher helical content after interacting with RAP74, a subunit of the TFIIF complex. We further show that this induced conformation in the AR AF1 is well-suited for its interaction with SRC-1.

The actions of androgens are mediated through the androgen receptor (AR), an intracellular receptor that belongs to the steroid nuclear receptor superfamily (*1*−*3*). A general outline of how AR mediates the biological effects of androgens is known. The unliganded receptor is associated with several heat shock and other proteins. When androgen binds to the AR, the receptor becomes activated, is released from these proteins, dimerizes, and is localized to the nucleus, where in the regulatory regions of target genes it binds to specific DNA sequences, and to other coregulatory proteins, to regulate the transcription of specific genes (*4*−*6*). However, the exact mechanism by which AR passes signals from a hormone to a specific gene is still unknown to a large extent. This in part is due to the lack of knowledge about the working three-dimensional structure of the AR.

The general structure of the AR is similar to that of other steroid receptors and, by comparison to other members of the nuclear hormone receptor (NHR) family, consists of a highly conserved DNA-binding domain (DBD), a less conserved ligand-binding domain (LBD), and a poorly conserved N-terminal domain (NTD). This fundamental domain model divides the primary sequence into N- and C-terminal regions, separated by the DBD, which specifies high-affinity binding to DNA at certain short, specific nucleotide sequences, or "response elements" (REs). The C-terminal region contains the LBD, which is responsible for androgen binding and ligand-dependent regulation of transcriptional activation (*2*, *3*).

Unique to the AR within the nuclear receptor family are glutamine, proline, and glycine homopolymeric sequences found in its N-terminal region. There is some degree of variability in the size of the AR due to polymorphic variations in the length of these regions, such as the poly-Q region in exon 1. The NTD contains a powerful transactivation domain AF1, which is constitutively active in the absence of the LBD (*7*). Protein structure algorithms predict that this region is mostly unstructured and highly negatively charged, similar to regions found in other acidic activation domains, including the AF1/tau1 activation domain of the glucocorticoid receptor (GR). In fact, a recent study has shown that this region is intrinsically unstructured in aqueous solutions, when expressed independently as a recombinant peptide (*8*). A naturally occurring solute, trimethylamine *N*-oxide (TMAO), or a heterologous binding partner has been shown to promote the folding of AR AF1 into a compact, protease resistant form (*8*). A similar kind of folding has also been reported for the conformation of the GR AF1 domain (*9*).

There are suggestions that an induced conformation or set of conformations occurs in AF1 so that it can carry out its transcription function. The question then is, what causes these conformational changes. An induced fit could occur only when the AF1 domain encounters its proper binding partner protein. In fact, recent studies have shown that several transcription factors' transactivation domains, including those of some steroid receptors, undergo a transition to a folded

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state upon interaction with coregulatory proteins (*8*, *10*−*13*).

With respect to AR AF1, interactions with the general transcription factor proteins TFIIF and TFIIH and coactivator proteins are likely to form the basis for the multiprotein AR-dependent transcription initiation complex (*14* and references therein). We hypothesize that such interactions induce or stabilize a structured conformation of AF1.

In this study, we have tested the hypothesis that an induced-fit mechanism leads to imposition of structure in the AR AF1 domain. Following up our previous work demonstrating that the AR AF1 domain can interact directly with the C-terminal domain of the RAP74 (RAP74-CTD) subunit of the TFIIF complex (*14*, *15*), we further tested the hypothesis that this interaction can induce secondary and/or tertiary structure in an otherwise mostly unstructured AR AF1 domain. Our results from Fourier transform infrared (FTIR) spectroscopy demonstrate that when it encounters RAP74, the AR AF1 region adopts more helical content. These results are in accordance with the hypothesis that an induced-fit mechanism induces more helical structure in AR AF1 when it encounters a specific binding partner. The induced helicity seems to be at the expense of β-sheet and an accumulation of small changes in bend, turn, and coil components. We further show that this induced structure in AR AF1 facilitates binding of p160 coactivator family member SRC-1.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification. Expression and purification of His−AF1 (amino acids 142−485), GST−AF1, and His−RAP74-CTD (amino acids 363−517) proteins have been described previously (*14*, *15*). Recombinant His-tagged proteins were expressed in *Escherichia coli* BLR (DE3) cells by induction with 1 mM isopropyl β-D-thiogalactoside (IPTG) for 1−2 h at 37 °C in LB medium and purified from the soluble fraction by Ni^{2+}−nitriloacetate (NTA)−agarose affinity chromatography. The GST−AF1 fusion protein was expressed by induction with 0.1 mM IPTG for 4−5 h at room temperature in 2× TY medium. The cells were lysed as described above, and the recombinant proteins were purified from the soluble fraction using glutathione−Sepharose 4B resin (Amersham Pharmacia Biotech). All the purified proteins were dialyzed against 25 mM HEPES (pH 7.9), 100 mM sodium acetate, 5% glycerol, and 1 mM dithiothreitol (DTT), and protein concentrations were estimated against BSA standards using the Bradford reagent (Bio-Rad).

