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EFFECT OF CHAOTROPIC ANIONS ON THE RATE OF DISSOCIATION OF ESTRADIOL-RECEPTOR PROTEIN COMPLEXES OF MOUSE UTERINE CYTOSOL[1]

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SUMMARY: The stability of mouse uterine cytosol receptor-[^{3}H]estradiol complex was evaluated in the presence of neutral salts of the Hoffmeister series. Marked increases in the rate of dissociation of the complex were observed with the more chaotropic anions (SCN^-, ClO_4^-, NO_3^-, Br^-), and the effects of these ions were greater at lower temperatures, where water assumes a more rigid structure. At higher temperatures F^- and CH_3COO^-, which tend to stabilize water structure, led to retardation of the rate of dissociation of the hormone-receptor complex. There was essentially no change in steroid specificity in the presence of the markedly chaotropic salts. The perturbation of water structure adjacent to the steroid binding site is a factor to be considered in the isolation of steroid receptor complexes.

In the regulation of gene expression by steroid hormones the primary step is the avid binding of the steroids to specific proteins in the cytosol. To isolate and characterize these receptors has proved difficult, since these important proteins are present in minute quantities and are exceedingly labile. The presence of estradiol receptors in target and nontarget tissues of the mouse has been reported from this and other laboratories (1-6).

During our attempts at purification of the mouse uterine receptor, we observed a correlation between the stability of the steroid-receptor complex and the influence of ions on water structure. We report here the effect of chaotropic anions on the rate of dissociation of estradiol from receptor complexes in mouse uterine cytosol. These ions provide an effective

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means of evaluating the role of water structure adjacent to the active site of a protein (7).

MATERIALS AND METHODS

Preparation of cytosol: Uteri from 35-day-old NYA:Nylar mice were used. After careful separation from fatty matter the excised uteri were minced with scissors and homogenized with 3 volumes of a cold solution containing 0.32 M sucrose, 0.1 mM dithiothreitol, and 0.01 M Tris-HCl buffer, pH 7.6, using a Kontes Duall 24 glass tube and a Teflon plunger. The homogenate was centrifuged in a Beckman J-21 centrifuge with a JA-20 rotor at 12,000 x g for 10 min. The supernatant was recentrifuged at 300,000 x g for 60 min in a Beckman preparative ultracentrifuge (L-75) with 75 Ti rotor. The resulting supernatant was designated the cytosol. Its protein concentration, determined by the method of Schaffner and Weissman (8), was about 5 mg/ml. Testosterone was added to a final concentration of 1.2 μM, and the suspension was stored at -20°C until used.

Assay of [^{3}H]estradiol dissociation from estrogen receptors: Uterine cytosol pretreated with testosterone was incubated with 7 nM [^{3}H]estradiol for 60 min at 4°C. Unlabeled estradiol was added at 0.4 μM (final concentration) in the presence (0.36 M final concentration) or absence of salts. The reaction mixture was then transferred to a regulated temperature bath (MGW/Lauda), and incubation was continued at 10, 20, or 30°C.

Aliquots (200 μl) were withdrawn at sequential times, and the amount of protein-bound [^{3}H]estradiol in each was measured by the gel filtration technique described by Penefsky (9). A plastic tuberculin syringe fitted with a porous polyethylene disc (70 μ pore size) was packed to the 0.8-ml mark with Sephadex G-25 medium previously swollen in 20 mM Tris-HCl buffer, pH 7.6. The column was placed in a test tube and spun in a tabletop centrifuge (International Equipment Corp.) with a swinging bucket rotor at full speed for 3 min. A sample of reaction mixture (200 μl) was placed on the top of the column, and centrifugation was repeated as before. The effluent was collected in a fresh test tube, then mixed with 0.8 ml of water and 10 ml of Aquasol (New England Nuclear Corp.), and the radioactivity was determined in a Beckman LS-250 scintillation counter. This method compares well with the technique of charcoal-dextran adsorption of free estradiol (10). In a typical assay of protein-bound estradiol in a reaction mixture, both methods gave almost identical values. However, the charcoal-dextran method is unreliable in the presence of high concentrations of certain salts, such as KCl, NaBr and $NaNO_3$ (11).

