Conformational Analysis of the Androgen Receptor Amino-terminal Domain Involved in Transactivation

INFLUENCE OF STRUCTURE-STABILIZING SOLUTES AND PROTEIN-PROTEIN INTERACTIONS*

Received for publication, January 30, 2002, and in revised form, March 12, 2002
Published, JBC Papers in Press, March 14, 2002, DOI 10.1074/jbc.M20103200

James Reid‡‡‡, Sharon M. Kelly¶, Kate Watt††, Nicholas C. Price¶, and Iain J. McEwan†

From the ‡Department of Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland, United Kingdom and ¶IBLS Division of Biochemistry and Molecular Biology, Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, Scotland, United Kingdom

The androgen receptor (AR) is a member of the nuclear receptor superfamily. Sequences within the large amino-terminal domain of the receptor have been shown to be important for transactivation and protein-protein interactions; however, little is known about the structure and folding of this region. In the present study we show that a 344-amino acid polypeptide representing the main determinants for transactivation has the propensity to form α-helical structure and that mutations which disrupt putative helical regions alter conformation. Folding of the AR was observed in the presence of the helix-stabilizing solvent trifluoroethanol and the natural osmolyte trimethylamine N-oxide (TMAO). TMAO resulted in the movement of two tryptophan residues to a less solvent-exposed environment and the formation of secondary/tertiary structure resistant to protease cleavage. Critically, binding to the RAP74 subunit of the general transcription factor TFIIF resulted in extensive protease resistance, consistent with induced folding of the receptor transactivation domain. These data indicate that this region of the AR is structurally flexible and folds into a stable conformation upon interactions with a component of the general transcription machinery.

The actions of the male sex hormones testosterone and dihydrotestosterone are mediated by the intracellular androgen receptor (AR)1 (reviewed in Refs. 1 and 2). The AR belongs to a large family of nuclear receptors, whose members mediate the actions of steroid and thyroid hormones, retinoic acid, vitamin D3, and fatty acid derivatives. The majority of these receptor actions of steroid and thyroid hormones, retinoic acid, vitamin D3, and fatty acid derivatives are mediated by the intracellular androgen receptor (AR).

The actions of the male sex hormones testosterone and dihydrotestosterone are mediated by the intracellular androgen receptor (AR). The AR belongs to a large family of nuclear receptors, whose members mediate the actions of steroid and thyroid hormones, retinoic acid, vitamin D3, and fatty acid derivatives. The majority of these receptor actions of steroid and thyroid hormones, retinoic acid, vitamin D3, and fatty acid derivatives are mediated by the intracellular androgen receptor (AR). This paper is available online at http://www.jbc.org

This paper is available online at http://www.jbc.org

Vol. 277, No. 22, Issue of May 31, pp. 20079–20086, 2002
Printed in U.S.A.


In the present study we have used a combination of circular dichroism (CD), fluorescence spectroscopy, and sensitivity to protease digestion to analyze the secondary and tertiary structure of the amino-terminal domain of the AR involved in transactivation. These studies reveal that the AR contains limited stable secondary structure in aqueous solution but adopts a more stable conformation in the presence of the secondary structure-stabilizing solvents TFE and TMAO. Of particular note is that binding of the AR transactivation domain to a target protein, i.e., the general transcription factor TFIIF, was found to induce a more stable conformation.

**EXPERIMENTAL PROCEDURES**

**Mutagenesis, Expression, and Purification of Recombinant Proteins**—AR4 mutant polypeptides M5, M8, and M9 were created by site-directed mutagenesis using the oligonucleotide primers described in Table I. For AR4-M5 the coding sequence of AR4 was first subcloned into Bluescript pBSK (+), and single-stranded DNA was prepared. AR4-M8 and -M9 were created using the QuikChange™ kit (Stratagene) and pET-AR4 (16) as template DNA. In the case of mutant M8, the parent DNA was first mutated with the L236P primers, and a second round of mutagenesis was then performed on the resulting mutant DNA using the L251P primers. The authenticity of all plasmids was confirmed by restriction enzyme digests and DNA sequencing of the insert. It should be noted that one additional change, a conservative serine to threonine, was found in AR4-M8 at position 255.

