Thermodynamics of steroid binding to the human glucocorticoid receptor

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The thermodynamics of the interaction of glucocorticoids with their receptor were studied in cytosol from human lymphoblastoid cells. The rate and affinity constants of dexamethasone and cortisol between 0°C and 25°C were calculated by curve-fitting from time-course and equilibrium kinetics. The data were consistent with a simple reversible bimolecular interaction. Arrhenius and Van't Hoff plots were curvilinear for both steroids. At equilibrium, the solution for the equation \( \Delta G = \Delta H - T \cdot \Delta S \) (eqn. 1) was (in kJ mol\(^{-1}\)) \(-47 = 36 - 83\) (dexamethasone) and \(-42 = -9 - 33\) (cortisol) at 0°C. Enthalpy and entropy changes decreased quasi-linearly with temperature such that, at 25°C, the respective values were \(-50 = -75 + 25\) and \(-43 = -48 + 5\). Thus, for both steroids, the interaction was entropy-driven at low temperature and became entirely enthalpy-driven at 20°C. Thermodynamic values for the transition state were calculated from the rate constants. For the forward reaction, eqn. (1) gave 45 = 84 – 39 (dexamethasone) and 46 = 60 – 14 (cortisol) at 0°C, and 44 = 24 + 20 (dexamethasone) and 46 = 28 + 18 (cortisol) at 25°C. These data fit quite well with a two-step model (Ross & Subramanian, 1981) Biochemistry 20, 3096–3102) proposed for ligand–protein interactions, which involves a partial immobilization of the reacting species governed by hydrophobic forces, followed by stabilization of the complex by short-range interactions. On the basis of this model, an analysis of the transition-state thermodynamics led to the conclusion that no more than half of the steroid molecular area is engaged in the binding process.

The receptor for glucocorticoid hormones is a DNA-binding protein, which, in the unbound state, is found in the cytosol of target cells (Rousseau, 1975). To approach the molecular mechanism of the receptor–steroid binding process, we have performed a thermodynamic analysis of the cell-free binding of steroids to the cytosolic receptor. Earlier studies yielded conflicting results (Koblinsky et al., 1972; Wolff et al., 1978; Jones et al., 1979; Snochowski et al., 1980), presumably owing to receptor thermolability in cytosol above 0°C and because higher temperatures accelerate (Higgins et al., 1973) the so-called transformation of the initial receptor–steroid complex into the DNA-binding conformation, thereby shifting the system away from a simple bimolecular interaction. Discrepancies could also be accounted for by our more recent finding that cell-free binding kinetics are not reproducible unless the concentration of free Ca\(^{2+}\) ions is kept under control (Rousseau et al., 1982) and protection of thiol groups is ensured (P. H. Eliard & G. G. Rousseau, unpublished work). To our knowledge, no information is available about the transition-state thermodynamics of glucocorticoid–receptor interactions, and no thermodynamic study on the human glucocorticoid receptor has been reported so far.

We have used here cytosol from cultured IM-9 cells, an established line of glucocorticoid-sensitive human lymphoblasts which contain steroid receptors for glucocorticoids, but not for other classes of steroid hormones. The binding studies were performed in the presence of a Ca\(^{2+}\)-chelating agent (EDTA), of a thiol-protecting agent (dithiothreitol), and of molybdate ions. Molybdate protects the cytosolic receptor against thermoinactivation (Nielsen et al., 1977) and blocks its transformation into the DNA-binding state (Leach et al., 1979). This enabled us to determine the transition-state and equilibrium thermodynamics of the binding of dexamethasone (9α-fluoro-16α-methyl-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione), which is a potent glucocorticoid agonist in

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this system (Rousseau et al., 1980), and of cortisol, the natural glucocorticoid in the human.

