McLean, M. J., Larson, J. E., Wohlrab, F., & Wells, R. D. (1987) *Nucleic Acids Res. 15*, 6917–6935.
McLellan, J. A., Palecek, E., & Lilley, D. M. J. (1986) *Nucleic Acids Res. 14*, 9291–9309.
Nickol, J., Behe, M. J., & Felsenfeld, G. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1771–1775.
Nordheim, A., Lafer, E. M., Peck, L. J., Wang, J. C., Stollar, B. D., & Rich, A. (1982) *Cell (Cambridge, Mass.) 31*, 309–318.
O'Connor, T. R., Kang, D. S., & Wells, R. D. (1986) *J. Biol. Chem. 261*, 13302–13308.
Pack, G. R., Prasad, C. V., Salafsky, J. S., & Wong, L. (1986) *Biopolymers 25*, 1697–1715.
Peck, J. L., & Wang, J. C. (1981) *Nature (London) 292*, 375–378.
Peck, L. J., & Wang, J. C. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 6206–6210.
Roy, K. B., & Miles, H. T. (1983) *Biochem. Biophys. Res. Commun. 115*, 100–105.
Ruggiero, J., Manzini, G., & Quadrifoglio, F. (1987) *Biopolymers 26*, 1975–1979.
Sarai, A. (1984) *Phys. Lett. A 103A*, 397–401.
Sarai, A., & Jernigan, R. L. (1985) *J. Biomol. Struct. Dyn. 2*, 767–784.
Singleton, C. K., & Wells, R. D. (1982) *Anal. Biochem. 122*, 253–257.
Singleton, C. K., Klysik, J., Stirdivant, S. M., & Wells, R. D. (1982) *Nature (London) 299*, 312–316.
Singleton, C. K., Klysik, J., & Wells, R. D. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 2447–2451.
Singleton, C. K., Kilpatrick, M. W., & Wells, R. D. (1984) *J. Biol. Chem. 259*, 1963–1967.
Stirdivant, S. M., Klysik, J., & Wells, R. D. (1982) *J. Biol. Chem. 257*, 10159–10165.
Strauss, F., Gaillard, C., & Prunell, A. (1981) *Eur. J. Biochem. 118*, 215–222.
Vologodskii, A. V., & Frank-Kamenetskii, M. D. (1984) *J. Biomol. Struct. Dyn. 1*, 1325–1333.
Wang, J. C. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 200–203.
Wang, R. Y.-H., Zhang, X.-Y., Khan, R., Zou, Y., Huang, L.-H., & Ehrlich, M. (1986) *Nucleic Acids Res. 14*, 9843–9860.
Zacharias, W., Larson, J. E., Klysik, J., Stirdivant, S. M., & Wells, R. D. (1982) *J. Biol. Chem. 257*, 2775–2782.
Zacharias, W., Larson, J. E., Kilpatrick, M. W., & Wells, R. D. (1984) *Nucleic Acids Res. 20*, 7677–7692.
Zhang, X.-Y., Ehrlich, K. C., Wang, R. Y.-H., & Ehrlich, M. (1986) *Nucleic Acids Res. 14*, 8387–8397.

DNA Binding Properties of Dioxin Receptors in Wild-Type and Mutant Mouse Hepatoma Cells†

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ABSTRACT: The current model of action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin) entails stimulation of target gene transcription via the formation of dioxin–receptor complexes and subsequent accumulation of the complexes within the cell nucleus. Here, we have analyzed the DNA binding properties of the dioxin receptor in wild-type mouse hepatoma (Hepa 1c1c7) cells and a class of nonresponsive mutant cells which fail to accumulate dioxin–receptor complexes within the nucleus in vivo. In vitro, both the wild-type and mutant [^{3}H]dioxin–receptor complexes exhibited low affinity for DNA–cellulose (5–8% and around 4% retention, respectively) in the absence of prior biochemical manipulations. However, following chromatography on heparin–Sepharose, the wild-type but not the mutant dioxin receptor was transformed to a species with an increased affinity for DNA (40–50% retention on DNA–cellulose). The gross molecular structure of the mutant, non DNA binding dioxin receptor did not appear to be altered as compared to that of the wild-type receptor. These results imply that the primary deficiency in the mutant dioxin receptor form may reside at the DNA binding level and that, in analogy to steroid hormone receptors, DNA binding of the receptor may be an essential step in the regulation of target gene transcription by dioxin.