The mutant AR4, RAP74-CTD (amino acids 363–517; Ref. 33), and LexADBD were expressed in Escherichia coli strain BL21 (pLysS) by induction with 1 mM isopropyl-β-D-thiogalactoside for 1–2 h at 37 °C in LB media. The cells were harvested by centrifugation and lysed by a process of freeze-thawing and incubation with 0.5 mg/ml lysozyme at 4 °C. The recombinant proteins were purified from the soluble fraction using Ni2+-nitriloacetate-agarose affinity chromatography resin (Qiagen).

A PCR product encoding AR4 was digested with BamHI and BgII and subcloned into pGEX-2TK (Amersham Biosciences), previously digested with BamHI, to generate pGEX-AR4. The GST-AR4 fusion protein was expressed in E. coli strain BLR(DE3) by induction with 0.1 mM isopropyl-β-D-thiogalactoside for 4–5 h at room temperature in 2×TY media (1.6% Bactotryptone, 1.0% yeast extract, 0.5% NaCl). The cells were lysed as before, and the recombinant proteins were purified from the soluble fraction using glutathione-Sepharose 4B resin (Amersham Biosciences). The purified proteins were dialyzed against 25 mM HEPES, pH 7.9, 100 mM sodium acetate, 5% glycerol, 1 mM dithiothreitol. Protein concentration was estimated against bovine serum albumin standards using the Bradford assay (34).

**Partial Proteolysis Assay**—Purified, recombinant AR4, AR4-M5, -M8, and -M9 were diluted to a final concentration of 50 pmol/10-μl reaction in proteolysis buffer (25 mM HEPES, pH 7.9, 10% glycerol, 0.2 mM EDTA, 5 mM MgCl2, 20 mM CaCl2, 60 mM KCl) and digested with 0.25 ng/μl trypsin for 2, 4, 6, 8, and 10 min at room temperature. Reactions were stopped by addition of 2× SDS-PAGE sample buffer and heating to 75 °C for 5 min. The samples were analyzed on a 15% SDS-PAGE gel, transferred to a nitrocellulose membrane, and probed with a mouse monoclonal anti-hexahistidine antibody (Sigma). GST-AR4 (50 pmol) in proteolysis buffer was partially digested alone or in the presence of LexADBD, ARDBD, or RAP74-CTD. The digestions were performed in a reaction volume of 25 μl at room temperature for the indicated times with 0.25 ng/μl trypsin, 2 ng/μl chymotrypsin, or 8 ng/μl endoproteinase Glu-C. GST-AR4 was also digested with each enzyme in the presence of TFE and/or TMAO for the times indicated at room temperature. All reactions were stopped by the addition of 2× SDS-PAGE sample buffer and heating to 75 °C for 5 min. After separation by SDS-PAGE, the digested proteins were detected by Western blotting using an anti-GST antibody (Sigma) and visualized by ECL.

**Enzymatic Assay of Trypsin**—The artificial trypsin substrate N-benzoyl-L-arginine ethyl ester (0.23 μM) was incubated with 0.25 ng/μl trypsin in 63 mM sodium phosphate, 0.06 mM EDTA, and 0.25 mM MgCl2 for 10 min at room temperature. The reaction was stopped by the addition of 2× SDS-PAGE sample buffer and heating to 75 °C for 5 min. After separation by SDS-PAGE, the digested proteins were detected by Western blotting using an anti-GST antibody (Sigma) and visualized by ECL.
RESULTS