Materials and methods

Chemicals and buffers

$[1,2,4-^3\text{H}]$Dexamethasone (46 Ci/mmol), $[1,2-^3\text{H}]$cortisol (36 Ci/mmol) and $[1,2,6,7(n)-^3\text{H}]$progesterone (96 Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. $[17a$-methyl$-^3\text{H}]$Methyltrienolone (R1881, 56 Ci/mmol) and $[17a$-methyl$-^3\text{H}]$promegestone (R5020, 87 Ci/mmol) were obtained from New England Nuclear Co., Boston, MA, U.S.A. Radiochemical purity was determined by t.l.c. with the solvent systems recommended by the supplier. Samples were evaporated to dryness immediately before use and redissolved at appropriate concentrations in incubation buffers. Non-labelled steroids were from Merck, Sharp and Dohme, Rahway, NJ, U.S.A. (dexamethasone), or from Steraloids (Wilton, NH, U.S.A.). Stock solutions at 1 mM in ethanol were stored at $-20^\circ\text{C}$. All chemicals were reagent grade from Merck, Darmstadt, Germany, or from Sigma Chemical Co., St. Louis, MO, U.S.A., unless mentioned otherwise. Homogenization buffer contained 20 mM-Tricine $\{N \cdot 2$ - hydroxy - 1, 1 - bis(hydroxymethyl)ethyl$\}$glycine] (Calbiochem, San Diego, CA, U.S.A.), pH 7.8 at 0°C, 10 mM-Na$_2$MoO$_4$, 1 mM-EDTA and 2 mM-dithiothreitol.

Cytosol preparation and binding studies

IM-9 cells were grown and harvested, and the cytosol was prepared as described by Rousseau et al. (1980). The concentration of cytosol protein, determined (Lowry et al., 1951) with bovine serum albumin as a standard, was adjusted with homogenization buffer to 5 mg/ml. Cytosol samples were stored at $-80^\circ\text{C}$, a procedure that preserves the receptor for several months. Binding of steroids to the receptor was determined as described by Rousseau et al. (1972). Incubations were conducted in homogenization buffer in water baths at temperatures controlled within 0.1°C, with cytosol contributing one-quarter of the volume. To determine bound steroid, 0.4 ml samples were removed and agitated for 5 s with 0.2 ml of an ice-cold aqueous charcoal suspension containing 25 mg of Norit A/ml and 5 mg of dextran (grade C, from BDH Chemicals, Poole, Dorset, U.K.)/ml. The mixture was centrifuged at 2500 g for 5 min at 4°C. Radioactivity was determined on 0.25 ml of the supernatant by scintillation counting in Picofluor (Packard, Downers Grove, IL, U.S.A.) with an efficiency of 48%. Quenching was corrected by the channels-ratio method. Non-specific binding was determined from incubations, conducted in parallel, which contained in addition 12.5 $\mu$M non-radioactive steroid or 50 $\mu$M-HgCl$_2$, a salt that inactivates the receptor (Rousseau, 1975). This value was subtracted from the amount of total bound steroid to yield receptor-bound steroid. All determinations were done in triplicate.

Calculations

All calculations were based on the initial assumption (Rousseau, 1975; Yeakley et al., 1980) that steroid binding to the glucocorticoid receptor in cytosol involves a simple and reversible biomolecular reaction, without interaction between sites such that

$$S + R \xrightarrow{k_{-1}} RS$$

where $S$ and $R$ refer to the concentrations of free steroid and free receptor respectively, $RS$ refers to the concentration of receptor-steroid complex, and $k_{+1}$ and $k_{-1}$ are the rate constants for association and dissociation. The equilibrium constant $K_A$ is defined by the Law of Mass Action:

$$K_A = \frac{RS}{R \cdot S} = \frac{k_{+1}}{k_{-1}} = \frac{1}{K_D}$$

(2)

The second-order association kinetics are expressed by

$$\frac{dRS(t)}{dt} = k_{+1} \cdot R(t) \cdot S(t) - k_{-1} \cdot RS(t)$$

(3)

where all concentrations are a function of time, $t$. The kinetics of dissociation are first-order:

$$\frac{dRS(t)}{dt} = -k_{-1} \cdot RS(t)$$

(4)

If neither the steroid nor the receptor is being degraded, their concentrations in eqn. (3) can be expressed as a function of their respective initial values at zero time ($t_0$), $S_0$ and $R_0$, such that

$$\frac{dRS(t)}{dt} = k_{+1} \cdot [R_0 - RS(t)] \cdot [S_0 - RS(t)] - k_{-1} \cdot RS(t)$$

(5)

and $RS(t_0) = 0$.