Fourier Transform Infrared Spectroscopy. FTIR spectra were recorded with a BOMEM (Quebec City, PQ) MB Series Fourier transform infrared spectrometer equipped with a dTGS detector and purged constantly with dry air generated by a Balston (Haverhill, MA) air-dryer. Protein samples were loaded in a liquid IR cell (Graseby) with CaF_2 windows and a path length of 6 μm. For each spectrum, a 200-scan interferogram was collected at the single-beam mode with a resolution of 4 cm^{-1}. Reference spectra were recorded under identical scanning conditions with only the corresponding buffer in the cell. Protein spectra were obtained according to previously established criteria using a subtraction procedure (*16*). The residual water vapor signals, if present, in the protein spectra were removed by subtracting the spectrum of gaseous water. Second-derivative spectra were obtained with a seven-point Savitsky−Golay derivative function. All second-derivative spectra were baseline-corrected and area-normalized as previously described (*17*). The secondary structure content of the protein was determined by curve fitting analysis of the inverted second-derivative spectrum from infrared second-derivative amide 1 spectra as described previously (*18*).

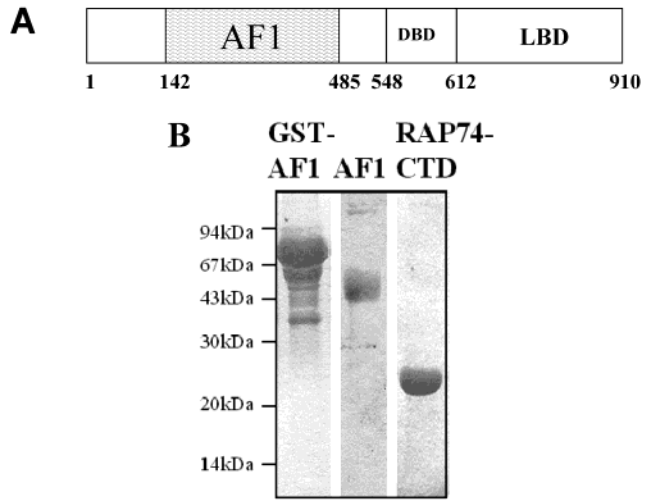

FIGURE 1: (A) Topological diagram of the human AR showing its major functional domain. The highlighted region represents the N-terminal activation domain, AF1. (B) Coomassie Blue-stained gel of purified AR AF1 and RAP74-CTD polypeptides.

Protein−Protein Interactions. GST−AF1 fusion protein or GST alone (100 pmol) was incubated for 30 min at 4 °C with 25 μL of a glutathione−Sepharose 4B bead slurry (Amersham Pharmacia Biotech), resuspended in 100 μL of ice-cold PBS. The immobilized protein was recovered by centrifugation and resuspended in 150 μL of pulldown buffer [20 mM HEPES-KOH (pH 7.9), 10% (v/v) glycerol, 100 mM NaCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 0.1% (v/v) Tween 20, 0.02 mg/mL BSA, 1 mM DTT, and 0.2 mM PMSF]. The reaction mixtures were then incubated for 2 h at 4 °C with 5 μL of radiolabeled SRC-CTD (amino acids 977−1441; Promega TNT Coupled Reticulocyte Lysate system). Folding of the AF1 was achieved by preincubating for 30 min with 2 M TMAO or a 2 M excess of RAP74-CTD. As controls, AR AF1 was preincubated with the nonbinding Lex-DBD protein (amino acids 1−87) for 30 min prior to the addition of radiolabeled SRC-CTD, or 2 M TMAO was added after the 2 h incubation with SRC-CTD and the binding reaction continued for a further 30 min. The beads and bound protein were recovered and washed five times with 200 μL of pulldown buffer, and the final Sepharose bead pellet was resuspended in 20 μL of 2× SDS sample. Samples were analyzed by SDS−PAGE.

RESULTS

RAP74−AR AF1 Interaction Leads to the Formation of Helical Structure in AR AF1. A topological diagram of the AR showing major functional domains is shown in Figure 1A. Three sets of purified recombinant protein samples (AF1, RAP74-CTD, and a 1:2 AF1/RAP74-CTD mixture) were used for FTIR experiments (Figure 1B). The second-