The polychlorinated aromatic hydrocarbon TCDD[1] is a potent inducer of aryl hydrocarbon hydroxylase, a cytochrome P-450 dependent enzyme activity involved in the oxidative metabolism of polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene. Evidence suggests that the induction of specific isozymes of cytochrome P-450 by TCDD (i.e., cytochromes P-450c and P-450d in the rat) is mediated by an intracellular, soluble receptor protein [for review, see Whitlock (1986) and Poland and Knutson (1982)]. Binding of TCDD causes an increase in the affinity of the receptor for nuclear target elements and a rapid increase in the rate of cytochrome P-450c transcription (Whitlock, 1986). The model of action

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[1] Abbreviations: TCDD and dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDF, 2,3,7,8-tetrachlorodibenzofuran; r$^-$, receptor-less phenotype; nt$^-$, nuclear transfer deficient receptor phenotype; nt^i, nuclear transfer increased receptor phenotype; R_s, Stokes radius; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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of TCDD described above is completely analogous to that described for steroid hormones. In particular, the molecular properties of the receptor proteins for TCDD and glucocorticoids are almost indistinguishable, representing highly asymmetrical molecules of $M_r \cong 90\,000$–$100\,000$ (Gustafsson et al., 1987). However, it should be emphasized that the dioxin and glucocorticoid receptors have no common ligand binding properties (Poellinger et al., 1985) and that the endogenous ligand for the dioxin receptor is not known.

The study of the mechanism of action of glucocorticoids was aided by the isolation of a variety of mutant lymphoid cell lines which are resistant to the cytolytic effect of the hormone (Bourgeois & Casson, 1985; Yamamoto et al., 1976; Stevens et al., 1983; Gehring, 1986). Three major classes of glucocorticoid-resistant mutants which contain altered hormone receptors have been described. "Receptorless" (r^-) mutants exhibit greatly reduced levels of specific steroid binding and immunoreactive material (Northrop et al., 1985). "Nuclear transfer increased" (nt^i) mutants express a truncated glucocorticoid receptor of $M_r \cong 40\,000$ which has normal hormone binding properties but exhibits an increased affinity for nonspecific DNA. "Nuclear transfer deficient" (nt^-) mutants, on the other hand, have normal hormone binding characteristics but do not bind to nonspecific DNA [see Yamamoto et al. (1976) and Gehring (1986) for reviews]. Characterization of these altered receptor forms as well as partial proteolysis of wild-type receptors has been valuable in the development of the theory of the domain structure of the glucocorticoid receptor [for a review, see Gustafsson et al. (1987)].

Mutant cell lines, which appear to be similar to the glucocorticoid-resistant cell lines discussed above, have been isolated from the mouse hepatoma wild-type cell line Hepa 1c1c7 (Hankinson, 1979). These mutants are deficient in the induction of cytochrome P-450 gene expression when exposed to TCDD. So far two classes of recessive mutants have been isolated. r^- Hepa 1c1c7 mutants exhibit reduced specific binding of dioxin (Legraverend et al., 1982) presumably due to either a reduced dioxin receptor number or the expression of a receptor with reduced affinity for the ligand. nt^- Hepa 1c1c7 mutant cells contain receptors with normal TCDD binding characteristics, but the ligand–receptor complexes are unable to accumulate in the nucleus (Legraverend et al., 1982) or have a much lower affinity for nuclear target elements as indicated by the low salt concentration required to extract these receptors from isolated nuclei (Whitlock & Galeazzi, 1984).

Somatic cell hybridization experiments have shown that, in contrast to the glucocorticoid receptor mutants, the defects in the dioxin receptor mutants can be assigned to two complementation groups (Hankinson, 1983). This suggests that a factor, in addition to the dioxin receptor, may be important in the transcriptional activation of the cytochrome P_1-450 gene.

We have previously shown that the dioxin receptor is a DNA binding molecule in vitro (Wilhelmsson et al., 1986) in analogy to steroid hormone receptors in general. In this paper we show that the Hepa 1c1c7 nt^- mutant dioxin receptor is deficient in DNA binding. Thus, it is possible to correlate in vitro DNA binding of the dioxin receptor with dioxin responsiveness in vivo, further emphasizing the analogies between the dioxin and glucocorticoid receptor models.