At a time $t$, when equilibrium has been reached, i.e. when $dRS(t)/dt = 0$, eqn. (5) can be solved as follows:

$$RS(t) = 0.5 \cdot [S_0 + R_0 + K_D] - \sqrt{(S_0 + R_0 + K_D)^2 - 4 \cdot S_0 \cdot R_0}$$

(6)

$R_0$ and $K_D$ values were calculated from equilibrium saturation curves obtained by incubating a fixed receptor concentration with various concentrations of steroid. For pilot experiments, the data were plotted by linear regression according to Scatchard (1949). For the thermodynamic studies, $R_0$ and $K_D$ values were determined by curve-fitting of the more precise plot of $RS$ against $S_0$.
to eqn. (6). Curve-fitting was performed by the least-squares method, by using the non-linear regression program MLAB (Knott, 1979), on a Tektronix 4051 terminal (Tektronix, Beaverton, OR, U.S.A.) linked to a DEC 20 computer (Digital Equipment Co., Boston, MA, U.S.A.).

For determination of the rate constants \( k_{+1} \) and \( k_{-1} \), a fixed concentration of receptor was incubated with a fixed concentration of steroid, and receptor-bound steroid was measured at various time points until equilibrium was reached. When plotting \( RS \) as a function of time \( t \), both \( k_{+1} \) and \( k_{-1} \) could be obtained by non-linear curve-fitting of the differential eqn. (5) after introducing \( S_0 \) and \( R_0 \) values (Ketelslegers et al., 1975). In some experiments, a 200-fold excess of non-labelled steroid was added to an incubation pre-equilibrated with labelled steroid, after which receptor-bound steroid was measured at various time points. The value of \( k_{-1} \) could then be determined directly, by using eqn. (4), from semi-logarithmic linear plots of the binding data.

**Results**

*Stability of the receptor sites under cell-free conditions*

Since the \( \Delta pK_a \) of Tricine buffer is \(-0.021^\circ C^{-1} \), increasing the temperature from 0 to 30°C decreased the pH of incubations by about 0.6 unit. We sought conditions under which such pH changes in themselves did not influence the binding properties of the receptor. It was found that the affinity and the binding capacity were constant between pH 7.1 and pH 8.2 at 0°C. We therefore chose to adjust the pH at 7.8 at 0°C such that the lowest pH values brought about by increases in temperature (maximum 30°C) would not be below 7.2. Next we assessed the thermostability of the receptor under our incubation conditions. Fig. 1(a) shows that the receptor was stable for at least 16 h between 0° and 20°C, and for at least 7 h at 25°C. At 30°C, however, inactivation approached 50% by 2 h.

Several steroids that interact with the glucocorticoid receptor behave as glucocorticoid antagonists. Earlier studies have shown that free or antagonist-bound receptor is less stable than agonist-bound receptor (Rousseau et al., 1972), thereby suggesting the existence of at least two receptor conformations. Having found conditions under which the unbound receptor is thermostable (Fig. 1a), we assumed that such conditions would allow a thermodynamic study of the binding of glucocorticoid antagonists. Cytosol was incubated at increasing temperatures with different concentrations of one of the three antagonists progesterone, methyltrienolone and promegestone. Scatchard plots were constructed from the concentrations of specifically bound steroid determined at apparent equilibrium. At 0°C, the \( K_D \) values for the three steroids were 30, 13 and 7 nm respectively. Unexpectedly, however, the receptor-site concentration calculated by extrapolation decreased with increasing temperature (Fig. 1b), as if the receptor occupied by the antagonists were less stable than the unoccupied receptor. This decrease was not due to steroid degradation, as verified by thin-layer radiochromatography of the steroids extracted.
from the incubations. In contrast, receptor occupied by either of the two agonists, dexamethasone and cortisol, was stable under those conditions (Fig. 1b). The Scatchard plots (not shown) obtained with these steroids were linear at 0°C, as in earlier work (Rousseau et al., 1980), and remained so at all temperatures tested, consistent with the simple model postulated above (eqn. 1). On the basis of these results, we had to exclude the antagonists from our thermodynamic study.

