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Photo-CIDNP study of the interaction between the glucocorticoid receptor DNA-binding domain and glucocorticoid response elements

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SUMMARY

Photo-CIDNP studies were performed on two protein fragments that both contain the double zinc-finger DNA-binding domain of the glucocorticoid receptor. In the absence of DNA, Tyr^{452} and Tyr^{474} are polarised in both fragments while Tyr^{497} is not. Addition of a palindromic glucocorticoid response element (GRE) results in the suppression of Tyr^{474} polarization while the polarization of Tyr^{452} is unaffected. The same result is observed upon adding a half GRE to the protein fragment indicating that the suppression of Tyr^{474} polarization is not due to protein-protein contacts but to interaction with DNA.

The glucocorticoid receptor (GR) is a 87 kDa gene regulatory protein belonging to the steroid/thyroid superfamily of nuclear transcription factors (Yamamoto, 1985; Gustafsson et al., 1987; Evans, 1988). When it is activated by a hormone it binds selectively to specific DNA sequences termed glucocorticoid response elements (GRE's) to regulate transcription. The GR is organized in several functionally distinct domains responsible for hormone binding, nuclear localization, DNA binding and transcriptional activation and repression (Beato, 1989). Mutational analysis has defined an 86-amino acid residue segment (residues 440–525 of the rat GR) required for specific DNA binding (Miesfeld et al., 1987). This region comprises two 'zinc-fingers' characteristic of this conserved superfamily of DNA binding proteins (Evans and Hollenberg, 1988; Freedman

et al., 1988). Protein fragments of about 100 amino acids encompassing residues 440–525 or 413–519 expressed in *E.coli* bind specifically to the same DNA sequences as the intact protein (Freedman et al., 1988; Dahlman et al., 1989).

In an attempt to further characterize the protein and its interaction with DNA at a molecular level, we have performed photochemically induced nuclear polarization (photo-CIDNP) experiments on two protein fragments encompassing 108 residues (DBD-108) and 93 residues (DBD-93), respectively, which have residues 440-510 of the rat GR in common (see Fig. 1). In the photo-CIDNP method nuclear spin polarization is created via the interaction with a photoexcited dye with the substrate. Enhanced absorption or emission is observed for a number of amino acids (His, Tyr, Trp, Met) if they are accessible to the dye (Kaptein, 1982; Stob and Kaptein, 1989). Photo-CIDNP has been widely used to determine the surface accessibility of the side chains of these amino acids in globular proteins. The accessibility of these amino acids can be modified by ligands interacting with the protein, resulting in a change in CIDNP intensity (Kaptein, 1982).

Photo-CIDNP of the glucocorticoid receptor DNA-binding domain

The ^{1}H resonance assignments of the aromatic residues of DBD-93 are shown in Fig. 2A. They were made as part of a more complete sequential assignment study of the glucocorticoid receptor DNA-binding domain (Härd et al., 1990a). The aromatic region of the photo-CIDNP difference spectra of both DBD-108 and DBD-93 (Figs. 2B and C) in the absence of DNA show the emission lines for the 3,5 protons of Tyr^{452} and Tyr^{474} while no polarization for Tyr^{497} and for the histidines was observed . The resonances of Tyr^{474} are only weakly polarised and these protons are probably less accessible to the dye. The relative intensity of the Tyr^{474} polarization is lower in DBD-108 as compared to DBD-93 indicating that this residue has a lower accessibility in DBD-108 than in DBD-93.

It is noteworthy that the His^{451} and His^{472} lines do not show up in the photo-CIDNP difference spectrum. Photo-CIDNP difference spectra recorded at pH 9.3, which is the optimal pH for histidine photo-CIDNP (Stob and Kaptein, 1989) also did not show any photo-CIDNP for the histidines. This lack of histidine polarization may be due to a competition effect. Both histidine residues are close to polarized tyrosine residues in the amino acid sequence (see Fig. 1). It is conceivable