The Androgen Receptor Transactivation Domain Can Adopt a Stable α-Helical Conformation—Despite the importance of the amino-terminal transactivation domain in the function of the AR, no three-dimensional structure for this region is currently available. We have therefore undertaken CD analysis of the AR transactivation domain (Fig. 1A, amino acids 142–485) to gain insight into the secondary structure content of this part of the receptor. The CD spectrum in the far UV range is sensitive to the conformation of the polypeptide backbone and can be used to estimate the proportions of secondary structure elements present and changes in the conformation under specific conditions. In aqueous solution, the AR polypeptide showed relatively little stable secondary structure. The dominant feature of the CD spectrum was a minimum at around 200 nm, which is characteristic of a non-ordered conformation (Fig. 2A). In contrast, in the presence of increasing amounts of the hydrophobic solvent TFE, the CD spectrum was characterized by minima at 208 and 222 nm, indicative of a significant proportion of α-helical structure (Fig. 2A). The increase in the value of the negative ellipticity at 222 nm with increasing TFE content was consistent with increasing α-helix content. We used the SELCON procedure (36) to give an indication of the changes in secondary structure content at increasing concentrations of TFE, although it should be noted that the deconvolution analysis has only been strictly calibrated for proteins in aqueous solutions. The analysis indicates that the helical content increased from 13 to 40% with a reduction in the turn and other structure contents from 32 to 20% and from 36 to 25%, respectively, as the TFE concentration increased, the quantum yield increased, and the quantum yield for tryptophan blue shifted. The CD spectrum resulted in selective tryptophan fluorescence. All spectra were corrected for the contribution of the buffer and solute concentrations. As a control, we measured the fluorescence spectrum of 1.6 μM N-acetyl-L-tryptophanamide in the absence and presence of 3 M TMAO, the highest concentration used in these experiments. There was a modest quenching of the N-acetyl-L-tryptophanamide signal (less than 15%) in the presence of TMAO (data not shown). Thus, we conclude the effects of TMAO on AR4 fluorescence are genuine effects on protein conformation.

Circular Dichroism Spectroscopy—Purified AR4 was dialyzed against 4 mM NaH2PO4, 6 mM Na2HPO4, 100 mM sodium sulfate, and 1 mM dithiothreitol for CD analysis. The far UV CD spectra for AR4 were measured at 20 °C in the presence or absence of trifluoroethanol on a Jacob J-600 spectropolarimeter calibrated with (1S)-(+)-10-camphorsulfonic acid. For UV CD spectra (195–260 nm) were measured using a cell of 0.02-cm path length. At the concentrations of protein obtainable in the presence of added solvents and solutes, it was not possible to record near UV CD spectra (260–320 nm) with suitable precision.

FIG. 3. Steady-state fluorescence spectroscopy of the AR transactivation domain. A, steady-state fluorescence emission spectra for AR4 in the absence or presence of increasing concentrations of TMAO after excitation (Ex.) at 278 nm. As TMAO concentration increased, the quantum yield increased, and the quantum yield for tryptophan blue shifted. B, acrylamide quenching of tryptophan fluorescence. Emission spectra for AR4 in the presence of increasing concentrations of acrylamide after excitation at 295 nm.
domain has the propensity to adopt a stable conformation with significant α-helical content.

TMAO Promotes Folding of the AR Transactivation Domain—Fluorescence emission spectra for proteins result from the presence of aromatic amino acids, with tryptophan fluorescence making the dominant contribution. The AR4 polypeptide contains 13 tyrosines and 2 tryptophan residues (Trp396 and Trp432) (Fig. 1A). Excitation at 278 nm results in fluorescence emission from tryptophan and tyrosine residues; in addition, there can be energy transfer from tyrosine to tryptophan. The spectrum thus provides information about the local conformation surrounding these residues. The fluorescence spectrum for AR4 is characterized by an emission maximum at 343 nm, due to the tryptophan residues, and a shoulder at 309 nm, resulting from tyrosine emission (Fig. 3A). The folding of the AR transactivation domain was investigated using TMAO, which has been shown to facilitate the folding of proteins into “native” conformations (see Ref. 37). In the presence of up to 3 M TMAO there was an increase in the quantum yield, the tryptophan emission maximum blue shifted to 336 nm, and the shoulder due to tyrosine fluorescence was lost (Fig. 3A). A similar trend was observed with 10 and 20% TFE (data not shown). These results indicate that the two tryptophan residues become less solvent-exposed, and there is an increase in the energy transfer from tyrosine to tryptophan residues, consistent with the AR polypeptide becoming more structured.