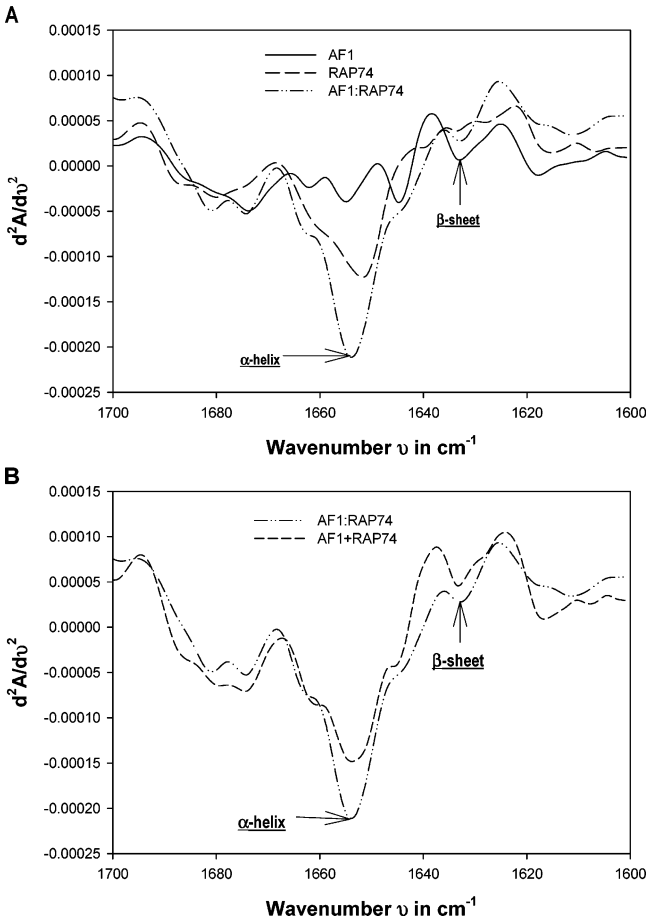

FIGURE 2: (A) Second-derivative FTIR spectra of AR AF1 (—), RAP74-CTD (– – –), and the AF1/RAP74-CTD mixture (—••—) recorded in 20 mM HEPES, 100 mM sodium acetate, and 1 mM DTT. (B) Spectra of the AF1/RAP74 mixture (—••—) and of additive independent AF1 and RAP74 (– – –). Averages of 200 scans. The protein concentrations were 0.2 mM AF1 and 0.4 mM RAP74.

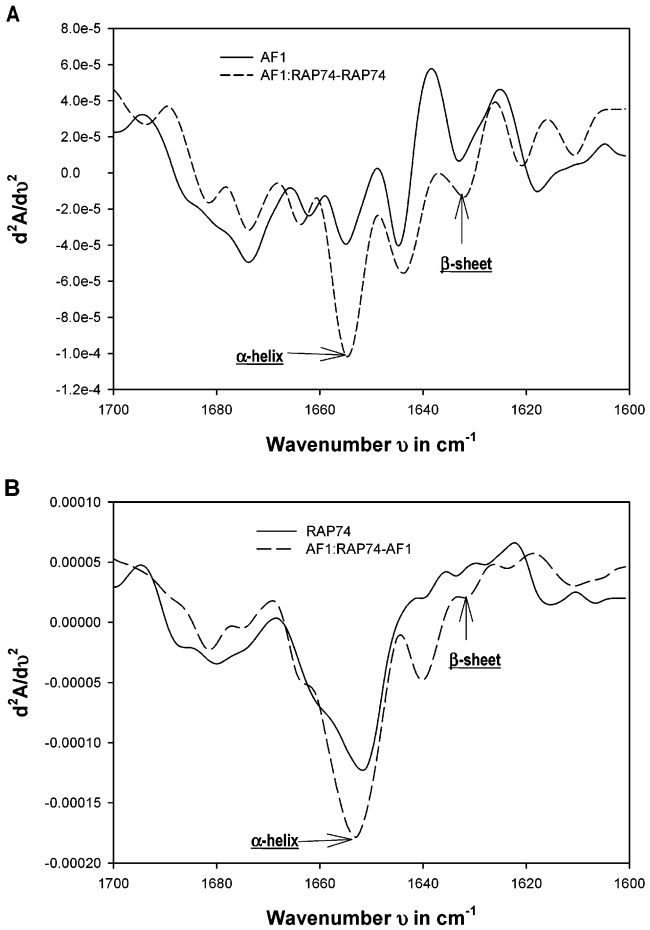

FIGURE 3: Second-derivative FTIR spectra of the AR AF1 domain. (A) Spectrum of the AF1 protein in buffer (—) and AF1 in the AF1—RAP74-CTD complex (– – –). (B) Spectrum of the RAP74-CTD protein in buffer (—) and RAP74-CTD in the AF1—RAP74-CTD complex (– – –). The spectra are an average of 200 scans. The spectrum of AF1 in the AF1—RAP74-CTD complex was plotted from the data obtained after the contribution of RAP74 from the complex had been subtracted, and similarly, the spectrum of RAP74-CTD was obtained after the contribution of AF1 from the complex had been subtracted.