First-order rate constants were calculated from regressions of the percent of protein-bound estradiol as a function of time. Each value was corrected for nonspecifically bound estradiol, which was determined by incubating the same amount of cytosol with a 100-fold excess of nonradioactive estradiol, in addition to radioactive estradiol, and measuring the amount of protein-bound radioactivity.

A semilogarithmic plot of the percent of residual protein-bound estradiol as a function of time resulted in a linear decay curve, which is characteristic of a first-order reaction.

Reagent-grade potassium salts of Cl^-, Br^-, and F^- and sodium salts of NO_3^-, ClO_4^-, SCN^-, and CH_3COO^- were used in this study.

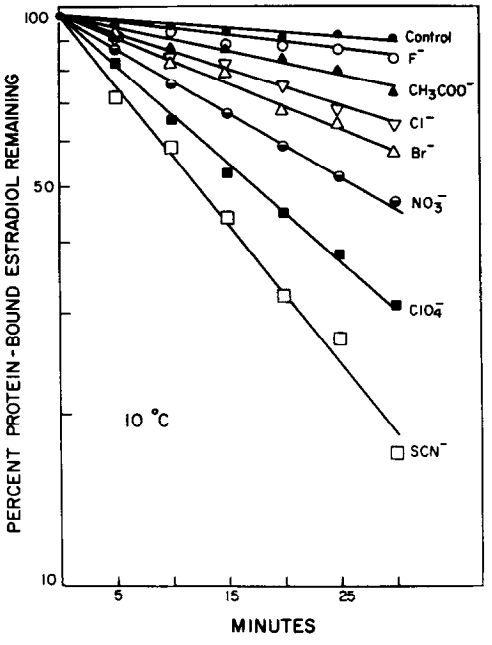

Figure 1: Rates of dissociation of mouse uterine estradiol-receptor complexes in the presence of neutral salts at 10°C. 100% represents ≃35 fmoles of bound estradiol per ≃0.73 mg cytosol protein. The concentration of ions was 0.36 M in all cases.

RESULTS AND DISCUSSION

The rate of dissociation of [^{3}H]estradiol-receptor protein complex in mouse uterine cytosol at 10°C in the absence and in the presence of various anions (0.36 M) of the Hoffmeister series is shown in Figure 1. The complex, once formed, is stable in the absence of any added neutral salt, so that the bound [^{3}H]estradiol exchanges at a very slow rate with the added excess of unlabeled estradiol. This observation is consistent with the reported stability of estradiol receptor complexes from other sources (12, 13).

High concentrations of neutral salts increased the rate of dissociation. Their relative effectiveness was as follows: $SCN^- > ClO_4^- > NO_3^- > Br^- > Cl^- > CH_3COO^- > F^-$, in good agreement with the reported effect of these ions on the structure and lipophilicity of water (14, 15). SCN^-, ClO_4^- and NO_3^- increase the water solubility of a variety of organic compounds which are sparingly soluble in water, and this effect has been attributed to water

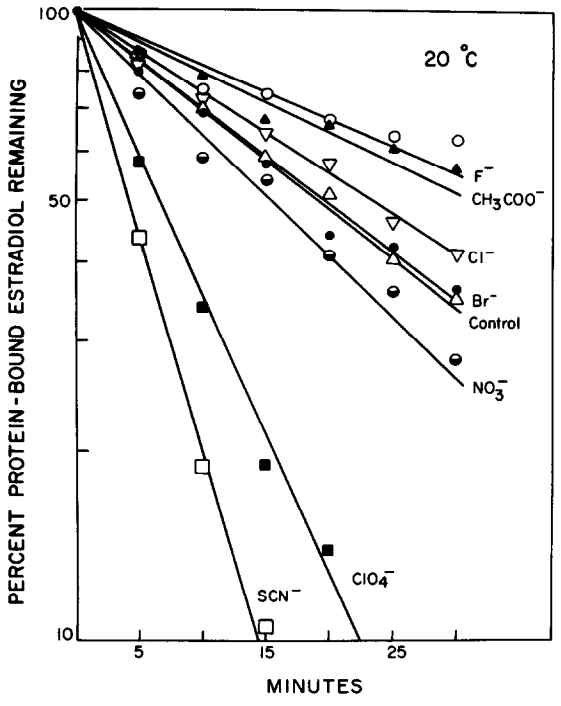

Figure 2: Rates of dissociation of mouse uterine estradiol-receptor complexes in the presence of neutral salts at 20°C.

structure-breaking properties of these ions, whereas CH_3COO^- and F^- have a stabilizing influence (15).

