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Unspecific DNA binding of the DNA binding domain of the glucocorticoid receptor studied with flow linear dichroism

Per Hagmar, Karin Dahlman+, Masayuki Takahashi*, Jan Carlstedt-Duke+, Jan-Åke Gustafsson+ and Bengt Nordén

Department of Physical Chemistry, Chalmers University of Technology, S-412 96 Gothenburg, +Department of Medical Nutrition and Center for Biotechnology, Karolinska Institute, Huddinge University Hospital, F82, S-141 86 Huddinge, Sweden and **Groupe de Cancérogenèse et de Mutagenèse Moléculaire et Structurale, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue René Descartes, F-670 84 Strasbourg cedex, France*

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The unspecific interaction between the DNA-binding domain of the human glucocorticoid receptor and DNA was studied using linear dichroism (LD) and circular dichroism (CD) spectroscopy. The amplitude of the LD signal was found to increase upon addition of protein at ionic strengths less than 60 nM Na^+, indicating an increased persistence length of the complex compared to uncomplexed DNA. Analysis of the LD spectrum suggests that the binding does not involve intercalation of tyrosine residues. Evidence of saturation is found at a binding stoichiometry of approximately 5 DNA base pairs per protein monomer.

DNA-protein interaction; Glucocorticoid receptor; Linear dichroism

1. INTRODUCTION

The glucocorticoid receptor belongs to a group of ligand-dependent transcriptional factors. Binding of ligand results in a conformational change in the receptor protein exposing its DNA-binding site. Cloning of the cDNA encoding the glucocorticoid receptor has facilitated structural and functional analysis of the molecule. It has been shown that the protein consists of several domains which appear to have the ability to fold and function independently (for a review see [1]). To initiate a detailed structural analysis of this molecule and in particular of its DNA-binding site, we have expressed the cDNA encoding the DNA-binding domain of the human glucocorticoid receptor in *E. coli*. We have purified the recombinant protein to homogeneity and shown that it has the same DNA-binding characteristics as the glucocorticoid receptor purified from rat liver [2].

In this work we examine the unspecific DNA binding of the DNA-binding domain of the human glucocorticoid receptor, DBD_r, by polarized-light spectroscopy, i.e., linear dichroism (LD) and circular dichroism (CD). While CD is sensitive to the local (secondary) structure of DNA and protein, LD may also detect changes in the global structure or hydrodynamical behavior of DNA (for a recent review cf. [3]).

2. MATERIALS AND METHODS

The DNA-binding domain of the human glucocorticoid receptor was expressed as a fusion protein with protein A in *E. coli*. The fusion protein was purified on IgG-Sepharose and the recombinant DNA-binding domain (DBD_r) was released from protein A using chymotrypsin as previously described [2]. The purity of the protein preparation was estimated to be at least 95% from NH_2-terminal sequence analysis and Coomassie staining of polyacrylamide gels.

Quantitative amino acid composition and spectral analysis were performed in order to determine an extinction coefficient for DBD_r. This gave a value of 5.6×10^3 $cm^{-1} \cdot M^{-1}$ at 280 nm

Correspondence address: P. Hagmar, Department of Physical Chemistry, Chalmers University of Technology, S-412 96 Gothenburg, Sweden

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which was used to determine protein concentrations. This value is in close agreement with $\epsilon_{280} = 5.8 \times 10^3$ cm$^{-1}\cdot$M^{-1} calculated from the predicted absorptive contributions of Trp and Tyr (zero and four, respectively, in DBD_r). The protein preparations were dialyzed against 20 mM phosphate buffer, pH 7.4, 50 mM NaCl, 1 mM DTT and stored at 4°C. Highly polymerized calf thymus DNA (Sigma, Type I, lot no. 66F-9645) was freshly dissolved in 20 mM phosphate buffer, pH 7.4, 50 mM NaCl and used without further purification. DNA concentrations were determined spectroscopically using ϵ_{260} = 13200 cm$^{-1}\cdot$M(bp)$^{-1}$.

Linear dichroism, $LD = A_\parallel - A_\perp$, and circular dichroism, $CD = A_l - A_r$, were measured on a Jasco J-500 spectropolarimeter as previously described [4,5]. The samples were oriented in a Couette flow cell, with an optical path length of 0.10 cm [6,7], using either a rotating inner or a rotating outer cylinder. The shear gradient was normally 900 s^{-1}, and the LD signals were found to be stable for at least 30 min. The reduced linear dichroism, $LD^r = LD/A_{iso}$, was calculated where A_{iso}, the isotropic absorbance, was measured on a Cary 2300 spectrophotometer. LD^r can be expressed as the product between an orientation factor, S, and an optical factor:

$$LD^r = S(3/2)(3\langle\cos^2\alpha\rangle - 1) \quad (1)$$

where α is the angle between the light absorbing transition moment and the hydrodynamical orientation axis (the helix axis of uncomplexed DNA). $\langle\ \rangle$ indicates an ensemble average. The DNA-protein complex was obtained by mixing of DNA and protein solutions. All measurements were performed at room temperature.