derivative amide I FTIR spectra indicating the nature of the secondary structure elements of these proteins are shown in Figure 2A. All spectra exhibited four basic band components that on the basis of previous infrared studies of proteins in aqueous solution (*19*) can be assigned to α-helix (∼1656 cm^{-1}), *β*-sheet (∼1635 cm^{-1}), *β*-turns (1685 and 1675 cm^{-1}), and random coil (1647 cm^{-1}). The curves show the greatest difference for the separate proteins in the range of wavenumber 1656 cm^{-1} (α-helix). Alone, AF1 shows little helical content, and RAP74-CTD displays an inverse peak, indicating some helical content. The spectrum of the AF1/RAP74-CTD mixture shows that the magnitude of the peak around 1656 cm^{-1} is significantly increased, suggesting that the complex has a greater helical content than either AF1 or RAP74 alone (Figure 2A). When the sum of the second-derivative spectra of AF1 and RAP74-CTD are compared with the spectrum of the AF1/RAP74-CTD mixture (Figure 2B), it is evident that the helical content is higher in the AF1/RAP74-CTD mixture than in the summation of the AF1 and RAP74-CTD spectra, suggesting that these changes are not just additive. Comparison of the second-derivative spectra of AF1 with and without RAP74-CTD present indicated a significant increase in helical content in AF1 when mixed with RAP74-CTD (Figure 3A), suggesting that interaction with RAP74-CTD leads to imposition of helical structure on the AF1 domain. Similar comparisons of RAP74-CTD spectra with and without AF1 complexed also showed an increase in the helical content of RAP74-CTD when it was complexed with AF1 (Figure 3B), though the changes are not as large as in AF1. The crystal structure of RAP74-CTD (amino acids 449—517) has been determined. The overall folding of this peptide is similar to that of the winged-helix motif protein (*20*). The approximate quantitative contributions of the four secondary structural elements calculated from these spectra are summarized in Table 1. It is evident from the data in Table 1 that the helical content in AF1 more than doubles after it binds to RAP74-CTD. This increased helical content comes at the expense of mostly *β*-sheet and a combination of bend and turn random coil conformations. The secondary structural elements in RAP74-CTD change slightly, upon mixing with AF1, and there is again an increase in the helical content at the expense of other forms. These changes in RAP74-CTD are difficult to interpret accurately due to the large changes observed in the helical content in AR AF1 in the complex. The secondary structure content of the protein was determined by curve fitting analysis of the inverted second-derivative spectrum from

Table 1: Summary of Secondary Structural Elements of AR AF1 with and without RAP74 Bound, in the Presence and Absence of TMAO[a]

	helix (%)	sheet (%)	bend (%)	torn (%)	coil (%)
AF1	16	24	19	17	24
AF1/RAP74−RAP74	35	15	17	14	21
RAP74	46	0.5	15	13	28
AF1/RAP74−AF1	55	0	13	10	25
AF1 in 3 M TMAO	37	17	11	11	24

[a] AF1/RAP74−RAP74 indicates the contribution of AF1 in the AF1−RAP74 complex, and AF1/RAP74−AF1 indicates the contribution of RAP74 in the complex.

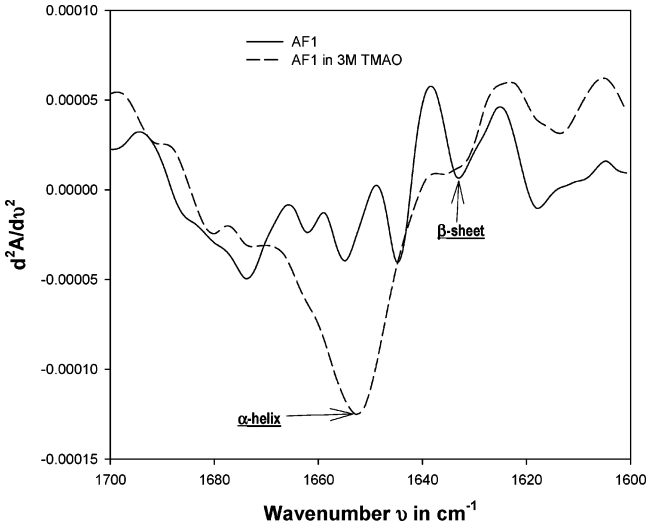

FIGURE 4: Second-derivative FTIR spectra of the AR AF1 domain: spectrum recorded in the absence (—) and presence (– – –) of 3 M TMAO. The spectra are an average of 200 scans. The protein concentrations were 0.2 mM.

infrared second-derivative amide 1 spectra, and is based on several assumptions; therefore, these quantitative analyses may not represent the exact picture, although quantitative structural determinations using this method can be quite reliable, if certain caveats are observed (*21*). These are addressed in the Discussion.

