A single d(GpG) cisplatin adduct on the estrogen response element decreases the binding of the estrogen receptor

Liliane Massaad-Massade[a,*], Charbel Massaad[a], Franck Legendre[b], Véronique Bas[b], Jean-Claude Chottard[b], Philippe Beaune[a], Robert Barouki[a]

[a] *Université René Descartes, Laboratoire de Toxicologie Moléculaire, U-490 INSERM, 45 rue des Saints-Pères, 75270 Paris Cedex, France*
[b] *Université René Descartes, UMR-8601 CNRS, 45 rue des Saints-Pères, 75270 Paris Cedex, France*

Received 23 November 1999

Edited by Jacques Hanoune

Abstract Both cisplatin and the estrogen receptor (ER) are known to bend DNA. The influence of the bending of sequences by the d(GpG)*cis*Pt adduct binding of ER to estrogen response element (ERE)-like sequences was examined. Three ERE-like oligonucleotides with different affinities for ER and which include a GG in the linker sequence were designed in order to form a single central d(GpG)*cis*Pt adduct. Using electrophoretic mobility shift assay and Scatchard analysis, it was shown that the presence of a single d(GpG)*cis*Pt adduct in the linker sequence decreases the ER affinity for DNA. These results do not support a critical role of a DNA bend in the initial recognition of ERE by ER. Then, the platination of DNA outside of the ERE half-sites decreases the interaction of ER with ERE.

Key words: Cisplatin adduct; Estrogen receptor; DNA bending

1. Introduction

cis-Diamminedichloroplatinum(II) (cisplatin or CDDP) is a chemotherapeutic agent widely used in the treatment of several types of human cancer [1]; its cytotoxicity is believed to be mediated by cisplatin-DNA adducts [2]. The in vitro as well as in vivo major adducts formed by CDDP are the N_7, N_7 d(GpG)*cis*Pt (≅65% of the platinum bound) and d(ApG)-*cis*Pt (≅25%) [3]. They alter the DNA structure [4], block replication and transcription [2,5] and activate a programmed cell death [6].

The d(GpG)*cis*Pt and d(ApG)*cis*Pt adducts bend DNA towards the major groove by 35–55° [7] and unwind it by 13° [8]. Recently, a number of cellular proteins have been described that interact preferentially with cisplatin-damaged DNA sites [9]. Cisplatin induces a DNA distortion which is recognized by high mobility group proteins (HMG) such as HMG1 [10] and HMG2 [11], human upstream binding factor (hUBF) [12], mitochondrial transcription factor [13], SSRP1 [14], testis HMG protein (tsHMG) [15] or sex determining region Y protein [16]. Proteins without an HMG domain motif, such as TBP, are also able to bind to the 1,2-intrastrand CDDP adducts [17]. It was not known whether the binding of other classes of nuclear proteins, in particular nuclear receptors, could be modified by cisplatin adducts.

The estrogen receptor (ER) is a member of a superfamily of nuclear receptors that have common structural and functional domains with two highly conserved regions: the central DNA binding domain and the C-terminal hormone binding domain [18]. The most highly conserved region among the members of this superfamily of receptors is the DNA binding domain. This region is responsible for the specific interaction of ER with an estrogen response element (ERE), a palindrome consisting of GGTCA half-sites separated by a linker sequence of 3 bp [19]. Nardulli et al. demonstrated that ER binding induces a 54° bending of the ERE-containing DNA fragments [20]. Following a hormonal stimulation, the ER binds to an ERE as a homodimer, leading to changes in transcription of hormone responsive genes; it could be implicated in the development of hormonally dependent cancers [21].

Since CDDP and ER are known to bend DNA to a similar extent, we assessed if DNA bending has a critical role in the recognition of ERE by ER. Therefore, we examine the changes in binding of ER to ERE modified by the major cisplatin d(GpG)*cis*Pt adduct in the linker sequence in order to induce a bend of the oligonucleotide able to mimic that of ERE upon ER binding. We selected oligonucleotides of high, intermediate and low ER affinities, all containing a single GG in the linker sequence. None of them contained a GG or an AG susceptible to give cisplatin adducts in the modified ERE half-sites. We show here that the presence of a single d(GpG)*cis*Pt adduct in the linker sequence decreases the ER affinity for DNA. Moreover, the platination of DNA sequences which are not ER binding sites, does not induce their recognition by this protein. These results do not support a critical role of a DNA bend in the initial recognition of ERE by ER.