Equilibrium thermodynamics

At 0°C, the equilibrium dissociation constant for dexamethasone is in the nanomolar range (the mean $K_D$ value, ±s.e.m., was 1.1 ± 0.1 nM in 12 experiments), i.e. equilibrium is strongly in favour of the steroid–receptor complex because of the markedly negative value of the Gibbs energy change of the system upon binding

$$\Delta G^0 = -RT \ln(1/K_D)$$

where $R$ and $T$ are the gas constant and the absolute temperature respectively. Changes in Gibbs energy value reflect changes in both the enthalpy ($\Delta H^0$) and the entropy ($\Delta S^0$) such that

$$\Delta G^0 = \Delta H^0 - T \cdot \Delta S^0$$

To evaluate the relative contributions of $\Delta H^0$ and $\Delta S^0$ to the binding energy, $K_D$ values for dexamethasone and cortisol were determined at five temperatures between 0 and 25°C by non-linear curve-fitting, by using eqn. (6). For both steroids, $K_D$ values varied with temperature, and Van’t Hoff plots were curvilinear (Fig. 2). Gibbs energy changes were calculated by using eqn. (7), and plots of $\Delta G^0$ against temperature also showed curvilinearity with one extremum, indicating a maximum stability of the complex (d$\Delta G^0$/dT = 0) at 20–25°C for both steroids (Fig. 3).

When $\Delta H^0$ and $\Delta S^0$ are independent of temperature, the Van’t Hoff plot is linear, and these parameters can be determined from the slope and the vertical axis intercept of the plot respectively. Because our plots were curvilinear, we resorted to the method of Osborne et al. (1976), which is to find a parabolic expression of $\Delta G^0$ as a function of temperature:

$$\Delta G^0 = A + B \cdot T + C \cdot T^2$$

where A, B, C are constants. The best fit of the experimental data for the two steroids is shown by the lines in Fig. 3, and coefficients of the parabolic expressions are given in the legend.

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Fig. 2. Van’t Hoff plots of the glucocorticoid–receptor interactions

Each point on the graph was derived by curve-fitting (eqn. 6) from an equilibrium binding experiment involving triplicate incubations with five different concentrations of steroid (see the Materials and methods section). $K_A$ is expressed in M$^{-1}$. The lines on the graph, which extend beyond the experimental points, were obtained by fitting a parabola.

Fig. 3. Influence of temperature on the change in Gibbs energy value at equilibrium

The symbols on the graph correspond to data obtained, by using eqn. (7), from the experimental points shown in Fig. 2. The lines correspond to the computer-drawn best fit of eqn. (9), with coefficients as follows. For dexamethasone (○), $\Delta G^0 = 609420 - 4510T + 7.70T^2$; for cortisol (△), $\Delta G^0 = 196210 - 1620T + 2.75T^2$. 

1984
From eqns. (8) and (9), it follows that
\[
\Delta H^0 = \frac{d(AG^0)}{dT} = A - C \cdot T^2
\] (10)
\[
\Delta S^0 = d\Delta G^0/dT = -B - 2 \cdot C \cdot T
\] (11)
\[
\Delta C_p^0 = d\Delta H^0/dT = -2C \cdot T
\] (12)
where \( \Delta C_p^0 \) is the heat capacity change. Note that \( \Delta C_p^0 \) values are obtained from the second derivative of experimental data and are therefore relatively imprecise.