This effect was more pronounced at higher temperatures. At 20°C and 30°C the estradiol-receptor complex became increasingly labile in the absence of any added salt (Fig. 2, Table 1). However, F^- and CH_3COO^- lowered the rate of dissociation considerably, and Cl^- and Br^- did so marginally, but NO_3^-, ClO_4^- and SCN^- ions led to a significant increase in the rate.

The lability of the estradiol-receptor complex at higher temperatures may be due in part to a heat-induced perturbation of water structure (7, 16) around the steroid binding site. Decrease of the dissociation rate by the structure stabilizing ions F^- and CH_3COO^- is consistent with this view (Fig. 2). Chaotropic effects are greater at lower temperatures, where the lattice of water molecules is more rigid. This is seen by a comparison at

TABLE 1

Half-lives ($t_{1/2}$) and kinetic rate constants of dissociation of the mouse uterine estradiol-receptor complex at three temperatures

Ion	10°		20°		30°	
	$t_{1/2}$ (min)	k (min^{-1})	$t_{1/2}$ (min)	k (min^{-1})	$t_{1/2}$ (min)	k (min^{-1})
None	185.0	0.0037	19.0	0.0364	4.25	0.163
F^-	142.0	0.0049	34.5	0.0201	6.0	0.115
CH_3COO^-	84.0	0.0082	21.0	0.0224	5.25	0.132
Cl^-	47.5	0.0147	23.25	0.0298	3.25	0.213
Br^-	37.0	0.0187	19.5	0.0355	3.75	0.185
NO_3^-	26.0	0.0270	15.25	0.0454	2.5	0.277
ClO_4^-	17.5	0.0396	6.5	0.106	1.75	0.397
SCN^-	12.5	0.0554	4.25	0.163	1.25	0.554

10, 20, and 30°C of the ratio $k_{ion}/k_{control}$, where k_{ion} is the first-order rate constant of the dissociation of the estradiol-receptor complex in the presence of 0.36 M salt and $k_{control}$ is the rate constant in the absence of additional salt (Table 2). At each temperature the ratio increased from F^- to SCN^- in the predicted sequence for perturbation of water structure by these ions. Furthermore, the ratios for the more chaotropic salts (SCN^-, ClO_4^-, NO_3^-, Br^-) were significantly higher at 10°C than at higher temperatures. The close correlation of the dissociation rate with the chaotropic activity of the salts, together with the amplification of this effect at lower temperatures, indicates that preservation of the water structure around the steroid binding site is an important factor in the stability of the complex.

TABLE 2

Ratios of dissociation of estradiol-receptor complexes ($k_{ion}/k_{control}$) at three temperatures

Ion	10°	20°	30°
None	1	1	1
F^-	1.3	0.55	0.67
CH_3COO^-	2.2	0.61	0.72
Cl^-	3.9	0.82	1.23
Br^-	5.0	0.97	1.07
NO_3^-	7.3	1.25	1.60
ClO_4^-	10.7	2.93	2.43
SCN^-	14.9	4.48	3.20

The ions did not alter the specificity of the binding (data not shown). Addition of testosterone, estren-17β-ol-3-one, estren-17-one did not promote the release of bound estradiol; but diethylstilbesterol, which binds to the estradiol site with a similar affinity (17), could stimulate this release, as did unlabeled estradiol. The dissociation rate constant at 10°C, using diethylstilbesterol, was 0.003 with F^- and 0.0505 with SCN^-, virtually identical to the corresponding rate constants observed with unlabeled estradiol (Table 1).