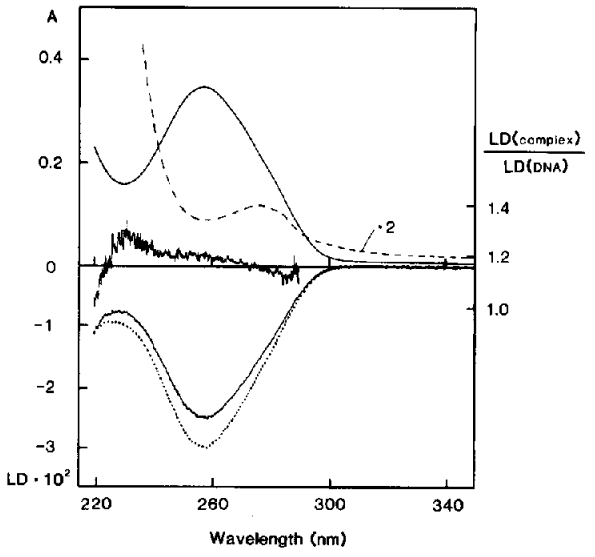

Fig.1. Normal absorption (A) and flow linear dichroism (LD) spectra of DNA (——), DBD_r (– – –) and DNA-DBD_r complex (●●●). The ratio LD(complex)/LD(DNA) is also shown in the center. DNA concentration was 25 μM bp in 50 mM NaCl and 20 mM phosphate, pH 7.4. DBD_r concentration 6.1 μM in 1 mM DTT, 50 mM NaCl and 20 mM phosphate, pH 7.4. Flow gradients 900 s^{-1}. All spectra are normalized to 1 cm optical path length.

3. RESULTS AND DISCUSSION

Fig.1 shows the normal UV absorption spectrum of calf thymus DNA and the DNA-binding domain of the glucocorticoid receptor (DBD_r) together with the LD of DNA and DNA-protein samples in 50 mM NaCl, 20 mM phosphate buffer, pH 7.4. The addition of DBD_r to DNA, at monovalent salt concentrations less than 60 mM, leads to a significant increase in the amplitude of the negative LD signal of DNA (figs 1 and 2), an effect which we interpret to be due to a stiffening of the DNA structure upon protein binding as will be discussed. No change in total absorption is observed when mixing the DBD_r and DNA solutions. The increase in LD is virtually proportional to the amount of protein added, up to 0.12 DBD_r/bp DNA (3 μM DBD_r, 25 μM DNA bp), where a plateau indicates saturation of the binding isotherm. (Upon additions exceeding 0.45 DBD_r/bp a further increase in the LD amplitude could frequently be observed.) The increase of the LD signal at the point of saturation is 30–50% compared to the LD signal of free DNA. (The value varied between different DBD_r preparations.) The protein itself did not exhibit any measurable flow LD, as expected for a small globular protein which cannot be hydrodynamically aligned by a mild shear gradient. This observation also excludes the possibility that the increase in LD amplitude, or the variations between different preparations, could originate from DNA contamination of the protein sample. Furthermore, the change in LD signal was found to require structured DBD_r: addition of urea-denatured DBD_r (up to 0.3 DBD_r/bp DNA) did not significantly alter the LD signal of DNA (fig.2).

The ratio between the LD spectrum of the DNA-protein complex and the pure DNA (also depicted in fig.1) decreases slightly in the region (274–285 nm) where the tyrosine residues contribute to the absorption, compared to the value at the DNA absorption maximum (260 nm). The protein light absorption at 274 nm is mainly caused by the four tyrosine residues of DBD_r (DBD_r contains no tryptophans). At 0.24 DBD_r/bp the protein is responsible for approximately 16% of the light absorption at 280 nm. If the protein were bound to

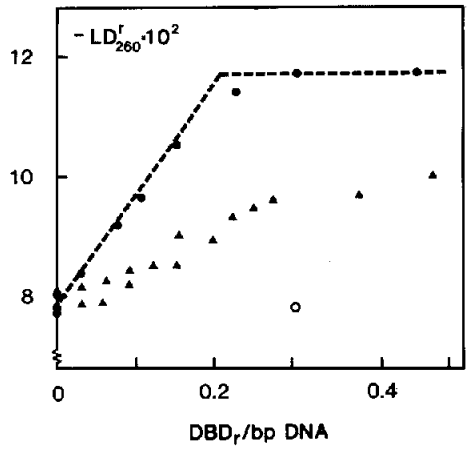

Fig.2. Reduced linear dichroism, $LD^r = LD/A$, at 260 nm, as a function of protein concentration. Filled circles and triangles denote two preparations representing typical extreme variations in LD behaviour. The isolated open circle shows the absence of LD change when adding denatured DBD_r. DNA concentration was 25 μM bp in 50 mM NaCl, 20 mM phosphate, pH 7.4. Flow gradient 900 s^{-1}.