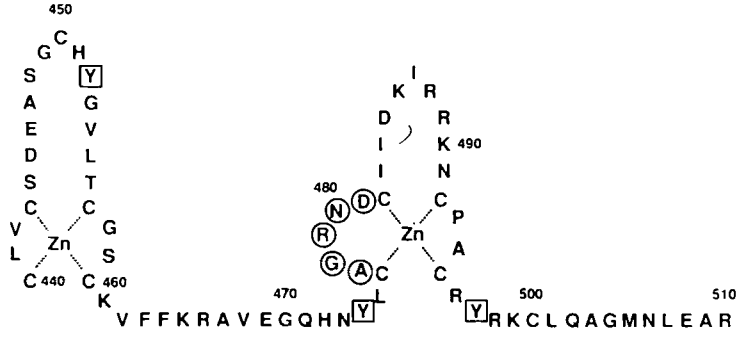

Fig. 1. Sequence of the glucocorticoid receptor DNA-binding domain encompassing the two 'zinc fingers' shared by DBD-93 and DBD-108. Residues implicated in protein-protein dimer formation in the protein-DNA complex are shown in circles, while the tyrosines are in squares. Zinc coordination is according to Severne et al. (1988).

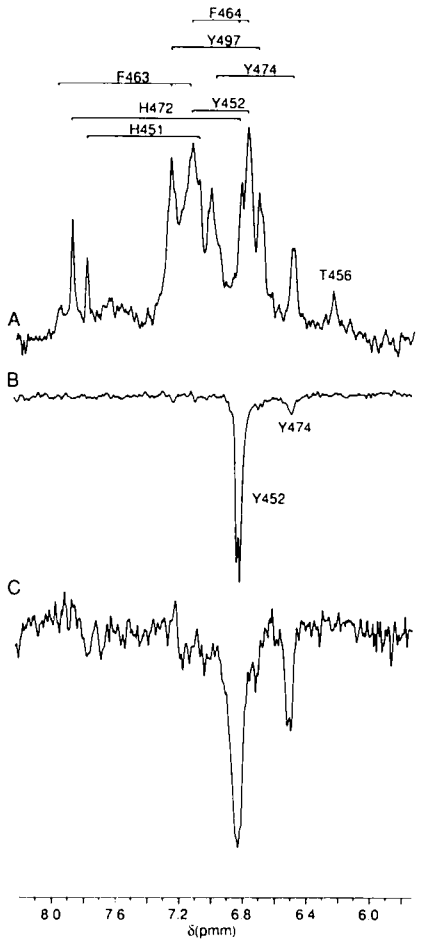

Fig. 2. (A) Aromatic region of a 0.5 mM solution of DBD-93, pH 6.7, in D_2O containing 50 mM phosphate and 200 mM NaCl at 300 K. DBD-93 and DBD-108 were prepared and purified as described previously (Freedman et al., 1988; Dahlman et al., 1989). (B) Photo-CIDNP difference spectrum of DBD-108 and of (C) DBD-93 under the same conditions showing the polarized resonances of Tyr^{452} and Tyr^{474}. Photo-CIDNP experiments were performed on a Bruker WM-360 spectrometer as described before (Kaptein, 1982; Stob et al., 1988) using a light pulse of 0.5 s and 5 W laser power. A line broadening of 3 Hz was applied in all cases.