2. Materials and methods

2.1. Expression of ER and its deleted fragments

Expression vectors for human ER were previously described by Green and Chambon, and Ylicomi et al. [22,23]. The cell extracts were prepared in COS-7 cells transfected with ER as described by Massaad et al. [24].

2.2. Oligonucleotides and platination

Five ERE-like oligonucleotides of 20 bp containing modified ERE half-sites were synthesized. Table 1 represents the sequences studied. ERE(1) is a classical ERE consensus sequence which consist of two half-sites separated by 3 bp (TGG). ERE(2) differs from the consensus sequence by 1 bp (substitution of the second G in the first half-site by T). ERE(3) differs from the consensus sequence by 2 bp (substitution of GG in the first half-site by TA). ERE(4) differs from the consensus sequence by 4 bp (substitution in the first and second half-site of both G and both C by TA).

The purity of the three ERE-like sequences (ERE(2), ERE(3) and

*Corresponding author. Fax: (33)-1-42 86 20 72.
E-mail: liliane.massade@biomedicale.univ-paris5.fr

PII: S0014-5793(99)01755-X

ERE(4)) which do not contain a GG in the half-sites was checked by high performance liquid chromatography and then allowed to react with the diaqua form of cisplatin as previously described by Reeder et al. [25]. After the single-stranded oligonucleotides platination, adducts were identified by enzymatic reactions of the products followed by MALDI mass spectroscopy analysis of the isolated fragments [26].

2.3. Preparation of competitors and radiolabelled probes

A 20 bp oligonucleotide containing a single, centrally located d(GpG)*cis*Pt adduct and its analogous unmodified fragments were used as both competitor DNAs and probes in electrophoretic mobility shift assay (EMSA). For EMSA and Scatchard studies, a high specific activity was required, the non-coding strand was 3′-end-labelled by using the Klenow fragment of DNA polymerase I in the presence of [α-^{32}P]dCTP (> 3000 Ci/mmol; 1 Ci = 37 GBq).

2.4. EMSA

The ability of the various platinated or untreated EREs to compete with radiolabelled consensus was investigated by competitive EMSA as previously described [24] using the recombinant ER-α (3.5 pmol) protein (Panvera Corporation, Madison, WI, USA). Free and bound forms of ERE were quantified by a phosphorimager (Image Quant Software). The ratio of the radioactivity included in the band over total radioactivity was calculated and results were expressed as percent of control.

The affinity of the ER to the platinated or unplatinated ERE was evaluated using Scatchard assays. 20 μl of ER-containing COS-7 extract was incubated with 2× reaction mix and an increasing amount of either labelled ERE(2) or [ERE(2)-Pt]. Free and bound forms were excised from the gel and counted in a scintillation counter. Scatchard plots were analyzed by graphpad inplot for estimation of the dissociation constant (K_d).

3. Results

3.1. Analysis of ER interaction with ERE-like sequences

We evaluated the ability of ER to interact with the ERE sequences listed in Table 1. We performed competitive EMSA assessing the binding of recombinant ER to a radiolabelled ERE(1) in the absence or presence of increasing amounts of unlabelled ERE(1), ERE(2), ERE(3) and ERE(4). In this assay, the binding of ER to an unlabelled ERE-like sequence resulted a decrease in the amounts of bound probe. As can be seen in Fig. 1A, ERE(2) and ERE(3) were able to compete for ER binding, whereas ERE(4) was not. After quantification, the relative affinities of the EREs to ER were as follows: ERE(1)≥ERE(2) > ERE(3) (10-fold)≫ERE(4) (Fig. 1B).