DNA by intercalation of the phenyl groups belonging to the four tyrosines, the tyrosine LD would have the same sign as the DNA LD, i.e. negative, and the ratio between the LD of the DNA-protein complex and of free DNA would be expected to increase in the tyrosine absorption region. In fact, an increase of the ratio would be obtained even if only one of the tyrosines were intercalated, provided that the aromatic planes of the remaining tyrosines were randomly distributed with respect to the hydrodynamical orientation axis, i.e. the DNA fiber axis. This indicates that the planes of the tyrosine phenyl groups are oriented more parallel than perpendicular to the DNA axis. The presence of a tyrosine residue in each of the two proposed 'zinc-fingers' in DBD_r [8,9] suggests the possibility of a DNA-protein binding favored by (conceivably sequence specific) intercalation of aromatic amino acid residues. Our results, however, thus indicate that the unspecific binding of the DNA-binding domain of the glucocorticoid receptor does not involve intercalation of the aromatic amino acids. The ratio between the LD spectra also shows a maximum at 230 nm, a feature which is more difficult to interpret since this absorption region corresponds to transitions in both DNA and the protein which are not limited to in-plane excitation of aromatic groups.

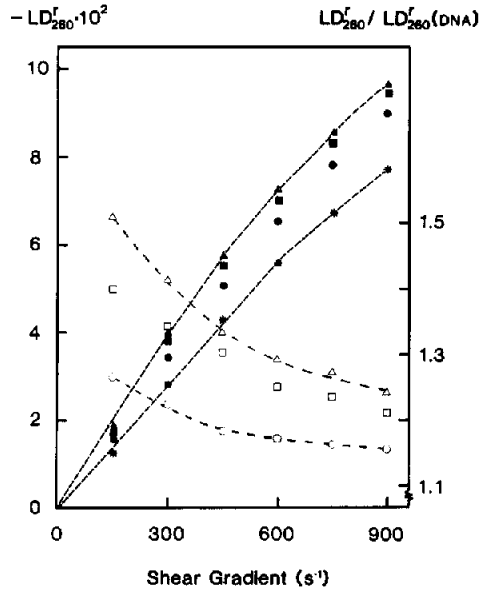

Fig.3. LD^r_{250} (filled symbols) and LD^r_{260}(complex)/LD^r_{260}(DNA) (open symbols) as a function of flow gradient. DBD_r concentrations were 0 (stars), 3.8 (circles), 6.1 (squares) and 9.1 μM (triangles). DNA concentration was 25 μM bp in 50 mM NaCl, 20 mM phosphate, pH 7.4.

The observed increase in LD amplitude can be due either to a larger value of the optical factor or an increase in the orientation factor S (eqn 1). The gradient dependence of the LD signal of the DNA-protein complex, and the ratio between the LD of the complex and the LD of DNA (fig.3), suggest that S is increased by stiffening of the DNA structure. As is seen in fig.3 the relative increase in LD is largest at small gradients. Since it is unlikely that the local structure of DNA (base tilt) is altered by the mild shear force (≤ 900 s^{-1}) the increase indicates a reduced flexibility of DNA upon protein binding, resulting in a stiffer and more easily oriented complex. The observed increase of the LD signal furthermore excludes the possibility of any significant formation of DNA loops or a larger bending of DNA, such as those observed upon specific binding of other transcription regulators [10] (and which also has been discussed in relation to the molecular mechanism of the present protein [11,12]). Since our results are limited to unspecific binding only, the absence of kinks might not be significant to the understanding of the specific DNA-protein interaction. However, in the case of

the cAMP receptor protein from *E. coli* (CRP or CAP), another transcription regulator, the LD signal was decreased upon both specific and unspecific binding ([13] and Takahashi, Kubista and Nordén, unpublished) which may indicate that the glucocorticoid receptor exerts its transcription regulation in a different manner. Future LD measurements on the specific DNA-DBD_r complex should provide further information.

CD spectra of DNA and DBD_r (results not shown) indicate no significant structural alterations upon complex formation. The resulting CD is virtually a pure linear combination of the CD of the two uncomplexed species indicating that no marked changes in secondary structure of either of the components are involved in the binding.