Experimental Procedures

Chemicals. [1,6-^{3}H]TCDD (28 Ci/mmol) was a generous gift from Dr. A. Poland (Madison, WI). Unlabeled 2,3,7,8-TCDF was kindly supplied by Dr. C. Rappe (Umeå, Sweden). DNA–cellulose was purchased from Sigma Chemical Co. (St. Louis, MO). All other materials (buffers, chromatographic matrices, and marker proteins) were from standard sources, as described previously (Cuthill et al., 1987; Wilhelmsson et al., 1986).

Cells. The Hepa 1c1c7 cells, a subclone of the mouse hepatoma line Hepa 1 (Hankinson, 1979), and the mutant line c4 derived from it (Legraverend et al., 1982; Hankinson, 1983) were kindly provided by Dr. O. Hankinson (University of California, Los Angeles, CA). Culture conditions for the Hepa 1c1c7 cell line have been described previously (Cuthill et al., 1987). c4 mutant cells were grown in minimum essential medium supplemented with 10% fetal calf serum (Gibco), 2 mM L-glutamine (Flow), 100 IU/mL bensylpenicillin (Astra, Sweden), and 0.1 mg/mL streptomycin (Novo, Copenhagen, Denmark). The cells were maintained as for wild-type Hepa 1c1c7 cells (Cuthill et al., 1987).

Buffers. The following buffers were used: ETGM buffer, 20 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid, 10% (w/v) glycerol, and 2 mM (2-hydroxyethyl)mercaptan, pH 7.4; EP buffer, 20 mM sodium phosphate, 1 mM ethylenediaminetetraacetic acid, and 150 mM NaCl, pH 7.4.

Chromatographic Analyses of Labeled Cytosol. Preparation and labeling of cell cytosol with [^{3}H]TCDD have been described previously (Cuthill et al., 1987) as have the procedures used in the determination of the chromatographic properties of the dioxin receptor on heparin–Sepharose and DNA–cellulose (Wilhemsson et al., 1986). Briefly, cytosol was labeled at 0–4 °C for 18 h prior to analysis on polyanionic exchange matrices. High-performance gel permeation chromatography of labeled cell cytosol was performed at 4 °C on a prepacked Superose 12 HR column (10 × 300 mm) connected to an automated fast protein liquid chromatography system (Pharmacia, Uppsala, Sweden). A total of 500 μL of labeled cytosol (1–2 mg of protein/mL) was applied to the column equilibrated in EP buffer and eluted at a flow rate of 36 mL/h. Relative molecular weights of the various receptor forms were calculated as described previously (Cuthill et al., 1987).

Safety Precautions. Since both TCDD and TCDF are extremely toxic compounds (Poland & Knutson, 1982), their use necessitated special handling procedures as outlined elsewhere (Poland et al., 1976). Contaminated disposable materials were sent away for high-temperature incineration.

Results and Discussion

DNA Binding Properties of the Wild-Type and Nuclear Transfer Deficient Dioxin Receptors. Chromatography of [^{3}H]TCDD-labeled receptor from wild-type and nt^- Hepa 1c1c7 cytosol on DNA–cellulose resulted in a very low degree of retention of labeled material on the column. In both cases more than 90% of the recovered protein-bound radioactivity was associated with the flow-through fraction of the column. A total of 5–8% of the protein-bound radioactivity from wild type cytosol was retained and eluted at a salt concentration of ~0.2 M KCl (Figure 1A) while ~4% of the nt^- recovered activity was retained only weakly and eluted at a salt concentration of ~0.02 M KCl (Figure 1B).

In contrast to the results obtained on DNA–cellulose, ~96% and 90% of the recovered protein-bound radioactivity was retained when wild-type and nt^- cytosols were chromatographed on heparin–Sepharose, respectively. These results are in agreement with results obtained for the non DNA binding and DNA binding forms of the rat hepatic dioxin receptor (Wilhelmsson et al., 1986). Similar results were obtained whether the retained material was eluted by a linear KCl gradient (Figure 2) or a single 0.5 M KCl washing step (data not shown). In three separate experiments the labeled wild-