Table 1
Nucleotide sequences of EREs

Element	Sequence[a]	
Consensus	5′-GGTCA **NNN** TGACC-3′	(strand I)
	3′-CCAGT **NNN** ACTGG-5′	(strand II)
ERE(1)	5′-GAAAGGTCA **TGG** TGACCTAC-3′	(strand I)
	3′-CTTTCCAGT **ACC** ACTGGATG-5′	(strand II)
ERE(2)	5′-GAAATGTCA **TGG** TGACCTAC-3′	(strand I)
	3′-CTTTACAGT **ACC** ACTGGATG-5′	(strand II)
ERE(3)	5′-GAAATATCA **TGG** TGACCTAC-3′	(strand I)
	3′-CTTTATAGT **ACC** ACTGGATG-5′	(strand II)
ERE(4)	5′-GAAATATCA **TGG** TGATATAC-3′	(strand I)
	3′-CTTTTAAGT **ACC** ACTATATG-5′	(strand II)

[a]Underlined bases are those that differ from consensus sequence, in bold the linker sequence.

3.2. Affinity of ER to cisplatin-modified sequences

ERE(1) was not a good candidate for platination because of the presence of a GG in the first half-site which would give an additional cisplatin adduct. For that reason, only ERE(2), ERE(3) and ERE(4) were platinated. The effect of platination on the ability of ERE(2), ERE(3) and ERE(4) to compete with radiolabelled consensus ERE for ER binding was examined. Recombinant ER was incubated with a radiolabelled

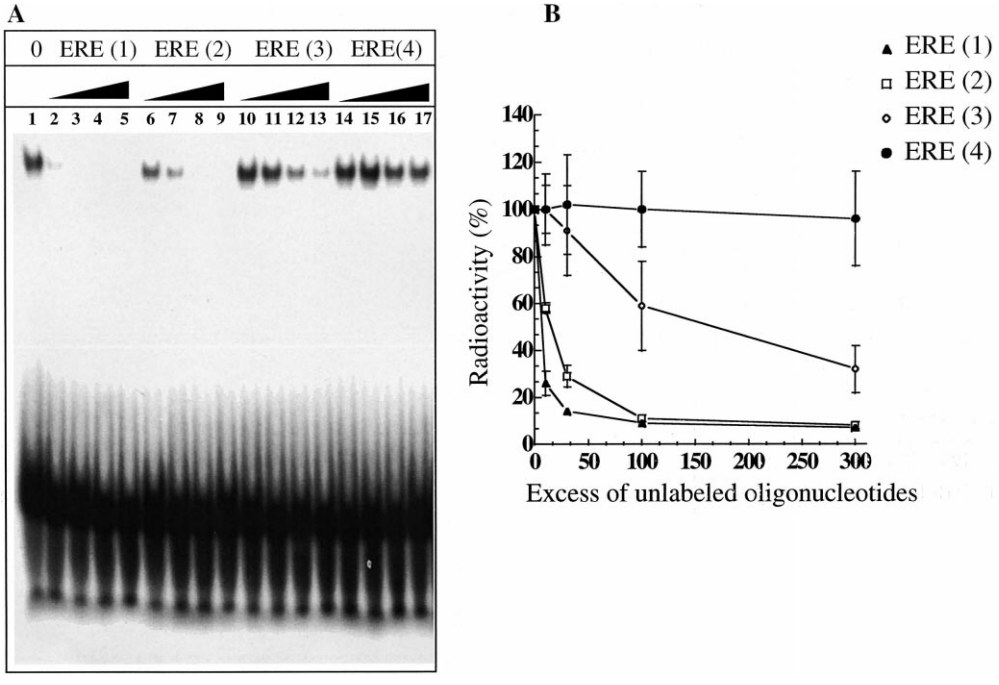

Fig. 1. Assessment of ER affinity for the ERE-like sequences. A: The binding of human recombinant ER (Panvera) to a radiolabelled double-stranded consensus ERE(1) (0.1 ng) was competed by increasing amounts of unlabelled double-stranded ERE(1) (lanes 2–5), ERE(2) (lanes 6–9), ERE(3) (lanes 10–13) and ERE(4) (lanes 14–17) (10-, 30-, 100- and 300-fold excess). No competitors were added in lane 1. B: The ratio of the radioactivity included in the band over total radioactivity was calculated and the results were expressed as percent of control. Each point represents the mean ± S.D. of four independent experiments.