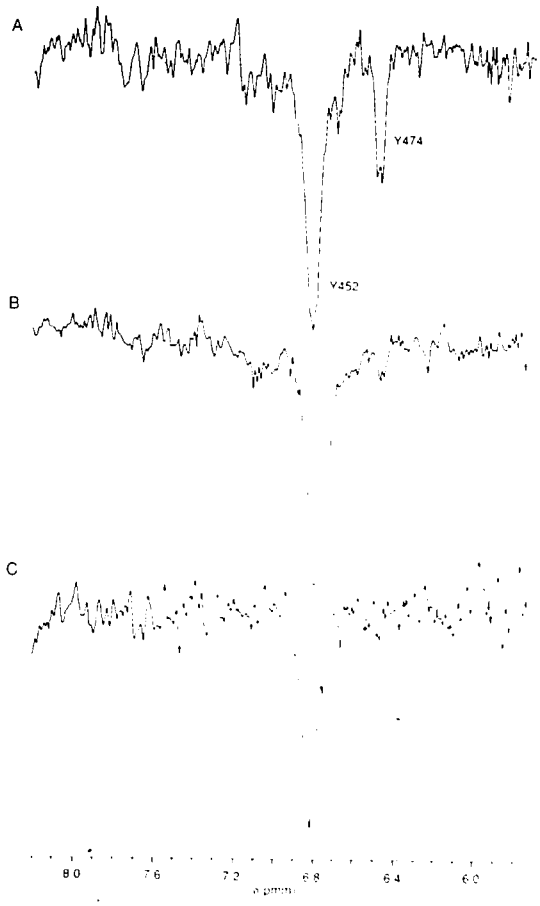

Fig. 3. (A) Aromatic region of the photo-CIDNP difference spectra of DBD-93 without DNA and (B) in the presence of 5 μl 15 mM 5'd(AGAACAGTCTGTTCT)3', a symmetrized GRE, showing the suppressed polarization of Tyr^{474}; (C) in the presence of 10 μl 13 mM 5'd(GCAGAACAGC)3'•5'd(GCTGTTCTGC)3', a half GRE. The DNA fragments were synthesized via an improved phosphotriester approach (van der Marel et al., 1981). Conditions are as in Fig. 2.

that when tyrosine and histidine side chains are in close proximity, the histidyl radical formed after reaction with triplet flavine rapidly reacts with tyrosine to yield effectively a tyrosyl-flavinyl radical pair. This is supported by the fact that the histidine residue in the photo-CIDNP difference spectrum of the His-Tyr dipeptide under the same conditions as in Fig. 1 has an intensity of only 5% of the tyrosine signal (data not shown).

To test if the difference in intensity of the photo-CIDNP signal of Tyr^{474} and Tyr^{452} in the free protein may be due to protein-protein interaction in the absence of DNA the concentration dependence of the photo-CIDNP of DBD-93 was investigated (Berliner and Kaptein, 1980). No concentration dependence was noted in the concentration range of 0.2-0.8 mM, indicating that the difference in polarization between Tyr^{452} and Tyr^{474} is not due to protein-protein interaction. It can be concluded that Tyr^{452} is situated on the surface of the protein, while Tyr^{474} is less accessible. Tyr^{497} is inaccessible to the dye and is apparently buried in the interior of the protein.

The photo-CIDNP effects of the tyrosine residues were compared with the solvent accessibility calculated from the solution structure of the DBD as reported by us recently (Härd et al., 1990b) according to the method described by Lee and Richards (1971). Surface accessibilities were calculated on a Silicon Graphics Personal Iris workstation using the 'Insight' program. The dot density was set at 1 and the sphere radius at 1.5Å. The surface accessibilities were determined by counting the number of dots representing the surfaces of the residues of interest. The 'buried' Tyr^{497} has an accessibility of 18 (arbitrary units) while values of 20 and 39 were calculated for Tyr^{452} and Tyr^{474} respectively. Tyr^{497} has the lowest calculated surface accessibility consistent with its absence in the photo-CIDNP difference spectrum. The calculated surface accessibilities of Tyr^{452} and Tyr^{474} are the opposite of what one would expect on the basis of the observed CIDNP intensities. Thus, there is only a fair agreement between the CIDNP results and the surface accessibility as calculated from the reported structure. This discrepancy might be due to the fact that the calculations are performed on a static structure disregarding the mobility of the tyrosine side chains of the protein during the experiment. Furthermore, the structure of the DBD used in the calculations is not sufficiently well determined to allow exact knowledge of the position of the side chains. An alternative explanation may be the effect of the proximity of the histidine residues mentioned before, resulting in altered 'effective accessibilities' for the two tyrosines.

The interaction with glucocorticoid response elements

Because the Tyr^{474} polarization in DBD-93 is stronger than in DBD-108 the former fragment was used to study the interaction with DNA. Figure 3B shows the photo-CIDNP difference spectrum of DBD-93 in the presence of the palindromic GRE sequence 5'd(AGAACAGTCTGTTCT)3', which is a symmetrised version of the high-affinity GRE half-site (Tsai et al., 1988) with a TT mismatch in the middle. It can be noted that the signal of Tyr^{474} is suppressed, while the resonance of Tyr^{452} is unaffected. The complex could not be dissociated by raising the NaCl concentration to 1 M in agreement with results from fluorescence measurements which indicated that the sequence-specific binding is not very sensitive to salt concentration (Härd et al., 1990c). Similar results were obtained with DBD-108 (data not shown).