The equilibrium thermodynamic parameters calculated for dexamethasone and cortisol are given in Table 1. These data were treated graphically as described by Waelbroeck et al. (1979) to see how the parameters of eqn. (8) vary with temperature. Data for dexamethasone are shown in Fig. 4(a). For both steroids, \( \Delta H^0 \) and \( T \cdot \Delta S^0 \) decreased quasi-linearly as temperature increased from 0 to 25°C; the interaction was entropy-driven (positive \( T \cdot \Delta S^0 \)) at low temperatures and became entirely enthalpy-driven (negative \( \Delta H^0 \)) between 20 and 25°C; \( \Delta C_p^0 \) was negative. The interpretation of these data is discussed below.

**Transition-state thermodynamics**

The foregoing data were obtained at equilibrium, and therefore yielded no information about the energy barrier (\( \Delta G^T \)) that must be overcome during the association or dissociation process. This information can be derived from a study of the temperature-dependence of the rate constants. By analogy with the transition-state theory of enzyme kinetics (Dixon & Webb, 1964), one may assume that an activated complex \( RS^T \) of higher energy is in equilibrium with the free ligands and \( RS \) such that

\[
R + S \rightleftharpoons RS^T \rightleftharpoons RS
\] (13)

('activation' should not be confused with the process by which \( RS \) acquires the property to interact with DNA; the latter process is called here 'transformation').

The free-energy change (\( \Delta G^T \)) of the transition state can then be expressed as

\[
\Delta G^T = \Delta H^T - T \cdot \Delta S^T = -RT \cdot \ln k + RT \cdot \ln (bT/h)
\] (14)

where \( k \) is the experimental rate constant (forward or reverse) and \( b \) and \( h \) are the Boltzmann and Planck constants, respectively.

The rate constants were calculated from plots of the time-course of binding conductance at different temperatures (Fig. 5). Mathematical curve-fitting using eqn. (5) yielded \( k_{+1} \) and \( k_{-1} \). The excellent fit obtained for both steroids (Fig. 5), together with the fact that \( k_{-1} \) values were in agreement with those obtained directly in cold-chase experiments
Fig. 4. Thermodynamic parameters of dexamethasone-receptor interactions
Computer-drawn graphs based on eqns. (10) and (11), by using equilibrium data (a) or data for the forward reaction (b).

Fig. 5. Time course of the binding of dexamethasone and cortisol to the glucocorticoid receptor
Representative experiments of binding kinetics at different temperatures. Receptor concentrations \( R_0 \) were as follows (in nM): dexamethasone, 2.1 (0°C), 1.3 (25°C); cortisol, 1.1 (0°C), 1.4 (10°C), 1.3 (20°C). Steroid concentrations \( S_0 \) were as follows (in nM): 29.1 (0°C), 1.3 (25°C); cortisol, 33.2 (0°C), 13.2 (10°C), 4.7 (20°C). The lines correspond to the computer-drawn best fit for eqn. (5).

(see Fig. 6b), and the finding that the rate constants were independent of ligand concentration (results not shown), are all consistent with the simple bimolecular model depicted in eqn. (1). A comparison of the rate and equilibrium constants for dexamethasone and cortisol (Table 1) shows that the lower affinity of cortisol is essentially due to a faster dissociation rate. At low temperatures at least, the latter more than compensates for the faster association rate.