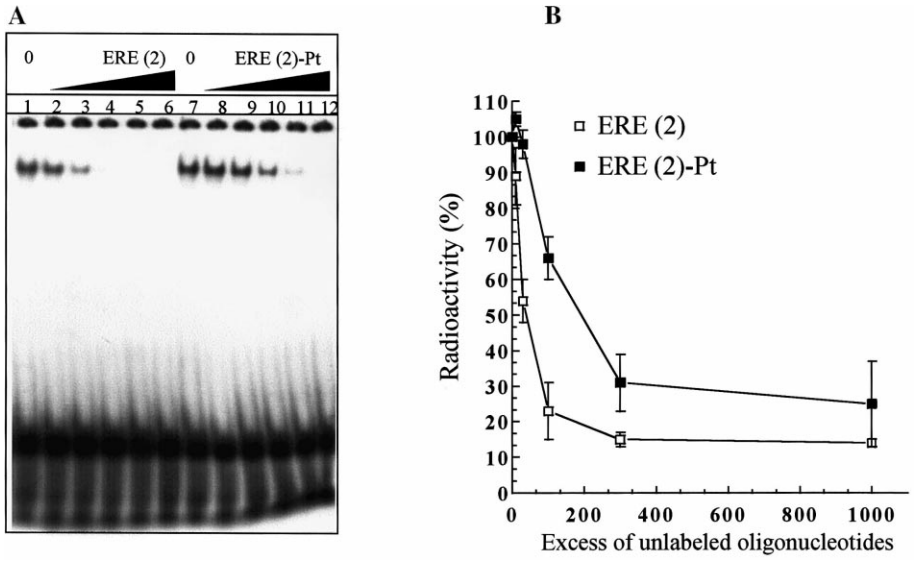

Fig. 2. Assessment of ER affinity for the ERE(2) and platinated ERE(2) sequences. A: EMSA was performed with purified human recombinant ER (Panvera) and the ERE(1) probe (0.1 ng) in the absence (lanes 1 and 7) or the presence of increasing amounts of unlabelled competitors (10-, 30-, 100-, 300- and 1000-fold excess) ERE(2) (lanes 2–6) or [ERE(2)-Pt] (lanes 8–12). B: The ratio of the radioactivity included in the band over total radioactivity was calculated and the results were expressed as percent of control. Each point represents the mean ± S.D. of three independent experiments.

ERE(1) and with increasing amounts of unmodified or cisplatin-treated oligonucleotides ERE(2), ERE(3) and ERE(4).

3.2.1. A d(GpG) cisplatin adduct decreases ER binding to ERE(2). ERE(2) was treated with *cis*$[Pt(H_2O)_2(NH_3)_2]^{2+}$, leading to a single centrally located 1,2-intrastrand d(GpG) chelate of the *cis*$[Pt(NH_3)_2]^{2+}$ moiety in the oligonucleotide [ERE(2)-Pt]. We used the ERE(1) sequence as a probe at 0.1 ng and as competitors either ERE(2) or [ERE(2)-Pt] at an excess of 10-, 30-, 100-, 300- and 1000-fold. As seen in Fig. 2, ERE(2) was about 4-fold more efficient than ERE(2)-Pt for ER binding.

In order to determine whether the cisplatin adduct modified the affinity of the receptor for the ERE(2) oligonucleotide, we performed Scatchard assays using ER prepared in COS-7 cells incubated with increasing amounts of either labelled ERE(2) or [ERE(2)-Pt]. Fig. 3A–C shows of such an experiment the corresponding Scatchard plot. For each oligonucleotide concentration, the amount of ER-ERE(2) complex was more