Estradiol receptors aggregate in a nonspecific manner (18, 19) under a variety of conditions, and chaotropic salts such as KBr and NaSCN have been used to prevent such aggregation (20, 21). It is likely that the surface is more ionic than the steroid-binding pocket, since the receptor protein binds strongly to anionic supports such as phosphocellulose (22), heparin Sepharose (23), DNA-cellulose (24), and blue Sepharose (unpublished observations) and high salt concentrations ($\geq$ 0.4 M) are required in each case to release the

receptor protein. Chaotropic salts such as NaSCN have also been used to release the protein (23). However, protein released from these anionic supports are often deficient in bound estradiol and seem unable to rebind estradiol (19). It has been our experience that receptor protein released from the blue Sepharose column by 0.4 M NaSCN lost much of the bound [^{3}H]-estradiol and was inactive in the oligo(dT) cellulose binding assay (6) (data not shown).

Our results indicate the importance of water structure in maintaining the estradiol binding site and should be considered in the choice of a neutral salt for the purification and characterization studies of estradiol-binding proteins.

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REFERENCES

1. Shyamala, G., Mori, T. and Bern, H. A. (1974) J. Endocrin. 63, 275-284.
2. Fox, T. O. and Johnston, C. (1974) Brain Res. 77, 330-337.
3. Bullock, L. P. and Bardin, C. W. (1975) Endocrinology 97, 1106-1111.
4. Detlefsen, M. A., Smith, B. C. and Dickerman, H. W. (1977) Biochem. Biophys. Res. Commun. 76, 1151-1158.
5. Bouton, M. M. and Raynard, J. P. (1978) J. Steroid Biochem. 9, 9-15.
6. Thanki, K. H., Beach, T. A. and Dickerman, H. W. (1978) J. Biol. Chem. (In press).
7. von Hippel, P. H. and Schleich, T. (1969) Structure and Stability of Biological Macromolecules, Timasheff, S. N. and Fasman, G. D., eds., pp. 417-574, Marcel Dekker, Inc., New York.
8. Schaffner, W. and Weissman, C. (1973) Anal. Biochem. 56, 502-514.
9. Penefsky, H. (1977) J. Biol. Chem. 252, 2891-2899.
10. Sandborn, B. M., Rao, B. R. and Korenman, S. G. (1971) Biochemistry 10, 4955-4960.
11. Peck, E. J. and Clark, J. H. (1977) Endocrinology 101, 1034-1043.
12. Troung, H. and Baulieu, E. E. (1971) Biochim. Biophys. Acta 237, 167-172.
13. Weichman, B. M. and Notides, A. C. (1977) J. Biol. Chem. 252, 8856-8862.
14. von Hippel, P. H. and Wong, K-Y. (1964) Science 145, 577-580.
15. Hatefi, Y. and Hanstein, W. G. (1969) Proc. Natl. Acad. Sci., USA 62, 1129-1136.
16. Walrafen, G. E. (1972) Water, Franks, F., ed., Vol. 1, pp. 151-214, Plenum Press, London.
17. Korenman, S. G. (1969) Steroids 13, 163-167.
18. Stancel, G. M., Leung, K. M. T. and Gorski, J. (1973) Biochemistry 12, 2130-2141.
19. Jensen, E. V. and DeSombre, E. R. (1972) Ann. Rev. Biochem. 41, 203-230.

20. Sica, V., Nola, E., Puca, G. A. and Bresciani, F. (1976) Biochemistry 15, 1915-1923.
21. Auricchio, F., Rotondi, A., Schiavone, E. and Bresciani, F. (1978) Biochem. J. 169, 481-488.
22. Schrader, W. T., Buller, R. E., Kuhn, R. W. and O'Malley, B. W. (1974) J. Steroid Biochem. 5, 989-999.
23. Molinari, A. M., Medici, N., Moncharmont, B. and Puca, G. A. (1977) Proc. Natl. Acad. Sci., USA 74, 4886-4890.
24. Irving, R. and Mainwaring, W. I. P. (1974) J. Steroid Biochem. 5, 711-716.