For both steroids, the Arrhenius plots for the forward (Fig. 6a) and the reverse (Fig. 6b) reactions were curvilinear, indicating that \( \Delta H^\ddagger \) and \( \Delta S^\ddagger \) varied with temperature. Since the expression of \( \Delta G^\ddagger \) in eqn. (14) is applicable to both the forward and the reverse reactions, \( \Delta G^\ddagger \) values for each of
these processes were calculated by introducing in eqn. (14) the appropriate rate constants \((k_{+}^{-1}\) or \(k_{-}^{-1}\)) and temperatures. Values for \(\Delta H^\ddagger\), \(\Delta S^\ddagger\) and \(\Delta C_P^\ddagger\) as a function of temperature could then be computed according to eqns. (10)–(12) (Fig. 4b and Table 1). The variation in the experimental rate constants observed at a given temperature affected the convergence of the curve-fitting algorithm, and this yielded results that were less precise than those derived from equilibrium studies. Still, in a given experiment, the \(K_p\) values derived from kinetic data \((k_{-}^{-1}/k_{+}^{-1})\) did not differ significantly from those determined at equilibrium (Table 1). The thermodynamic profiles in Fig. 4b show that at low temperature the energy barrier of the transition state was mainly due to a largely positive enthalpy change, which is partially compensated for by the favourable entropy change. Around 15°C, the entropy change became unfavourable while the enthalpy change kept decreasing, such that the energy barrier \((\Delta G^\ddagger)\) was about the same at 25° as at 0°C.

All the thermodynamic data are summarized in energy diagrams (Fig. 7) which depict the quantitative and qualitative energy changes that are thought to govern the association and dissociation processes for dexamethasone and cortisol.

**Discussion**

The results obtained at equilibrium show that, at 0°C, the glucocorticoid–receptor interaction is partially (for cortisol) or totally (for dexamethasone) under entropic control, as suggested by Munck et al. (1972). This points to the importance of hydrophobic interactions, namely the partial withdrawal of non-polar groups from water, resulting in the entropically favourable dispersion of previously ordered water molecules. The way in which temperature influenced the results obtained at equilibrium is consistent with that interpretation. First, the entropy change decreased with increasing temperature, in keeping with the notion that the organization of water molecules is adversely influenced by temperature. Second, the \(\Delta C_P^\ddagger\) was negative, as expected for hydrophobic interactions. Note that the \(\Delta C_P^\ddagger\) values calculated here are within the range of the values reported for ligand–protein interactions (Sturtevant, 1977).

Independent evidence for strong hydrophobic bonding stems from our earlier observation (van Bohemen & Rousseau, 1982) that non-steroidal compounds structurally unrelated to glucocorticoids bind to the receptor and competitively inhibit steroid binding to the receptor site. These compounds do not display glucocorticoid-agonist activity, suggesting that, as expected, hydrophobic interactions are not specific, and that other forces, presumably dependent on the stereochemical properties of the steroid molecule, stabilize the receptor–steroid complex. The effects of temperature on the binding parameters described here are also consistent with this interpretation. Indeed, at
25°C, neither $\Delta H^\circ$ nor $\Delta S^\circ$ was positive, as would be the case for a purely hydrophobic interaction. This suggests that hydrogen bonds, formed in the low dielectric environment of the receptor protein, and van der Waals (stacking) forces contribute significantly to the binding energy (Ross & Subramanian, 1981). Concerning hydrogen bonds, the importance of proton-acceptor and proton-donor functions on the steroid molecule has been discussed elsewhere (Munck et al., 1972; Rousseau et al., 1979, 1983).

The thermodynamic profile of cortisol binding differs qualitatively from that described for its interaction with transcortin, which is enthalpy-driven and entropically unfavourable at 4°C (Mickelson et al., 1981). This suggests that the binding of steroids to plasma transport proteins and to intracellular receptors involves different molecular mechanisms.

Our data fit nicely with the two-step thermodynamic model proposed by Ross & Subramanian (1981) for ligand–protein interactions. According
to this model, the reacting species become partially immobilized in a primarily hydrophobic association step, the mutual penetration of hydration layers causing a disorder of the solvent. The second step involves short-range interactions, which stabilize the complex and account for the negative enthalpy change seen at high (25°C) temperatures. Consistent with this model, our analysis of the transition state shows that the energy barrier is mainly enthalpic, and stays that way at all temperatures, as predicted from an initial hydrophobic step. At equilibrium, the negative enthalpy contribution of short-range interactions compensates for the positive enthalpy change owing to the hydrophobic contacts and, predictably, outweighs the latter at high temperatures (Table 1).