Acrylamide is known to act as a dynamic quencher of tryptophan fluorescence and can therefore be used to investigate the accessibility of tryptophan residues. Tryptophan residues on the surface will be more readily quenched than those buried within the protein structure. Fig. 3B shows that in the presence of increasing amounts of acrylamide the fluorescence intensity for the tryptophan maximum after excitation at 295 nm was reduced. Using the Stern-Volmer equation (“Experimental Procedures”), a plot of $F/F_0$ against acrylamide concentration was obtained, where $F$ and $F_0$ are the fluorescence intensities in the absence and presence of the quenching agent, respectively. From the linear plot, a Stern-Volmer constant ($K_{SV}$) of 10.8 M$^{-1}$ was calculated, indicating that both tryptophans have a high degree of exposure to solvent and behave in an identical fashion (35). In the presence of 3 M TMAO, a $K_{SV}$ of 9.7 M$^{-1}$ was observed (data not shown). This is consistent with the folding of the AR polypeptide, leading to the tryptophan residues being less susceptible to the quenching agent. Taken together the spectroscopy analysis indicates that the AR transactivation domain is structurally flexible and capable of a adopting a more folded conformation in the presence of the alcohol TFE or the natural osmolyte TMAO.

The AR Transactivation Domain Does Contain Local Regions of Secondary Structure—The AR transactivation domain is predicted to contain four α-helical regions, giving an α-helical content of about 20% (Fig. 4), comparable with the values calculated from CD analysis. Therefore, to investigate the possible existence of local structural elements, the fluorescence spectrum for AR4 after denaturation was measured. In the presence of 6 M urea the tryptophan emission maximum red-shifted to 350 nm, indicating the tryptophan residues have become even more fully exposed to solvent (Fig. 5). In addition, a clear peak of tyrosine fluorescence at 309 nm was observed. Data represent the average of three independent experiments.

Fig. 4. Secondary structure predictions for AR4 wild-type or mutant polypeptides (M5, M8, and M9) were obtained from Network Protein Sequence Analysis (phil.ibcp.fr/NPSA). Secondary structure elements include β-strand (short bars) and α-helix (tall bars). Mutation M5 was originally described by Chamberlain et al. (7) for the corresponding residues in the rat AR as disrupting transactivation activity of the full-length receptor. Helical regions 1–3, targeted by mutagenesis, are highlighted.

Fig. 5. The fluorescence emission spectrum for AR4 in the absence or presence of 6 M urea after excitation (Ex.) at 278 nm. A λmax for tryptophan was observed at 342 nm in the absence of urea. In the presence of urea, the λmax for tryptophan increased to 350 nm, and a clear peak of tyrosine fluorescence at 309 nm was observed. Data represent the average of three independent experiments.
To investigate further the local conformation of the AR transactivation domain, we used limited proteolytic digestion and site-directed mutagenesis. The first three helical regions were targeted with double point mutations, which were predicted to disrupt the local helical conformation (Fig. 4). AR4 was digested with trypsin for different times, and fragments retaining the amino-terminal histidine tag were specifically detected using an anti-His antibody. Trypsin digestion of AR4 led to four distinct amino-terminal fragments (Fig. 6, bands 2–5). Strikingly, point mutations M5 and M8, predicted to disrupt helical regions 1 or 2, respectively, led to a loss or reduction in bands 4 and 5 (Fig. 6). Furthermore, fluorescence spectroscopy of all three mutant polypeptides (M5, M8, and M9) revealed an increase in the signal due to tyrosine emission, consistent with the loss of local structure and an increase in the distance between tyrosine and tryptophan residues (data not shown). Taken together, these studies provide strong support for the existence of local structural elements within the AR4 polypeptide, which can be disrupted by helix-breaking mutations.