Assuming a two state model, and noting that the LD change at low protein concentrations is almost proportional to the concentration of complex, the site size n (number of base pairs of DNA covered by the binding of one protein molecule) can be determined from the LD saturation level. The equilibrium constant can in principle be determined in the same manner. Such determinations, however, are rather uncertain because of a sensitivity to measuring errors and variations between different sample preparations. We estimated the site size to be $n \approx 4.5$–6 bp/protein. This result agrees fairly well with the value obtained from studies of specific DNA-binding of the glucocorticoid receptor using footprinting and methylation interference assays, which suggest that 15 bp of DNA are covered by each protein dimer, $n \approx 7.5$ [14].

Preliminary results show that the change in LD amplitude depends on the ionic strength. The increase in LD amplitude compared to the LD signal of DNA is reduced with increasing salt concentration. For example, in 60–70 mM NaCl and 20 mM phosphate (pH 7.4) there is no measurable change of LD upon addition of DBD_r, and at even higher ionic strength the amplitude of the LD signal is decreased instead (results not shown). However, protein and gradient titrations at higher salt concentrations (150 mM NaCl, 20 mM phosphate, pH 7.4), as well as at lower salt concentrations (10 mM NaCl, 20 mM phosphate, pH 7.4) show features that are similar to those reported in fig.2 (50 mM NaCl, 20 mM phosphate, pH 7.4). In a corresponding gradient dependence study at 150 mM NaCl, 20 mM phosphate, pH 7.4 (results not shown), the relative change in LD signal is still largest at low flow gradient but, as mentioned, the change is a decrease in LD amplitude. This observation also indicates that the DNA-DBD_r complex is more rigid and easier to orient than uncomplexed DNA but also suggests that the complex has a smaller optical factor. This may be consistent with a slight bend of DNA around the protein; the bending is probably not pronounced (kink) since the alignment of the complex would then have been reduced.

In conclusion, our results provide the following information about the unspecific binding of DBD_r to DNA. The interaction leads to a detectable change of the DNA LD owing to an increased persistence length of DNA at low ionic strengths. The protein associates to DNA mainly by electrostatic interaction, without involving intercalation of the aromatic amino acid residues and without any marked change of either protein or DNA conformation. At higher ionic strengths the LD behavior may be consistent with a slight coil of the DNA around the protein. A characteristic site size was estimated to about one protein per 5 bp of DNA.

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REFERENCES

[1] Gustafsson, J.-Å., Carlstedt-Duke, J., Poellinger, L., Okret, S., Wikström, A.-C., Brönnegård, M., Gillner, M., Dong, Y., Fuxe, K., Cintra, A., Härfstrand, A. and Agnati, L. (1988) Endocr. Rev. 8, 185–234.

[2] Dahlman, K., Strömstedt, P.-E., Rae, C., Jörnvall, H., Flock, J.-I., Carlstedt-Duke, J. and Gustafsson, J.-Å. (1989) J. Biol. Chem. 264, 804–809.

[3] Nordén, B. and Kubista, M. (1988) NATO ASI series C 242, 133–165.

[4] Davidsson, Å. and Nordén, B. (1976) Chem. Scr. 9, 49–53.

[5] Nordén, B. and Seth, S. (1985) Appl. Spectrosc. 39, 647–655.

[6] Wada, A. and Kozawa, S. (1964) J. Polym. Sci. 2, 853–864.

[7] Nordén, B. and Tjerneld, F. (1976) Biophys. Chem. 4, 191–198.

[8] Klug, A. and Rhodes, D. (1987) Trends Biochem. Sci. 12, 464–469.
[9] Hollenberg, S., Weinberger, C., Ong, E., Cerelli, G., Oro, A., Lebo, R., Thompson, B., Rosenfeld, M. and Evans, R. (1985) Nature 318, 635–641.
[10] Wu, H.-M. and Crothers, D.M. (1984) Nature 308, 509–513.
[11] Ptashne, M. (1986) Nature 322, 697–701.
[12] Buc, H., Amouyal, M., Buckle, M., Herbert, M., Kolb, A., Kotlartz, D., Menendez, M., Rimsky, S., Spassky, A. and Yeramian, E. (1987) in: RNA Polymerase and the Regulation of Transcription (Reznikoff, W.S. et al. eds) pp.115–125.
[13] Pörschke, D., Hillen, V. and Takahashi, M. (1984) EMBO J. 3, 2873–2878.
[14] Tsai, S., Carlstedt-Duke, J., Weigel, N., Dahlman, K., Gustafsson, J.-Å., Tsai, M.-J. and O'Malley, B. (1988) Cell 55, 361–369.