If this model is valid, then, the question as to how much of the surface of the steroid molecule is engaged in its receptor site can be re-evaluated. This problem had been approached by Wolff et al. (1978), using equilibrium measurements, on the basis of the relationship (see Chothia & Janin, 1975) \( \Delta G = \Delta G_r + \Delta G_s \), where \( \Delta G \) is the observed free energy of the hydrophobic interaction, \( \Delta G_r \) is the free energy associated with the loss of entropy when the ligands become associated, and is therefore equal to \( -T \cdot \Delta S \), and \( \Delta G_s \) is proportional to the surface area involved in hydrophobic bonding and is equal to \( \Delta G_s \) (steroid) + \( \Delta G_s \) (receptor). As pointed out by Ross & Subramanian (1981), this relationship holds true only when van der Waals interactions and hydrogen bonds can be thermodynamically neglected. This would be the case at low temperature during the first step of the model proposed above, namely for the process corresponding to the transition state at 0°C. Using for \( \Delta S_r \) a value of \(-76\) cal (i.e. \(-318 J/\text{K} \cdot \text{mol}^{-1}\)) and for the calculation of \( \Delta G_s \) a value of \(-2250\) cal (i.e. \(-9410 J/\text{nm}^2\)) (see Wolff et al., 1978), we find the following value for the steroid surface area (SA) presumably involved in receptor binding:

\[
SA = \frac{-T \cdot \Delta S_r - \Delta G_s^\dagger}{- \Delta G_s} = \frac{(273 \times 0.318) - 45.6}{2 \times 9.41} = 2.2 \text{ nm}^2
\]

The surface area of the cortisol molecule accessible to the solvent has been estimated as 5.7 nm\(^2\) (Schmit & Rousseau, 1978). It thus seems unlikely that the steroid molecule becomes 'buried' in the receptor protein. This interpretation is consistent with the \( \Delta C_p^\dagger \) value observed for cortisol at 25°C. According to Sturtevant (1977), when short-range interactions and conformational changes can be neglected, the \( \Delta C_p \) resulting from hydrophobic forces at 25°C, \( \Delta C_p \) (hydro), can be expressed as:

\[
\Delta C_p \text{(hydro)} = \frac{1.05 \cdot \Delta C_p - \Delta S}{1.31}
\]

These conditions would be met during the first step in the model proposed, which we have assumed to be described by our analysis of the transition state. By introducing in eqn. (15) the values for \( \Delta C_p^\dagger \) and \( \Delta S^\dagger \) given in Table 1, one obtains a value for \( \Delta C_p \) (hydro) of \(-10171 \cdot \text{K}^{-1} \cdot \text{mol}^{-1}\). Assuming that each mol of water dispersed contributes 5.6 cal (i.e. 23.4 J/\text{K} \cdot \text{mol}^{-1}) to the \( \Delta C_p \) (hydro) at 25°C (Sturtevant, 1977), this value would correspond to about 20 molecules of water lost per complementary molecular surface area. On the basis of a van der Waals radius of 0.14 nm for the water molecule (Chothia, 1975), the interaction would involve about 2.5 nm\(^2\) of steroid surface, which is in agreement with the value (2.2 nm\(^2\)) calculated from the \( \Delta C_p^\dagger \) value.

Finally, our experiments show that receptors bound by antagonists are thermolabile, or become undetectable by our assay procedure when temperature increases (Jones & Bell, 1982). Receptor thermolability has been ascribed to oxidation of thiol groups and to a molybdate-sensitive (dephosphorylation?) process (Houslay et al., 1982). The presence of dithiothreitol and molybdate in the incubation mixture would prevent such mechanisms from taking place, and indeed agonist-bound and free receptors remained stable under our experimental conditions. Thus it is likely that, on binding an antagonist, the receptor assumes a conformation that differs from that engaged in agonist binding or from the unbound state.

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