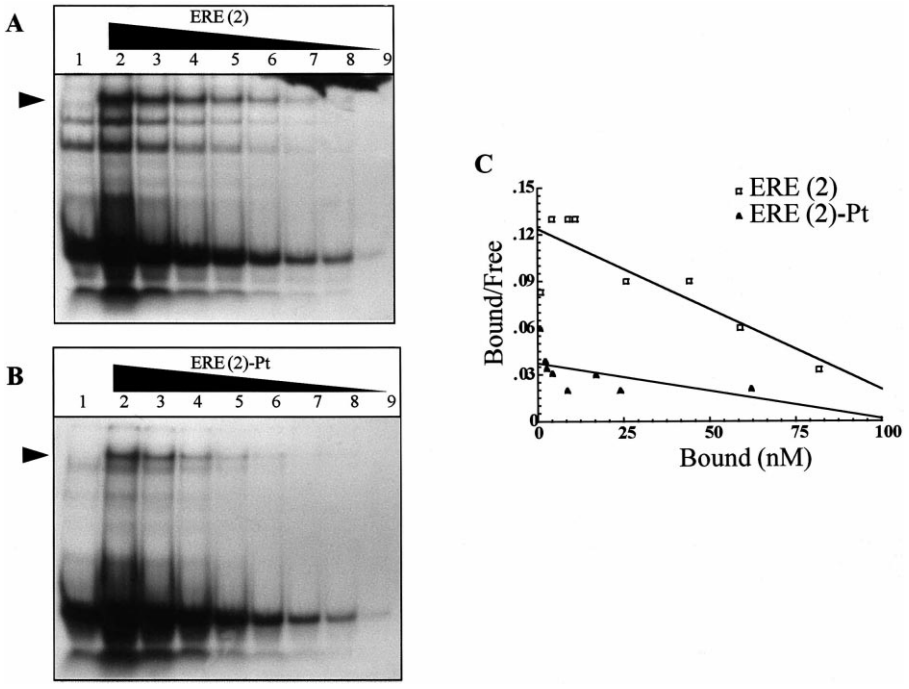

Fig. 3. Determination of the dissociation constant (K_d) of ERE(2) and [ERE(2)-Pt] by Scatchard analysis. A and B: EMSA was performed using HER overexpressed in COS-7 cells and increasing amounts of radiolabelled ERE(2) (A) or [ERE(2)-Pt] (B) (0.025×10^6 cpm to 2.5×10^6 cpm). C: Complexes and free probes were cut off the gel and counted. The figure shows a representative experiment that was repeated four times.

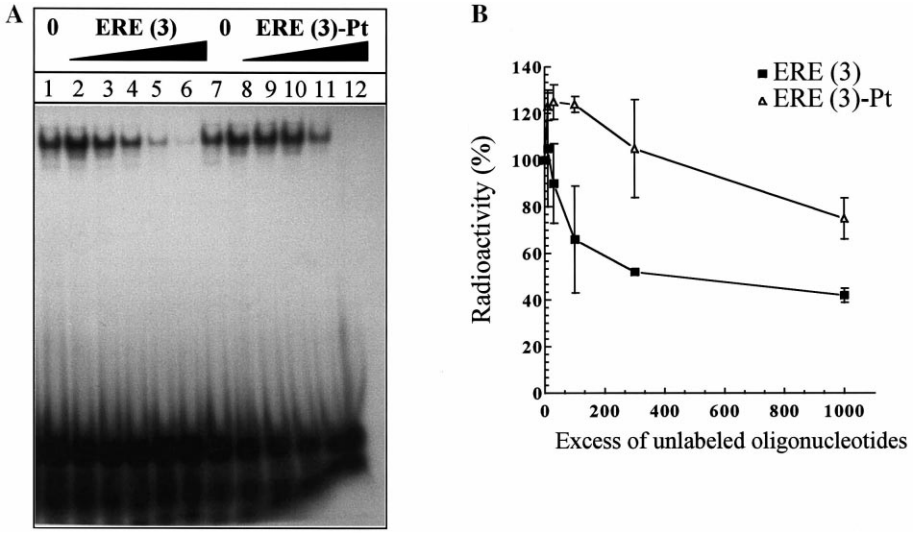

Fig. 4. Assessment of ER affinity for the ERE(3) and platinated ERE(3) sequences. A: EMSA was performed with purified human recombinant ER (Panvera) and the ERE(1) probe (0.1 ng) in the absence (lanes 1 and 7) or the presence of increasing amounts of unlabelled competitors (10-, 30-, 100-, 300- and 1000-fold excess) ERE(3) (lanes 2–6) or [ERE(3)-Pt] (lanes 8–12). B: The ratio of the radioactivity included in the band over total radioactivity was calculated and the results were expressed as percent of control. Each point represents the mean ± S.D. of three independent experiments.

abundant than that of the complex ER-[ERE(2)-Pt]. After quantification, Scatchard analysis revealed that the dissociation constant (K_d) value was 4-fold lower for ERE(2) ($K_d = 0.93$ nM) than for [ERE(2)-Pt] ($K_d = 3.77$ nM) (Fig. 3C) while the number of total sites was similar. This result indicates a decreased affinity of ER for [ERE(2)-Pt].