Protein-Protein Interactions Induce Folding of the AR Transactivation Domain—The function of the AR is critically dependent upon protein-protein interactions (see the Introduction). Among such interactions, we have previously demonstrated binding of the AR4 polypeptide to the general transcription factor TFIIF, which was sufficient to relieve squelching by the receptor transactivation domain (16). To investigate the role of protein-protein interactions on AR transactivation domain folding, a polypeptide containing the carboxyl-terminal 155 amino acids of RAP 74 (RAP74-CTD), which contains the receptor binding site, was used. Because RAP74-CTD has a carboxyl-terminal histidine tag, a GST-AR4 fusion protein was used in these studies to permit specific detection of AR polypeptides using an anti-GST monoclonal antibody. The GST-AR4 fusion protein was extremely sensitive to proteolytic cleavage, and treatment with trypsin, chymotrypsin, or endoproteinase Glu-C resulted in the generation of a series of stable fragments of relative molecular mass 24,000–30,000 Da, representing the GST moiety (Figs. 7 and 8). In the presence of RAP74-CTD (amino acids 363–517), the AR polypeptide was markedly less susceptible to cleavage by trypsin (Fig. 7A) and chymotrypsin (Fig. 7B). In addition to the full-length protein, fragments of 55,000 Da (band 2) and 34,000–37,000 Da (bands 3–4) or 59,000 Da (band 2) were protected from cleavage with trypsin or chymotrypsin, respectively. Total protein concentration was kept constant in all samples using the LexA DNA or AR DNA as nonspecific proteins of comparable molecular mass to RAP74-CTD. RAP74-CTD was less efficient at protecting the AR polypeptide from endoproteinase Glu-C cleavage, although limited protection of the full-length and a 57,000-Da fragment was seen (Fig. 8C, bands 1 and 2).

Resistance to protease digestion upon binding of RAP74-CTD could result from the masking of cleavage sites, induced folding of the AR transactivation domain, or a combination of the two. Because RAP74-CTD binding resulted in extensive protection of the AR polypeptide from digestion, it seemed likely that a conformational change was playing some role. To investigate the effects of protein folding on protease sensitivity, the effects of the hydrophobic solvent TFE and the natural osmolyte TMAO were examined. In the presence of increasing concentrations of TMAO or TFE, AR4 became less susceptible to trypsin cleavage (Fig. 7B and C). TFE, and to lesser degree TMAO, also resulted in the generation of 34,000–37,000-Da fragments (bands 3 and 4) and 55,000 Da (band 2) fragments, as seen with RAP74-CTD binding. Cleavage of the synthetic substrate, N-benzoyl-L-arginine ethyl ester, was monitored at 253 nm to ensure that TFE and TMAO did not inhibit trypsin enzyme activity. In fact, in the presence of 10% TFE or 3 M TMAO there was a modest increase in trypsin activity of 10–15% after a 20-min incubation (data not shown). In the presence of TMAO the AR transactivation domain was dramatically less susceptible to chymotrypsin and endoproteinase Glu-C digestion (Fig. 8B and D). The pattern of chymotrypsin protection seen in the presence of TMAO was very similar to that seen upon RAP74-CTD binding (compare Fig. 8A with 8B and D). In contrast, the pattern of protection with endoproteinase Glu-C digestion was more complex, with TMAO having a much more dramatic impact upon protease sensitivity (Fig. 8C and D). We interpret these results as providing evidence for at least in part a conformational change in the AR4 polypeptide upon binding of RAP74-CTD, which leads to a protease resistant state. The folding of the AR4 appears similar to structures induced and/or stabilized by the solutes TFE and TMAO, as revealed by trypsin and

*J. Reid, R. Betney, I. Murray, K. Walt, and I. J. McEwan, manuscript in preparation.*
chymotrypsin cleavage, but is clearly not identical, resulting in differences in sensitivity to the endoproteinase Glu-C cleavage.