The Conformations in AR AF1 Induced by Binding RAP74 and by Osmolyte TMAO Appear To Be Similar in Nature. Our previous studies have shown that TMAO can induce a more compact structure in the AR AF1 domain (*8*), as assessed by fluorescence emission spectroscopy and resistance to protease digestion. Here we tested whether the presence of TMAO and binding of RAP74-CTD induce similar conformations in the AR AF1 domain. To test this, we recorded the FTIR spectrum of the AR AF1 recombinant protein in the presence of 3 M TMAO, a concentration that causes maximal AF1 folding (*8*), and compared it with the spectrum of AF1 in the absence of TMAO. As shown in Figure 4, in 3 M TMAO there is a large change in the peak at ~1656 cm^{-1}, consistent with greatly increased helical content. It is evident from the quantitative analysis of this spectrum that the proportions of secondary structural elements are similar to those observed in AF1 after it binds to RAP74-CTD (Table 1), suggesting that both the presence of TMAO and RAP74-CTD binding may induce similar secondary structures in the AF1. However, it remains to be

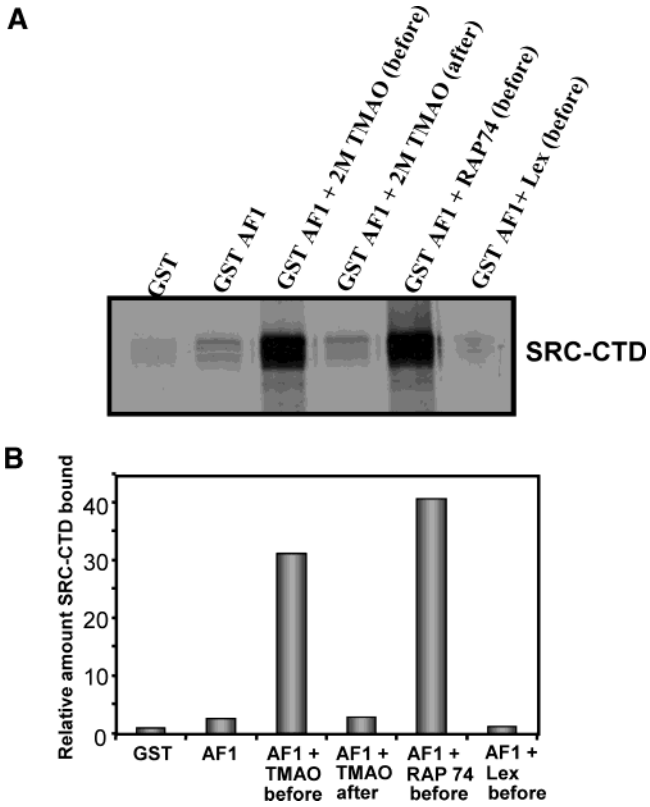

FIGURE 5: Induced folding of AF1 enhances binding of the coactivator protein SRC-1a. (A) SDS−PAGE analysis of radiolabeled SRC-CTD (amino acids 977−1441) bound to GST alone (lane 1) or the GST−AF1 fusion protein (lanes 2−6). AF1 was preincubated for 30 min with 2 M TMAO (lane 3), a 2 M excess of RAP74-CTD (lane 5), or the control protein LEX-DBD (lane 6); as an additional control, 2 M TMAO was added after the incubation of AF1 with SRC-CTD, and the binding reaction continued for 30 min (lane 4). (B) Quantitative analysis of amounts of SRC-CTD bound under different experimental conditions. The relative intensity of bound SRC-CTD was measured using a Fujifilm FLA-3000 densitometer and AIDA 2.0 software. The binding studies were repeated three times, and the data that are shown are from a representative experiment.

seen whether overall folding in the AF1 under both of these conditions is identical.

AF1−SRC-1 Interaction Is Facilitated by RAP74 Binding or the Presence of TMAO. Previously, we have shown that induced folding of AF1 of the GR enhanced protein−protein interactions with the TATA-binding protein (TBP), the CREB-binding protein (CBP), and a p160 coactivator (*9*). To test whether induced folding of AR AF1 similarly enhanced coactivator binding, we carried out a series of "pulldown" experiments with the GST−AF1 fusion protein and the radiolabeled SRC-1 C-terminal domain (amino acids 977−1441, SRC-CTD). The GST−AF1 protein exhibited an ~3-fold increase in the level of binding of SRC-CTD relative to GST alone (Figure 5, columns 1 and 2). Incubation of the GST−AF1 protein with 2 M TMAO, prior to binding of SRC-CTD, led to a dramatic increase in the level of binding (31-fold); this increase was not observed if the TMAO was added after binding was allowed to occur (Figure 5, columns 3 and 4). Strikingly, a similar enhancement (40-fold) in the level of SRC-CTD binding was observed after preincubation of the GST−AF1 protein with RAP74-CTD. This increase was not observed after preincubation with a nonreceptor binding protein, the LexA DNA-binding domain (Figure 5,

columns 5 and 6). These results strongly suggest that induced folding of the AR AF1 domain by TFIIF binding or the presence of the natural osmolyte TMAO leads to a conformation that enhances subsequent binding of the p160 coactivator protein SRC-1a.