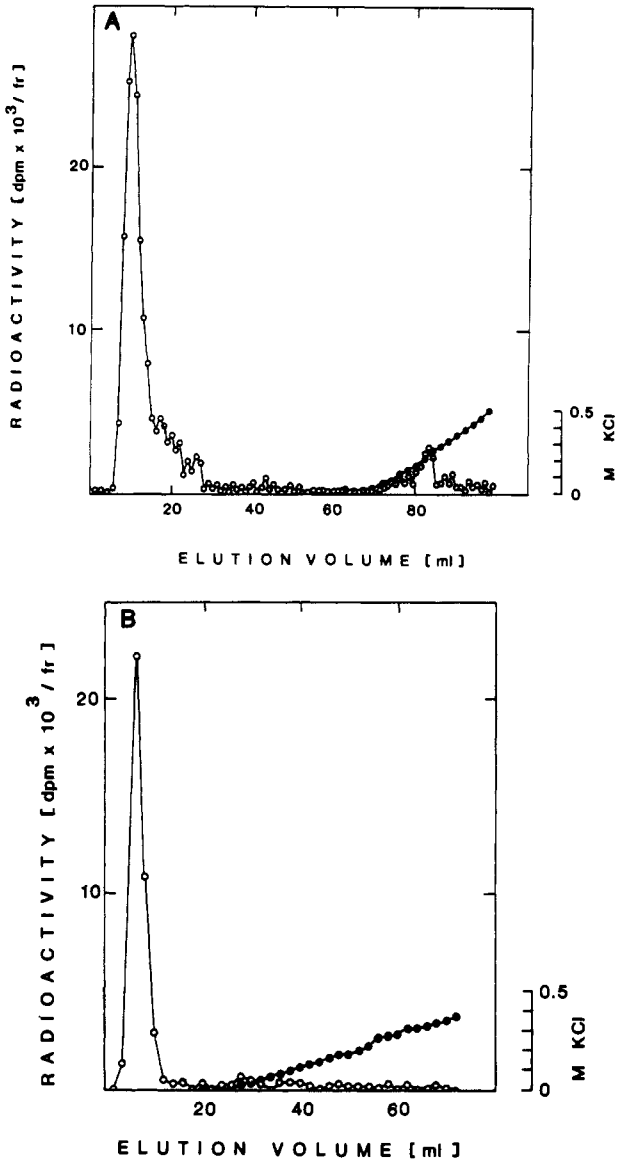

FIGURE 1: DNA–cellulose chromatography of [^{3}H]TCDD-labeled cytosol from wild-type and nt$^-$ mutant mouse hepatoma cells. (A) Wild-type Hepa 1c1c7 cytosol (2 mL, ~2 mg of protein/mL) was labeled with 3 nM [^{3}H]TCDD at 0–4 °C for 18 h and chromatographed on DNA–cellulose (1.5 × 5 cm) at a flow rate of ~10 mL cm^{-2} h^{-1}. (B) Under identical experimental conditions, c4 mutant mouse hepatoma cell cytosol (2 mL, ~2 mg of protein/mL) was labeled with 3 nM [^{3}H]TCDD and analyzed by DNA–cellulose chromatography. Fractions were assayed for radioactivity (○) and conductivity (●).