3.2.2. A d(GpG) cisplatin adduct abolishes ER binding to ERE(3) and does not affect the ER binding to ERE(4). The ability of unmodified or platinated ERE(3) to compete with radiolabelled ERE(1) for binding to the ER protein. We performed an EMSA using as probe the ERE(1) sequence at 0.1 ng and as competitors ERE(3) or [ERE(3)-Pt] at 1, 3, 10, 30 100 ng. As seen in Fig. 4, ERE(3) competes approximately 10-fold better than [ERE(3)-Pt] for ER binding, suggesting that the affinity of ER for [ERE(3)-Pt] is very low. This was confirmed by saturation studies showing that while a complex was formed between ER and ERE(3), no association was detected between ER and [ERE(3)-Pt] even at high concentration of the probe (data not shown). In the case of ERE(4), no binding was observed with either the platinated or unplatinated oligonucleotide, thus the structural modification elicited by the cisplatin adduct does not increase the ER affinity to this oligonucleotide.

4. Discussion

We analyzed the effect of cisplatin adduct on the binding of ER to various modified EREs. The rationale supporting this work was the observation that both cisplatin adducts and ER binding trigger a similar bending of DNA at least in some studies. We therefore used three different oligonucleotides, ERE(2), ERE(3) and ERE(4), displaying a high, medium and very low activity for the ER. All these sequences contained a single GG in the linker sequence and were platinated on this GG in order to induce a bend of the oligonucleotide able to mimic that of ERE upon ER binding. None of the sequences studied contained a GG or an AG susceptible to give cisplatin adducts in the modified ERE half-sites.

Following GG platination, the affinity of ER for all the modified sequences either decreased or was completely abolished. In the case of ERE(3), the affinity of ER for the platinated sequence is decreased about 4-fold. ER binding to [ERE(4)-Pt] is completely abolished and that to [ERE(3)-Pt] remains very weak at best. The data show that the DNA modifications induced by the d(GpG)*cis*Pt adduct do not favor the binding of ER despite the similar bends induced by the platinum chelate and for an ER bound DNA. Furthermore, although unlikely, we cannot exclude that decreased affinity of ER for platinated sequences could reflect a steric hindrance caused by the d(GpG)*cis*Pt adduct which could inhibit the ability of the two ER monomers to maintain contact and form a stable ER dimer.

The d(GpG)*cis*Pt adduct leads to both DNA bending towards the major groove and unwinding of the double helix together with an extension of the minor groove. Several biochemical and biophysical studies have assessed the structural changes caused by cisplatin adducts [27–30]. Electrophoretic mobility studies gave a 32–34° range for bending [27], whereas NMR associated to molecular modeling gave a 58–78° range for bending and 13–25° unwinding [28,29]. For the same platinated oligonucleotide, analyzed by the same group, different values were found by X-ray diffraction [30] and solution NMR investigations [28]. These structural differences could depend on the stacking of the oligonucleotide in the crystal structure.

In the case of the ER, the structural and biochemical studies have revealed that ER DBD interacts with the major groove of the DNA helix and occupies both half-sites of the palindromic ERE sequence [31–33]. ERE is bent toward the major groove by about 54° when ER is bound [34–36]. No unwinding has been reported. It was suggested that the structural modification of DNA by ER is a consequence of ER binding, and that such a structure could be a critical feature of the efficiency of ER binding [37]. The decrease or absence of ER binding to GG-*cis*Pt-containing ERE-like sequences may be due to a configuration or flexibility of the platinum

adduct inappropriate for efficient ER recognition. This observation can be related to the results of Kim et al., which demonstrated that prebending of the ERE toward the minor groove reduced the binding of ER DNA to its specific site [38]. They concluded that the interaction of a eukaryotic transcription factor with its recognition sequence can be strongly influenced by altering the topology through prebending of the DNA. Thus, while the direction of DNA bending by cisplatin is similar to that induced by ER, other structural modifications are probably different.