Photo-CIDNP was previously used to study the non-specific and specific interaction between *lac* repressor headpiece and DNA (Buck et al., 1980; Stob et al., 1988). It was concluded that those resonances, that were suppressed by the addition of operator DNA, were involved in DNA binding. This conclusion is in agreement with the model of a *lac* repressor headpiece-DNA complex derived from 2D NMR data (Boelens et al., 1987). In the case of the DBD, however, the analysis is more complicated since the protein is a monomer in solution, but binds to DNA as a dimer. Biophysical studies employing fluorescence spectroscopy have shown that DBD-108 binds cooperatively to DNA (Härd et al., 1990c). Site-directed mutagenesis has identified amino acids 477–481 at the base of the second zinc finger to be responsible for the recognition of half-site spacing

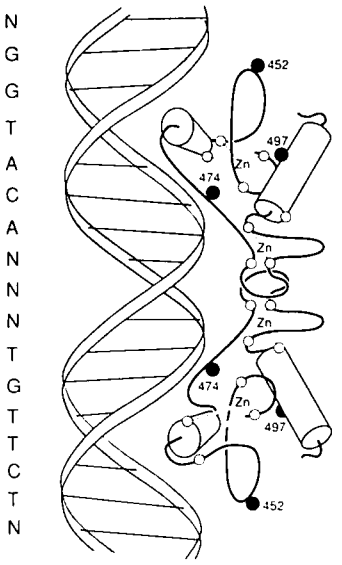

Fig. 4. Schematic representation of a structural model of the dimeric complex between the glucocorticoid receptor DBD and a GRE (Härd et al., 1990b). The positions of the tyrosines residues are indicated. Tyr^{452} is not involved in either protein-protein or DNA-protein contacts while Tyr^{474} is part of the protein-DNA interface in the major groove of the DNA in the dimeric complex. Tyr^{497} is buried in the interior of the protein.

and cooperativity via protein-protein interaction (Umesono and Evans, 1989; Dahlman-Wright et al., 1991). Therefore, the inaccessibility to the dye of Tyr^{474} in DBD complexed with the palindromic GRE could be due to either protein-protein or protein-DNA contacts. To distinguish between the two possibilities we looked at the photo-CIDNP of DBD complexed with a half GRE as well. Addition of the half GRE 5'd(GCAGAACAGC)3'•5'd(GCTGTTCTGC)3' also results in the suppression of the Tyr^{474} signal (Fig. 3C). The inaccessibility of Tyr^{474} in the complex with the half GRE, in which no protein-protein contacts are present, clearly demonstrates that Tyr^{474} is part of the protein-DNA contact region. Tyr^{452} is neither involved in DNA binding nor protein-protein contacts. In principle, it is conceivable that the suppression of Tyr^{474} polarization is due to dimer formation induced by a half GRE. Band-shift assays using mutant GRE's, however, have shown that the DBD binds to a half GRE as a monomer (Dahlman-Wright et al., 1990).

The results of our experiments are consistent with a model for the dimeric DBD-GRE complex as proposed by us recently on the basis of the three-dimensional structure obtained from 2D NMR data combined with genetic and biochemical information (Härd et al., 1990b). This model is shown schematically in Fig. 4. In this model, Tyr^{474} is in a favourable position to contact the phosphate backbone of the GRE, while the accessibility of Tyr^{452} is affected neither by protein-DNA nor by protein-protein contacts. Mutation of Tyr^{474} to a histidine (Schena et al., 1989) did not result in altered DNA-binding activity. This can be explained by the fact that histidine and tyrosine can interact with DNA in similar ways. In the case of the *lac* repressor, for instance, it was found that replacement of Tyr^{17} in the recognition helix by a histidine residue did not lead to changes in repression (Lehming et al., 1990). The involvement of a tyrosine residue in DNA binding is also consistent with results of fluorescence measurements on the DBD-GRE interaction which showed a quenching of tyrosine fluorescence upon binding to the DNA (Härd et al., 1990c).

Currently, further ^{1}H-NMR studies are in progress in order to determine the structure of the complex between the DNA-binding domain of the glucocorticoid receptor and GRE's.

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