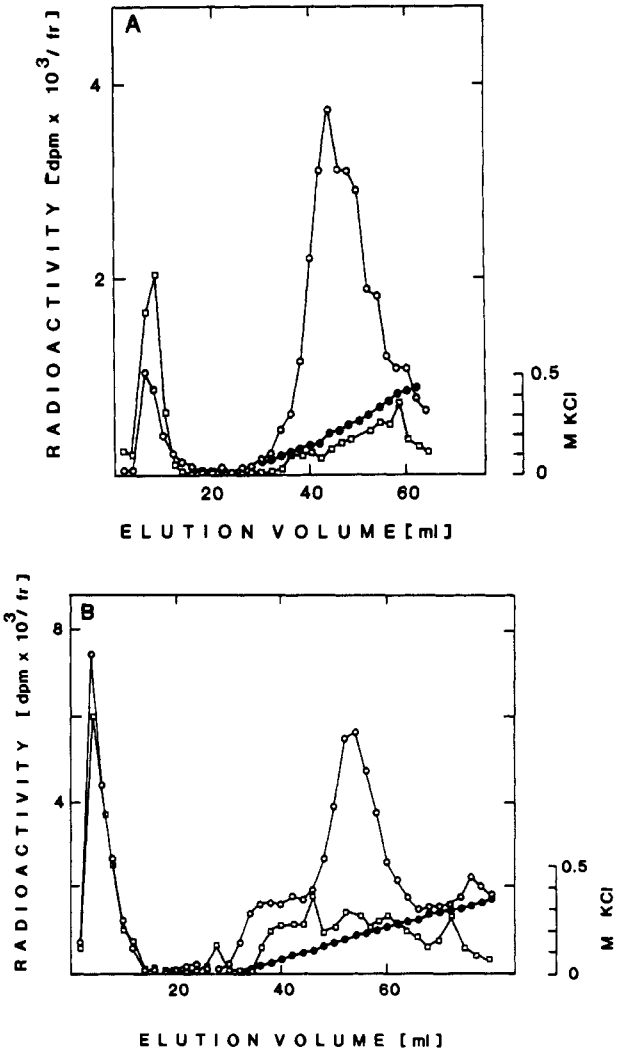

FIGURE 2: Heparin–Sepharose chromatography of the wild-type and nt$^-$ mutant dioxin receptor. (A) Wild-type Hepa 1c1c7 cytosol (2 mL, ~2 mg of protein/mL) was labeled with 3 nM [^{3}H]TCDD at 0–4 °C for 18 h in the absence (○) or presence (□) of a 200-fold molar excess of unlabeled TCDF and analyzed by heparin–Sepharose chromatography on 1.5 × 3 cm columns. (B) Similarily, c4 mutant mouse hepatoma cell cytosol (2 mL, ~2 mg of protein/mL) was labeled with 3 nM [^{3}H]TCDD in the absence (○) or presence (□) of a 200-fold molar excess of unlabeled TCDF and chromatographed on heparin–Sepharose. Fractions were analyzed for radioactivity and conductivity (●).

type and nt$^-$ dioxin receptors eluted from heparin–Sepharose at salt concentrations of 0.2–0.3 and 0.15–0.2 M KCl, respectively (Figure 2).

Chromatography of the labeled rat hepatic dioxin receptor on heparin–Sepharose is known to transform[2] the receptor from a predominantly non DNA binding form to a DNA binding form in vitro (Wilhelmsson et al., 1986). In analogy to these observations, chromatography of labeled cytosol from wild-type Hepa 1c1c7 cells on heparin–Sepharose resulted in an increase in retention of protein-bound radioactivity on DNA–cellulose from ~5–8% (cf. above) to 43% (Figure 3A,B). In contrast, however, under these conditions there is little, if any, detectable binding of the nt$^-$ dioxin receptor to DNA–cellulose (Figure 3C,D).

In summary it can be concluded that the nt$^-$ variant dioxin receptor has a reduced affinity for nonspecific DNA in vitro relative to the wild-type receptor. The affinity for heparin–Sepharose of the nt$^-$ mutant dioxin receptor is in parity with the affinity for the same matrix of the nonliganded, non DNA binding forms of the rat hepatic dioxin and glucocorticoid receptors (Hannah et al., 1986; Wilhelmsson et al., 1986). The difference in affinity for heparin–Sepharose of the wild-type and nt$^-$ dioxin receptor forms, in turn, is in good agreement with the difference in salt concentration required to extract these receptor forms from cell nuclei (Whitlock & Galeazzi, 1984). However, it is not known whether the low salt concentration required for the extraction of the unoccupied dioxin receptor reflects nuclear localization of the receptor (Denison et al., 1986; Gudas et al., 1986). It is of interest to note, however, that the DNA binding properties of the receptor may

[2] Receptor activation and transformation are terms generally used for the process whereby steroid hormone receptors acquire the ability to bind to target elements within cell nuclei in vivo and to DNA–cellulose in vitro. Due to the general similarities between the glucocorticoid and dioxin receptor systems, the same terminology will be used for the dioxin receptor.