DISCUSSION

It has been recognized in recent years that significant portions of many proteins contain large regions that are intrinsically or natively unstructured (*10*−*13*). Structural and functional characterization of these intrinsically unstructured proteins or regions, which alone do not automatically fold into their fully folded, functional structures, represents a major challenge. Examples of such protein regions include the N-terminal transactivation domain (NTD) of several steroid receptors, including the AF1 domains of the GR, ER, and AR (reviewed in ref *10*). The NTDs of steroid receptors have little primary sequence homology. It has been previously reported that the AR AF1 domain makes physical and functional interactions with coregulatory proteins that by multiple mechanisms influence the effect of the AR on transcription (*22*−*26*). Several coregulatory proteins are known to interact with the AR, including SRC-1, TIF2, CBP/p300, RIP140, SMRT, ART-27, and the TFIIF complex (*14*, *22*−*26*). The AF1 domain of the AR is known to play an important role for many of these interactions. However, it remains to be determined how these interactions influence the AR AF1 function and consequently gene regulation.

There are reports that intrinsically unfolded regions of a number of proteins adopt a folded conformation when they interact with their proper binding partner(s) (*27*−*30*). In this study, we have tested the hypothesis that the AR AF1 domain adopts a functional folded conformation when AF1 encounters its known binding partner, the RAP74 subunit of the TFIIF complex (*13*, *14*). Our results indicate that the AF1 domain has little helical structure until it encounters RAP74. This interaction leads to formation of more helical conformation in AR AF1. This appears to come as a result of the loss of β-sheet along with an accumulation of small losses of turns, bends, and coil. It is a general belief that in many transcription factors, activation domains work by an induced folding and binding mechanism, suggesting that they may be only partially structured until they encounter their proper partner proteins. In a number of cases, the intrinsically unstructured transactivation domains have adopted a folded structure upon interaction with a target protein (*27*−*30*). Induced folding events associated with target factor binding have not previously been demonstrated directly for AR AF1, though our previous proteolysis studies strongly suggested a conformational change upon binding of RAP74 to AR AF1.

Our findings herein, showing that AR AF1 adopts a more helical conformation when bound to RAP74, clearly indicate that AF1 can acquire helical structure through an induced-fit mechanism, when it encounters a specific binding partner. It remains to be seen whether this occurs with other known coregulatory proteins that bind AR AF1, but we hypothesize that this will be so. Similar changes in secondary structural elements were induced in AR AF1 both by the osmolyte TMAO and by RAP74 binding. TMAO is known to cooperatively fold intrinsically unstructured proteins (*9*, *31*−*33*), and cooperative folding of proteins is associated with "native-like" conformations (*34*, *35*). This result suggests that the induced AF1 conformation(s) may be physiologically relevant.

Although qualitatively it seems clear that significantly more helical content occurs in AF1 upon binding of RAP74, FTIR alone may not be sufficient to quantify exactly the secondary structures within a protein (*21*). Consistent with our results here, however, quantitative analysis of structural elements in uncomplexed AF1 by circular dichroism (*8*) gave similar results. Our FTIR results clearly show an increase in helical content after RAP74 binding, though the exact amount will require further studies. We note that examination of the amide range absorption spectra (not shown) showed no differentials in broad major peaks that became obscured by the second-derivative plots. Also, examination of the 1700−1800 cm^{-1} range in the second-derivative plots (not shown) revealed no sharp absorptions, suggesting that the spectra were free of water vapor (*21*).

To test the functional significance of the induced helical structure in AR AF1, we examined the interaction of AR AF1 with SRC-1. SRC-1 is known to make physical (*36*) and functional (*37*, *38*) contact with the AR AF1 domain during *in vivo* regulation of transcription. We carried out a pulldown experiment to study protein−protein interactions between SRC-1 and a fusion protein, GST−AF1. Our data clearly show that the presence of TMAO and binding of RAP74 each induce a conformation in the AR AF1 domain, which facilitates AF1's interaction with SRC-1. It is interesting to note that addition of TMAO after prior incubation of AR AF1 and SRC-1 did not result in any measurable increase in the level of SRC-1 binding, suggesting that folding of AR AF1 may be impeded by nonspecific interactions with the coactivator. Further studies will be necessary to resolve this issue. However, it should be emphasized that the main conclusion from the protein−protein interaction data is that preincubation with the binding partner TFIIF (RAP74), but not a noninteracting protein, significantly enhanced the interaction of SRC-CTD. We conclude that conditional folding of the AR AF1 domain is a prerequisite for tight binding of SRC-1 to AF1. It will be interesting to see whether this is true for other AR AF1 coregulatory proteins as well.