Several authors reported that the cisplatin-DNA adducts cause structural distortions in DNA that mimic the conformation of the preferred natural binding sites of some nuclear proteins luring them away from their natural binding sites and decreasing their biological effects. The proteins described could be classified into two groups, the repair proteins and the architectural proteins. The repair proteins, such as xeroderma pigmentosum group A and E, UV-damage recognition protein, T4 endonuclease VII [39–44], recognize cisplatin adducts and are involved in damage recognition as a first step of the repair pathways. The architectural proteins, such as HMG1/2, hUBF, SSRP1 or tsHMG, are generally abundant nuclear or chromatin proteins that have an architectural role in the formation of functional higher order protein/DNA or protein/protein complexes (for review, see [45]). Since the binding of those proteins to DNA induces a deformation of DNA structure which is important for proper promoter architecture, the structural distortions caused by cisplatin adducts may create a key geometric feature that favors the binding of these proteins. In contrast with those proteins, the ER DBD binds in the major groove of the DNA, giving sequence-specific H-bonds with bases comprising part of the ERE palindrome, and forming extensive contacts with the phosphate backbone [32,33]. In this complex, the DNA is bent [20] toward the major groove [36].

In conclusion, this study demonstrates that the d(GpG)*cis*Pt adduct decreases or abolishes ER ability to interact with ERE-like sequences and does not increase the affinity of the ER for DNA sequences that are not normally specific binding sites for it. The most likely hypothesis is that the conformation or flexibility of the cisplatin adduct is inappropriate for ER recognition.

Acknowledgements: This work was supported by 'ARC' Grant 1808 and by the 'Région Ile de France' through the SESAME program. We thank Dr. L. Tora and Prof. P. Chambon for providing the hER expression vector.