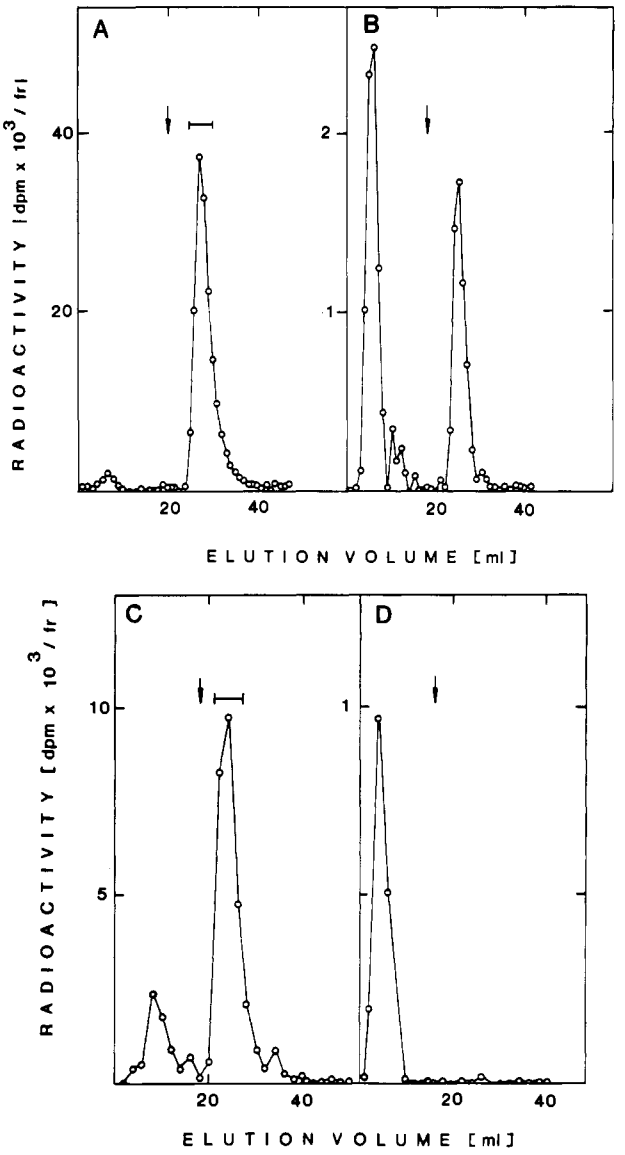

FIGURE 3: Sequential chromatography on heparin–Sepharose and DNA–cellulose of wild-type and nt⁻ mutant cytosol labeled with [³H]TCDD. (A) Wild-type Hepa 1c1c7 cytosol (2 mL, ~2 mg of protein/mL) was labeled with 3 nM [³H]TCDD at 0–4 °C for 18 h and applied to heparin–Sepharose. Retained material was eluted with ETGM buffer containing 0.5 M KCl (arrow). Receptor-containing fractions were pooled (pool indicated by a bar), desalted by Sephadex G-25 chromatography, and chromatographed on DNA–cellulose. Retained material was again eluted with ETGM buffer containing 0.5 M KCl (B). (C) c4 mutant mouse hepatoma cell cytosol (2 mL, ~2 mg of protein/mL) was labeled with 3 nM [³H]TCDD and subjected to sequential chromatography on heparin–Sepharose and (D) DNA–cellulose as described above.

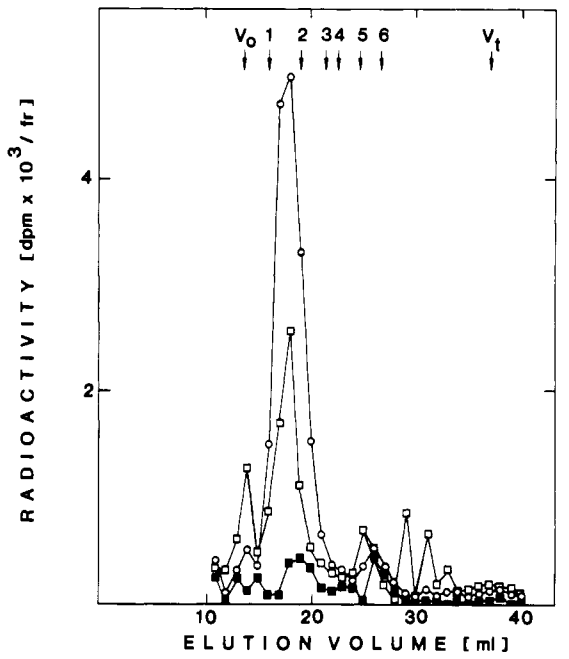

FIGURE 4: High-performance gel permeation chromatography of wild-type and nt⁻ mutant dioxin receptors. Cytosol from wild-type Hepa 1c1c7 [(○) ~2 mg of protein/mL] or c4 mutant mouse hepatoma cells [(□) ~2 mg of protein/mL] was labeled with 3 nM [³H]TCDD at 0–4 °C for 18 h. In a parallel experiment, c4 mutant cytosol was labeled with [³H]TCDD in the presence of a 200-fold excess of unlabeled TCDF (■). Labeled material (500 μL) was analyzed on a Superose 12 column as described under Experimental Procedures. The column was calibrated with blue dextran (V_0), ^3H_2O (V_t), and the following proteins (R_s values in parentheses): 1, thyroglobulin (8.6 nm); 2, ferritin (6.1 nm); 3, aldolase (4.8 nm); 4, bovine serum albumin (3.5 nm); 5, ovalbumin (3 nm); 6, myoglobin (2.0 nm).

not be correlated with the nuclear uptake of the protein since it has been suggested that the putative nuclear localization signal of steroid receptors is close to but distinct from the DNA binding domain (Wolff et al., 1987).