In sum, our data indicate that the interaction of the AR AF1 domain with the TFIIF complex is more than a simple tethering of molecules. This interaction imposes a functional conformation in the AF1 domain, which facilitates the formation of an assembly of proteins with AF1. These preliminary studies may prove to be an important step in determining the physical and functional roles of coregulatory proteins in gene regulation by the AF1 domain of the steroid receptors.

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REFERENCES

1. Eder, I. E., Culig, Z., Putz, T., Nessler-Menardi, C., Bartsch, G., and Klocker H. (2001) Molecular biology of the androgen

receptor: from molecular understanding to the clinic, *Eur. Urol. 40*, 241−251.
2. Kallio, P. J., Pakvimo, J. J., and Janne, O. A. (1996) Genetic regulation of androgen action, *Prostate Suppl. 6*, 45−51.
3. Hiipakka, R. A., and Liao, S. (1998) Molecular Mechanism of Androgen Action, *Trends Endocrinol. Metab. 9*, 317−324.
4. Callewaert, L., Verrijdt, G., Christiaens, V., Haelens, A., and Claessens, F. (2003) Dual function of an amino-terminal amphipatic helix in androgen receptor-mediated transactivation through specific and nonspecific response elements, *J. Biol. Chem. 278*, 8212−8218.
5. Lindzey, J., Kumar, M. V., Grossman, M., Young, C., and Tindall, D. J. (1994) Molecular mechanisms of androgen action, *Vitam. Horm. 49*, 383−432.
6. Berrevoets, C. A., Doesburg, P., Steketee, K., Prapman, J., and Brinkmann, A. O. (1998) Functional interactions of the AF-2 activation domain core region of the human androgen receptor with the amino-terminal domain and with the transcriptional coactivator TIF2 (transcriptional intermediary factor 2), *Mol. Endocrinol. 12*, 1172−1183.
7. Jenster, G., van der Korputm, H. A., Trapman, J., and Brinkmann, A. O. (1995) Identification of two transcription activation units in the N-terminal domain of the human androgen receptor, *J. Biol. Chem. 270*, 7341−7346.
8. Reid, J., Kelly, S. M., Watt, K., Price, N. C., and McEwan, I. J. (2002) Conformational analysis of the androgen receptor amino-terminal domain involved in transactivation. Influence of structure-stabilizing solutes and protein−protein interactions, *J. Biol. Chem. 277*, 20079−20086.
9. Kumar, R., Lee, J. C., Bolen, D. W., and Thompson, E. B. (2001) The conformation of the glucocorticoid receptor AF1/tau1 domain induced by osmolyte binds co-regulatory proteins, *J. Biol. Chem. 276*, 18146−18152.
10. Kumar, R., and Thompson, E. B. (2003) Transactivation functions of the N-terminal domains of nuclear hormone receptors: protein folding and coactivator interactions, *Mol. Endocrinol. 17*, 1−10.
11. Warnmark, A., Wikstrom, A., Wright, A. P. H., Gustafsson, J. A., and Hard, T. (2001) The N-terminal regions of estrogen receptor alpha and beta are unstructured in vitro and show different TBP binding properties, *J. Biol. Chem. 276*, 45939−45944.
12. McEwan, I. J., Dahlman-Wright, K., Ford, J., and Wright, A. P. H. (1996) Functional interaction of the c-Myc transactivation domain with the TATA binding protein: evidence for an induced fit model of transactivation domain folding, *Biochemistry 35*, 9584−9593.
13. Shen, F., Triezenberg, S. J., Hensley, P., Porter, D., and Knutson, J. R. (1996) Transcriptional activation domain of the herpesvirus protein VP16 becomes conformationally constrained upon interaction with basal transcription factors, *J. Biol. Chem. 271*, 4827−4837.
14. Reid, J., Murray, I., Watt, K., Betney, R., and McEwan, I. J. (2002) The androgen receptor interacts with multiple regions of the large subunit of general transcription factor TFIIF, *J. Biol. Chem. 277*, 41247−41253.
15. McEwan, I. J., and Gustafsson, J. A. (1997) Interaction of the human androgen receptor transactivation function with the general transcription factor TFIIF, *Proc. Natl. Acad. Sci. U.S.A. 94*, 8485−8490.
16. Dong, A., and Caughey, W. S. (1994) Infrared methods for study of hemoglobin reactions and structures, *Methods Enzymol. 232*, 139−175.
17. Dong, A., Prestrelski, S. J., and Carpenter, J. C. (1995) Infrared spectroscopic studies of lyophilization- and temperature-induced protein aggregation, *J. Pharm. Sci. 84*, 415−424.