DISCUSSION

The major determinants for androgen receptor-dependent gene activation have been mapped to the amino-terminal domain of the receptor. Studies involving deletion constructs (3–5, 8), fusion proteins (6), and point mutations (7) have highlighted the importance of amino acids 142–485 and suggested a modular nature for the transactivation domain. In the present study we show that this region of the AR-NTD undergoes a conformational change during interactions with the RAP74 subunit of TFIIH and in the presence of solvents that stabilize secondary structure. The isolated AR transactivation domain in aqueous buffer at neutral pH generally lacked stable structure but had the propensity to adopt an α-helical conformation in water-TFE solutions. Furthermore, fluorescence spectroscopy and limited proteolysis provided evidence for local secondary and/or tertiary structure elements. There are potentially up to 17 cleavage sites within AR4 for trypsin, yet digestion with this enzyme resulted in a series of discrete fragments, suggesting limited resistance to cleavage. The simplest explanation is that some sites are less accessible to enzyme cleavage as a result of protein structure (Fig. 6C). Introduction of point mutations with the intention of disrupting regions of predicted α-helix altered the pattern of fragments generated, consistent with the loss of local structure. Significantly, the AR transactivation domain was extensively protected from protease digestion in the presence of the RAP74 subunit of the AR-binding protein TFIIH or the solutes TFE and TMAO, compounds known to stabilize the α-helical structure (38, 39) or to enable polypeptides to fold into native conformations (Ref. 37 and references therein), respectively. Fig. 9 shows a summary of the amino-terminal fragments generated by trypsin, chymotrypsin, and endoproteinase Glu-C digestion. The extensive protection of the full-length AR polypeptide together with amino-terminal fragments of 55,000–59,000 Da from cleavage with different proteases suggested a conformational change upon RAP74-CTD binding. This is further supported by the fact that in the presence of the structure-stabilizing solutes TMAO and TFE, very similar patterns of protection are seen. This was most obvious with trypsin and chymotrypsin cleavage. However, even with endoproteinase Glu-C, where TMAO resulted in a more complex pattern of protection, the full-length AR transactivation domain was less susceptible to cleavage in the presence of RAP74-CTD. Taken together, the partial proteolysis results suggest that (i) TFE- and TMAO-induced folding of the AR transactivation domain leads to an overall more protease-resistant conformation and (ii) RAP74 binding in part leads to a conformation that shares some similarity with that induced by the two solutes but is not necessarily identical. The formation of secondary/tertiary structure, as judged by resistance to proteases and the movement of tryptophan residues to a less solvent-exposed environment, in the presence of TMAO is likely to reflect the folding of the AR transactivation function into a “native” state, in which it possesses stable three-dimensional structure.

The AR transactivation domain shares a number of structural properties with both the GR and progesterone receptors (PR), two other members of the same subfamily of steroid receptors, as well as the estrogen receptors α and β. The GR-AF-1/r1 transactivation domain (amino acids 77–262) shows little evidence for stable structure in aqueous solution (29, 30, 40) but undergoes a coil to helix transition in the presence of TFE or TMAO (30). Significantly, the ability to form an α-helical conformation correlated with the ability of a 41-amino acid polypeptide, constituting the r1 core (amino acids 187–244), to activate a reporter gene (32). More recently, Thompson and co-workers (40) report that in the presence of TMAO, GR-AF-1/r1 adopted a more compact conformation that was resistant to protease cleavage. Our findings with the AR transactivation domain are in good agreement with the above studies. However, we find evidence for local structural elements (Figs. 4–6), which were lost by the introduction of helix-breaking mutations (Figs. 4 and 6). In contrast to the GR-AF-1/r1, the full-length NTD of the GR was found to contain some structure when contiguous with the DBD (41). Furthermore, there was a clear increase in secondary structure content upon binding to a specific glucocorticoid response element (41). The NTD of the progesterone-A form was also found to be structured when linked with the DBD (42). However, although the DBD stabilized the structure of the NTD, there was no significant alter-
Significantly, the CD spectrum for the estrogen receptor was altered in the presence of the binding partner TATA-binding protein, consistent with a conformational change in the receptor polypeptide. Thus, the picture that is emerging is that, although the NTD of the steroid receptor shares little if any amino acid sequence identity, it does share a number of structural properties. These include (i) a general lack of stable structure in aqueous solution and (ii) the ability to undergo coil to helix transitions or to become more structurally ordered in the presence of hydrophobic solvents. Recent NMR spectroscopy analysis of the 73-amino acid transactivation domain of the tumor suppressor p53 revealed the presence of an α-helix and two β-turns in solution. The transactivation domain of the transcription factor ATF-2 consists of a structured subdomain containing two β-strands and an α-helix and a non-ordered domain.