In mutant mouse lymphoma cells, glucocorticoid receptors of the nt⁻ type are defective in nuclear uptake in vivo and exhibit low affinity for DNA–cellulose in vitro, in contrast to the wild-type receptor (Yamamoto et al., 1976; Gehring, 1986). The classical model for steroid hormone action as well as the model for dioxin action postulates that ligand is required for nuclear translocation, i.e., high affinity for nuclear components (Yamamoto, 1985; Whitlock, 1986). In vitro, certain manipulations are necessary for transforming both receptors from non DNA binding to DNA binding forms following ligand binding (Yamamoto & Alberts, 1976; Hannah et al., 1986; Wilhelmsson et al., 1986). However, it does not seem to be possible to in vitro transform the nt⁻ glucocorticoid or dioxin receptor mutants. Thus, binding of ligand appears to be a necessary but not sufficient criterion for DNA binding in vitro of both the glucocorticoid and dioxin receptors. The biochemical mechanism(s) underlying the so-called transformation step of the wild-type receptors is (are) still unknown.

Molecular Properties of Wild-Type and nt⁻ Mouse Hepatoma Dioxin Receptors. In the rat and mouse, the dioxin receptor is a highly asymmetrical molecular of $M_r \cong 100\,000$. However, we have shown that the dioxin receptor in wild-type Hepa 1c1c7 cells has a pronounced tendency to form stable aggregates of $M_r \approx 300\,000$, even at high ionic strength (Cuthill et al., 1987). A possible candidate for a receptor-associated factor contained within this large aggregate is the $M_r \approx 90\,000$ heat-shock protein which, under certain conditions, readily associates with several steroid hormone receptors (Catelli et al., 1985). When wild-type cells were homogenized in buffer containing sulfhydryl-reducing agents and analyzed on Superose columns in the absence of glycerol, the wild-type dioxin receptor eluted as an $R_s \cong 6.6$ nm species (Figure 4). Under identical conditions, gel permeation chromatography of the nt⁻ variant receptor revealed a similar predominating $R_s \cong 6.6$ nm entity (Figure 4). On sucrose density gradients, both receptors sedimented around ~4 S (data not shown). Thus, under certain conditions, it is possible to dissociate both the nt⁻ and wild-type forms of the dioxin receptor into monomers with similar gross structural properties (calculated $M_r \approx 100\,000$).

The nt⁻ glucocorticoid receptor mutant is similar in size to the wild-type receptor, i.e., $M_r \cong 90\,000$–$100\,000$ (Gehring & Hotz, 1983). Sequence comparisons (Weinberger et al., 1985; Krust et al., 1986; Miesfeld et al., 1986) in combination with

mutation analyses (Giguère et al., 1986; Godowski et al., 1987) of wild-type human and rat glucocorticoid receptors have defined two conserved domains in the carbon-terminal half of the protein carrying determinants for DNA and hormone binding. The nt⁻ mutant glucocorticoid receptor in S49 mouse lymphoma cells has recently been shown to exhibit a single amino acid substitution within the putative DNA binding domain of the receptor protein (Danielsen et al., 1986). In analogy, the nt⁻ mutant dioxin receptor does not contain any detectable deletion of the protein. It is also shown that partial proteolytic digestion of the monomeric $M_r \approx 100\,000$ rat hepatic dioxin receptor results in a loss of DNA binding without any concomitant change in gross structure (Hannah et al., 1986; Wilhelmsson et al., 1986). Hence, a similar model of functional domain structure as formulated for the glucocorticoid receptor [for a review, see Gustafsson et al. (1987)] may be applicable to the dioxin receptor system. However, as discussed in the introduction, it is possible that a factor in addition to the dioxin receptor may also be involved in the mechanism of dioxin action. This leaves open the possibilities that the nt⁻ dioxin receptor either carries a defect in its DNA binding domain and/or is deficient in its interaction with a putative regulatory factor.