18. Dong, A., Caughey, B., Caughey, W. S., Bhat, K. S., and Coe, J. E. (1992) Secondary structure of the pentraxin female protein in water determined by infrared spectroscopy: effects of calcium and phosphorylcholine, *Biochemistry 31*, 9364−9370.
19. Susi, H., and Byler, D. M. (1986) Resolution-enhanced Fourier transform infrared spectroscopy of enzymes, *Methods Enzymol. 130*, 290−311.
20. Kamada, K., De Angelis, J., Roeder, R. G., and Burley, S. K. (2001) Crystal structure of the C-terminal domain of the RAP74 subunit of human transcription factor IIF, *Proc. Natl. Acad. Sci. U.S.A. 98*, 3115−3120.
21. Jackson, M., and Mantsch, H. H. (1995) The use and misuse of FTIR spectroscopy in the determination of protein structure, *Crit. Rev. Biochem. Mol. Biol. 30*, 95−120.
22. Ikonen, T., Palvimo, J. J., and Janne, O. A. (1997) Interaction between the amino- and carboxyl-terminal regions of the rat androgen receptor modulates transcriptional activity and is influenced by nuclear receptor coactivators, *J. Biol. Chem. 272*, 29821−29828.
23. Bevan, C. L., Hoare, S., Claessens, F., Herry, D. M., and Parker, M. G. (1999) The AF1 and AF2 domains of the androgen receptor interact with distinct regions of SRC1, *Mol. Cell. Biol. 19*, 8383−8392.
24. Heery, D. M., Hoare, S., Hussain, S., Parker, M. G., and Sheppard, H. (2001) Core LXXLL motif sequences in CREB-binding protein, SRC1, and RIP140 define affinity and selectivity for steroid and retinoid receptors, *J. Biol. Chem. 276*, 6695−6702.
25. Markus, S. M., Taneja, S. S., Logan, S. K., Li, W., Ha, S., Hittelman, A. B., Rogatsky, I., and Garabedian, M. J. (2002) Identification and characterization of ART-27, a novel coactivator for the androgen receptor N terminus, *Mol. Biol. Cell 13*, 670−682.
26. Dotzlaw, H., Moehren, U., Mink, S., Cato, A. C. B., Iniguez-Lluhi, J. A., and Baniahmad, A. (2002) The amino terminus of the human AR is target for corepressor action and antihormone agonism, *Mol. Endocrinol. 16*, 661−673.
27. Uversky, V. N., Gillespie, J. R., and Fink, A. L. (2000) Why are "natively unfolded" proteins unstructured under physiologic conditions? *Proteins 41*, 415−427.
28. Lee, H., Mok, K. H., Muhandiram, R., Park, K. H., Suk, J. E., Kim, D. H., Chang, J., Sung, Y. C., Choi, K. Y., and Han, K. H. (2000) Local structural elements in the mostly unstructured transcriptional activation domain of human p53, *J. Biol. Chem. 275*, 29426−29432.
29. Radhakrishnan, I., Perez-Alvarado, G. C., Parker, D., Dyson, H. J., Montminy, M. R., and Wright, P. E. (1997) Solution structure of the KIX domain of CBP bound to the transactivation domain of CREB: a model for activator:coactivator interactions, *Cell 91*, 741−752.
30. Uesugi, M., Nyanguile, O., Lu, H., Levine, A. J., and Verdine, G. L. (1997) Induced alpha helix in the VP16 activation domain upon binding to a human TAF, *Science 277*, 1310−1313.
31. Baskakov, I. V., and Bolen, D. W. (1998) Forcing thermodynamically unfolded proteins to fold, *J. Biol. Chem. 273*, 4831−4834.
32. Burg, M. D. (1995) Molecular basis of osmotic regulation, *Am. J. Physiol. 268*, F983−F996.
33. Bolen, D. W. (2001) Protein stabilization by naturally occurring osmolytes, *Methods Mol. Biol. 168*, 17−36.
34. Baldwin, R. L., and Rose, D. G. (1999) Is protein folding hierarchic? II. Folding intermediates and transition states, *Trends Biochem. Sci. 24*, 26−33.
35. Bai, C., Biwersi, J., Verkman, A. S., and Matthay, M. A. (1998) A mouse model to test the *in vivo* efficacy of chemical chaperones, *J. Pharmacol. Toxicol. Methods 40*, 39−45.
36. Ueda, T., Mawji, N. R., Bruchovsky, N., and Sadar, M. D. (2002) Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells, *J. Biol. Chem. 277*, 38087−38094.
37. Shang, Y., Myers, M., and Brown, M. (2002) Formation of the androgen receptor transcription complex, *Mol. Cell 9*, 601−610.
38. Louie, M. C., Yang, H. Q., Ma, A.-H., Xu, W., Zou, J. X., Kung, H.-J., and Chen, H.-W. (2003) Androgen-induced recruitment of RNA polymerase II to a nuclear receptor-p160 coactivator complex, *Proc. Natl. Acad. Sci. U.S.A. 100*, 2226−2230.

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