Phosphorylation of the Androgen Receptor Transactivation Domain

Fig. 8. Limited proteolysis of GST-AR4 with chymotrypsin and endoproteinase Glu-C. A, chymotrypsin cleavage of GST-AR4 in the absence or presence of a 5 mM excess of the AR-binding protein TFIIF (RAP74-CTD) for 2, 5, 10, or 20 min. Proteolytic fragments were resolved by SDS-PAGE, and amino-terminal fragments were identified using an anti-GST antibody. The arrows indicate the full-length protein (band 1) and major fragments at 59,000 Da (band 2) and 33,000 Da (band 3) and a stable fragment at 26,000 Da (band 4). B, chymotrypsin cleavage of GST-AR4 in the presence of 3 mM TMAO. C, endoproteinase Glu-C cleavage of GST-AR4 in the absence or presence of a 5 mM excess of the AR-binding protein TFIIF (RAP74-CTD) for 10, 30, 60, 90, or 120 min. Proteolytic fragments were resolved by SDS-PAGE, and amino-terminal fragments were identified using an anti-GST antibody. The arrows indicate the full-length protein (band 1) and major fragments at 57,000 Da (band 2), 54,000 Da (band 3), 49,000 and 47,000 Da (bands 4 and 5) and a stable fragment at 32,000 Da (band 6). D, endoproteinase Glu-C cleavage of GST-AR4 in the presence of 3 mM TMAO.

Fig. 9. Schematic representation of the AR4 polypeptide showing the position of predicted secondary structure elements (cylinders, α-helix; arrows, β-strand) and trypsin cleavage sites (i.e. arginine and lysine residues (white lines)). Based on the size of the fragments observed, cleavage of GST-AR4 by trypsin gave rise to three major amino-terminal fragments, Arg355, Arg358/Lys360 (band 2), Arg371/373, Arg382 (band 3), and Lys374 (band 4). Chymotrypsin cleavage gave rise to two major amino-terminal fragments, Tyr263/265 (band 2) and Phe168 (band 3). Endoproteinase Glu-C gave rise to four major amino-terminal fragments, Asp260 (band 2), Glu272 (band 3), Glu285/Asp292 (band 4), Glu296 (band 5), and Asp327/330 (band 6).

Initial studies of the transactivation domain structure emphasized the non-ordered nature of these polypeptides and the ability to undergo coil to helix transitions or to become more structurally ordered in the presence of hydrophobic solvents (29, 30, 44–49) or target proteins (48, 50–52). However, although it remains accepted that transactivation domains lack stable tertiary structures in the absence of protein-protein interactions, there is growing evidence that elements of secondary structure may exist. Recent NMR spectroscopy analysis of the 73-amino acid transactivation domain of the tumor suppressor p53 revealed the presence of an α-helix and two β-turns in solution. The transactivation domain of the transcription factor ATF-2 consists of a structured subdomain containing two β-strands and an α-helix and a non-ordered domain. Similarly, the pKID domain of the cAMP-binding protein (CREB) consists of a stable α-helix and a region that undergoes a coil to helix transition upon interactions with the KIX domain of the CREB-binding protein (49, 51). In contrast, the transactivation domain of c-Myb adopts a helical conformation even in the absence of the binding to KIX (55). Therefore, depending on
the transcription factor and possibly the target factor, protein-protein interactions may lead to the induction and/or stabilization of α-helical conformation. Thermodynamically, interactions involving folding are enthalpy-driven, relying on a high degree of specific interactions (hydrogen bonding, electrostatic) between the activator and the target protein for high affinity binding (see Refs. 55 and 56). It is interesting, therefore, that TMAO-induced folding of the GR-AF1/2 domain enhanced the interaction of this domain with the target proteins TATA-binding protein, cAMP-binding protein (CREB)-binding protein, and SRC-1 (40). It will be important to test if these factors alone are capable of inducing folding of the GR transactivation domain. The present study we show that binding of the Rap74 subunit of TFIIF results in folding of the AR transactivation domain. It will be interesting to test if this is unique to TFIIF and whether such a conformational change alters the interaction of the AR with other potential binding partners. Taken together these data suggest a model for a possible hierarchy of protein-protein interactions, with high affinity binding inducing one or more surfaces that permit subsequent interactions.