In conclusion, it is possible to correlate DNA binding in vitro of the dioxin receptor with dioxin responsiveness in vivo which indicates strong similarities between the glucocorticoid and dioxin receptor models.

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Registry No. TCDD, 1746-01-6.

REFERENCES

Bourgeois, S., & Casson, J. C. (1985) *Biochem. Actions Horm. 12*, 311–351.

Catelli, M. G., Binart, N., Jung-Testas, I., Renoir, J. M., Baulieu, E. E., Feramisco, J. R., & Welch, W. J. (1985) *EMBO J. 4*, 3131–3135.

Cuthill, S., Poellinger, L., & Gustafsson, J.-Å. (1987) *J. Biol. Chem. 262*, 3477–3481.

Danielsen, M., Northrop, J. P., & Ringold, G. M. (1986) *EMBO J. 5*, 2513–2522.

Denison, M. S., Harper, P. A., & Okey, A. B. (1986) *Eur. J. Biochem. 155*, 223–229.

Gehring, U. (1986) *Mol. Cell. Endocrinol. 48*, 89–96.

Gehring, U., & Hotz, A. (1983) *Biochemistry 22*, 4013–4018.

Giguère, V., Hollenberg, S. M., Rosenfeld, M. G., & Evans, R. M. (1986) *Cell (Cambridge, Mass.) 46*, 645–652.

Godowski, P. J., Rusconi, S., Miesfeld, R., & Yamamoto, K. R. (1987) *Nature (London) 325*, 365–368.

Gudas, J. M., Kirenlampi, S. O., & Hankinson, O. (1986) *J. Cell. Physiol. 128*, 441–448.

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., & Agnati, L. (1987) *Endocr. Rev. 8*, 185–234.

Hankinson, O. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 373–376.

Hankinson, O. (1983) *Somat. Cell Genet. 9*, 497–514.

Hannah, R. H., Lund, J., Poellinger, L., & Gustafsson, J.-Å. (1986) *Eur. J. Biochem. 156*, 237–242.

Krust, A., Green, S., Argos, P., Kumar, V., Walter, P., Bornert, J.-M., & Chambon, P. (1986) *EMBO J. 5*, 891–897.

Legraverend, C., Hannah, H., Eisen, H., Owens, I., Nebert, D., & Hankinson, O. (1982) *J. Biol. Chem. 257*, 6502–6507.

Miesfeld, R., Rusconi, S., Godowski, P. J., Maler, B., Okret, S., Wikström, A.-C., Gustafsson, J.-Å., & Yamamoto, K. R. (1986) *Cell (Cambridge, Mass.) 46*, 389–399.

Northrop, J. P., Gametchu, B., Harrison, R. W., & Ringold, G. M. (1985) *J. Biol. Chem. 260*, 6398–6403.

Poellinger, L., Lund, J., Gillner, M., & Gustafsson, J.-Å. (1985) in *Molecular Mechanism of Steroid Hormone Action* (Moudgil, V. K., Ed.) pp 755–790, de Gruyter, New York.

Poland, A., & Knutson, J. (1982) *Annu. Rev. Pharmacol. Toxicol. 22*, 517–554.

Poland, A., Glover, E., & Kende, A. S. (1976) *J. Biol. Chem. 251*, 4936–4946.

Stevens, J., Stevens, Y.-W., & Haubenstock, H. (1983) *Biochem. Actions Horm. 10*, 383–446.

Weinberger, C., Hollenberg, S. M., Rosenfeld, M. G., & Evans, R. M. (1985) *Nature (London) 318*, 670–672.

Whitlock, J. P., Jr. (1986) *Annu. Rev. Pharmacol. Toxicol. 26*, 333–369.

Whitlock, J. P., Jr., & Galeazzi, D. (1984) *J. Biol. Chem. 259*, 980–985.

Wilhelmsson, A., Wikström, A.-C., & Poellinger, L. (1986) *J. Biol. Chem. 261*, 13456–13463.

Wolff, B., Dickson, R. B., & Hanover, J. A (1987) *Trends Pharmacol. Sci. 8*, 119–121.

Yamamoto, K. R. (1985) *Annu. Rev. Genet. 19*, 209–252.

Yamamoto, K. R., & Alberts, B. (1976) *Annu. Rev. Biochem. 45*, 721–746.

Yamamoto, K. R., Gehring, U., Stampfer, M. R., & Sibley, C. H. (1976) *Recent Prog. Horm. Res. 32*, 3–32.