References

[1] Loehrer, P.J. and Einhorn, L.H. (1984) Ann. Intern. Med. 100, 704–713.
[2] Brown, S.J., Kellett, P.J. and Lippard, S.J. (1993) Science 261, 603–605.
[3] Fichtinger-Schepman, A.M., van der Veer, J.L., den Hartog, J.H., Lohman, P.H. and Reedijk, J. (1985) Biochemistry 29, 707–713.
[4] Takahara, P.M., Rosenzweig, A.C., Frederick, C.A. and Lippard, S.J. (1995) Nature 377, 649–652.
[5] Kane, S.A. and Lippard, S.J. (1996) Biochemistry 35, 2180–2188.
[6] Chu, G. (1994) J. Biol. Chem. 269, 787–790.
[7] Rice, J.A., Crothers, D.M., Pinto, A.L. and Lippard, S.J. (1988) Proc. Natl. Acad. Sci. USA 85, 4158–4161.
[8] Bellon, S.F., Coleman, J.H. and Lippard, S.J. (1991) Biochemistry 30, 8026–8035.
[9] Toney, J.H., Donahue, B.A., Kellett, P.J., Bruhn, S.L., Essigmann, J.M. and Lippard, S.J. (1989) Proc. Natl. Acad. Sci. USA 86, 8328–8332.
[10] Pil, P.M. and Lippard, S.J. (1992) Science 256, 234–237.
[11] Chow, C.S., Barnes, C.M. and Lippard, S.J. (1995) Biochemistry 34, 2956–2964.
[12] Treiber, D.K., Zhai, X., Jantzen, H.-M. and Essigmann, J.M. (1994) Proc. Natl. Acad.Sci. USA 91, 5672–5676.
[13] Chow, C.S., Whitehead, J.P. and Lippard, S.J. (1994) Biochemistry 33, 15124–15130.
[14] Bruhn, S.L., Housman, D.E. and Lippard, S.J. (1993) Nucleic Acids Res. 21, 1643–1646.
[15] Ohndorf, U.M., Whitehead, J.P., Raju, N.L. and Lippard, S.J. (1997) Biochemistry 36, 14807–14815.
[16] Trimmer, E.E., Zamble, D.B., Lippard, S.J. and Essigmann, J.M. (1998) Biochemistry 37, 352–362.
[17] Vichi, P., Coin, F., Renaud, J.P., Vermeulen, W., Hoeijmakers, J.H., Moras, D. and Egly, J.M. (1997) EMBO J. 16, 7444–7456.
[18] Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E. and Chambon, P. (1989) Cell 59, 477–487.
[19] Klein-Hitpass, L., Ryffel, G.U., Heitlinger, E. and Cato, A.C.B. (1988) Nucleic Acids Res. 16, 647–663.
[20] Nardulli, A.M., Greene, G.L. and Shapiro, D.J. (1993) Mol. Endocrinol. 7, 331–340.
[21] Brandt, M. and Vickery, L. (1997) J. Biol. Chem. 272, 4843–4849.
[22] Green, S. and Chambon, P. (1987) Nature 335, 75–78.
[23] Ylicomi, T., Bocquel, M.T., Berry, M., Gronemeyer, H. and Chambon, P. (1992) EMBO J. 11, 3681–3694.
[24] Massaad, C., Coumoul, X., Sabbah, M., Garlatti, M., Redeuilh, G. and Barouki, R. (1998) Biochemistry 37, 6023–6032.
[25] Reeder, F., Guo, Z., Murdoch, P.D., Corazza, A., Hambley, T.W., Berners-Price, S.J., Chottard, J.C. and Sadler, P.J. (1997) Eur. J. Biochem. 249, 370–382.
[26] Gonnet, F., Kocher, F., Blais, J.C., Bolbach, G. and Tabet, J.C. (1990) Biophys. Chem. 35, 179–188.
[27] Bellon, S.F. and Lippard, S.J. (1990) Biophys. Chem. 35, 179–188.
[28] Gelasco, A. and Lippard, S.J. (1998) Biochemistry 37, 9230–9239.
[29] Herman, F., Kozelka, J., Stoven, V., Guittet, E., Girault, J.P., Huynh-Dinh, T., Igolen, J., Lallemand, J.Y. and Chottard, J.C. (1990) Eur. J. Biochem. 194, 119–133.
[30] Takahara, P.M., Frederick, C.A. and Lippard, S.J. (1996) J. Am. Chem. Soc. 118, 12309–12321.
[31] Klein-Hitpass, L., Tsai, S.Y., Greene, G.L., Clark, J.H., Tsai, M.J. and O'Malley, B.W. (1989) Mol. Cell. Biol. 9, 43–49.
[32] Schwabe, J.W., Neuhaus, D. and Rhodes, D. (1990) Nature 348, 458–461.
[33] Schwabe, J.W., Chapman, L., Finch, J.T. and Rhodes, D. (1993) Cell 75, 567–578.
[34] Nardulli, A.M. and Shapiro, D.J. (1992) Mol. Cell. Biol. 12, 2037–2042.
[35] Nardulli, A.M. and Shapiro, D.J. (1993) Receptor 3, 247–255.
[36] Nardulli, A.M., Grobner, C. and Cotter, D. (1995) Mol. Endocrinol. 9, 1064–1076.
[37] Petz, L.N., Nardulli, A.M., Kim, J., Horwitz, K.B., Freedman, L.P. and Shapiro, D.J. (1997) J. Steroid Biochem. Mol. Biol. 60, 31–41.
[38] Kim, J., de Haan, G., Nardulli, A.M. and Shapiro, D.J. (1997) Mol. Cell. Biol. 17, 3173–3180.
[39] Jones, C.J. and Wood, R.D. (1993) Biochemistry 32, 12096–12104.
[40] Kuraoka, I., Morita, E.H., Saijo, M., Matsuda, T., Morikawa, K., Shirakawa, M. and Tanaka, K. (1996) Mutat. Res. 362, 87–95.
[41] Chu, G. and Chang, E. (1988) Science 242, 564–567.
[42] Vaisman, A. and Chaney, S.G. (1995) Biochemistry 34, 105–114.
[43] Vaisman, A., Keeney, S., Nichols, A.F., Linn, S. and Chaney, S.G. (1996) Oncol. Res. 8, 7–12.
[44] Murchie, A.I.H. and Lilley, D.M.J. (1993) J. Mol. Biol. 233, 77–85.
[45] Zlatanova, J., Yaneva, J. and Leuba, S.H. (1998) FASEB J. 12, 791–799.