The results of the present study demonstrate that the AR transactivation domain exists in a partially unfolded conformation with regions of secondary structure. Upon binding the Rap74 subunit of the general transcription factor TFIIF, the AR transactivation domain adopts a more folded conformation, possibly involving significant α-helix formation. We propose that the folded AR-NTD could then serve as a platform for further protein-protein interactions, leading to the assembly of a transcriptionally competent complex, including interactions with other basal transcription factors and bridging factors. The composition of such a complex could in turn be regulated by the architecture of the DNA response elements. It remains a task of future work to map the conformational changes in more detail by analysis of the fragments produced by proteolysis.

Acknowledgments—We are grateful to the following for the gift of plasmids: Drs. A. O. Brinkmann (Erasmus University, Rotterdam) and Z. Burton (Michigan State University, East Lansing), and to I. Hunter (University of Aberdeen) for critical reading of the manuscript.

REFERENCES

1. Cata, A. C. B., and Peterziel, H. (1998) Trends Endocrinol. Metab. 9, 150–154
2. Hiippakka, R. A., and Liao, S. (1998) Trends Endocrinol. Metab. 9, 317–324
3. Simental, J. A., Sar, M., Lane, M. V., French, P. S., and Wilson, E. M. (1991) J. Biol. Chem. 266, 510–518
4. Jenster, G., van der Korput, H. A., van Vroonhoven, C., van der Kwast, T. H., Trappmann, J., and Brinkmann, A. O. (1991) Mol. Endocrinol. 5, 1396–1404
5. Jenster, G., de Ruiter, P. E., van der Korput, H. A., Kuiper, G. G., Trappman, J., and Brinkmann, A. C. O. (1994) Biochemistry 33, 14064–14072
6. Jenster, G., van der Korput, H. A., Trappman, J., and Brinkmann, A. O. (1995) J. Biol. Chem. 270, 7341–7346
7. Chamberlain, N. L., Whitacre, D. C., and Miesfeld, R. L. (1996) J. Biol. Chem. 271, 26772–26778
8. Gas, T., Marcelli, M., and McPhaul, M. J. (1996) J. Steroid Biochem. Mol. Biol. 59, 9–20
9. Langley, E., Zhou, Z. X., and Wilson, E. M. (1995) J. Biol. Chem. 270, 29983–29990
10. Deoos, P., Kull, C. H., Berrevoets, C. A., Stekate, K., Faber, P. W., Mulder, E., Brinkmann, A. O., and Trappman, J. (1997) Biochemistry 36, 1052–1064
11. Ikonen, T., Palvimo, J. J., and Janne, O. A. (1997) J. Biol. Chem. 272, 28921–28928
12. Langley, E., Kemppainen, J. A., and Wilson, E. M. (1998) J. Biol. Chem. 273, 92–101
Conformational Analysis of the Androgen Receptor Amino-terminal Domain Involved in Transactivation: INFLUENCE OF STRUCTURE-STABILIZING SOLUTES AND PROTEIN-PROTEIN INTERACTIONS
James Reid, Sharon M. Kelly, Kate Watt, Nicholas C. Price and Iain J. McEwan

J. Biol. Chem. 2002, 277:20079-20086.
doi: 10.1074/jbc.M201003200 originally published online March 14, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M201003200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 56 references, 33 of which can be accessed free at http://www.jbc.org/content/277/22/20079.full.html#ref-list-1