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IMPORTANCE OF STEROIDS 17-α-HYDROXYL IN THE HYDROGEN BOND FORMATION WITH HUMAN CORTICOSTEROID BINDING GLOBULIN

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SUMMARY

The activation energy for the association of human corticosteroid binding globulin (C.B.G.) with cortisol, corticosterone, 17-α-hydroxy-11-desoxycorticosterone and 11-desoxycorticosterone has been determined by measurement of the activation energy for dissociation and the enthalpy change of these complexes. Values of 3 kcal M^{-1} and 2.7 kcal M^{-1}, weaker than the minimum value due to hydrogen bonding and, within the limits expected for Van Der Waals forces, are obtained for desoxycorticosterone and corticosterone. Those for C.B.G.-cortisol and C.B.G.-17-α-hydroxy-11-desoxycorticosterone reach the value of hydrogen bonding 8.1 and 9.9 kcal M^{-1} respectively. The interaction between C.B.G. and cortisol may take place on the alpha side of the steroid molecule where the 17-α-hydroxy group could be involved in a specific hydrogen bonding function.

INTRODUCTION

Reversible binding of steroids to protein has been studied more widely with human serum albumin than with other proteins [1]. In 1958, Westphall[2] concluded from his studies that the interactions between steroids and human serum albumin take place on the rear side (alpha side) of the steroid molecule which is less sterically hindered and thus more capable of interaction. Extrapolating these conclusions, he explained the important physiological activity of certain subsituents on the alpha side, specially 17-alpha and 9-alpha, attributing a dominant role of these "contact points" in the interaction between steroid and protein.

Recently, corticosteroid-binding-globulin (C.B.G.) has been isolated and purified [3–6] and many authors have measured the relative binding affinities between steroids and C.B.G. of different species [7]. No systematic study of the internal mechanism of binding had been made. After isolation of C.B.G. by affinity chromatography, Rosner[5] studied the kinetics of the dissociation of the C.B.G.–cortisol complex in a flowing system at different temparatures and measured the activation energy of the dissociation [8].

In preliminary work, based on Rosner's technique, we demonstrated that it is possible to obtain values matching those found by this author, using human plasma as the C.B.G.-source of the interference of albumin is taken into account. We also obtained values of the equilibrium constants and the dissociation rates for several steroids of increasing polarity with human or rat C.B.G. (unpublished data).

Rosner[8] has suggested that the "association of C.B.G. and cortisol" proceeds through a transition state in which one hydrogen bond has been ruptured. We have tried to find which of the three hydroxyl groups of the cortisol molecule could be responsible for the hydrogen bond. We have calculated and compared the activation energy for association of C.B.G. and steroids sterically related to cortisol in relation to the number and the position of these hydroxyl groups.

EXPERIMENTAL

Material

Labelled steroids. [1,2,6,7 ^{3}H]-cortisol (specific radioactivity 93 Ci/mmol) was obtained from Radiochemical Center Amersham. [1,2 ^{3}H]-11-desoxycorticosterone (DOC 50 Ci/mmol), [1,2 ^{3}H]-corticosterone (10.5 Ci/mmol) [1,2 ^{3}H]-17-hydroxy-11-desoxycorticosterone (47 Ci/mmol) were purchased from I.R.E. (Belgium). Samples were tested by chromatography on a Sephadex LH 20 column [9].

Radioactivity was determined in Packard Tricarb Scintillation Spectrometer model 3380 allowing for quench correction by external standard. The efficiency was approximatively 30% for samples containing one ml of buffer and 10 ml of scintillation solution [5 ml Triton X100–toluene 1:1, 5 ml of P.P.O. (8.25 g/l) and P.O.P.O.P. (0.15 g/l) in toluene].

C.B.G. source. Heparinized blood samples (100 ml) were drawn from four healthy men at 3 p.m. The plasma were collected after centrifugation, frozen in batches (5 ml) and stored at −20°C until analyzed. The endogenous steroids were removed from the plasma by adsorption on charcoal. In half of the

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sample, the C.B.G. was destroyed by heating at 60°C for 20 min (unpublished data).

Methods

Determination of the equilibrium affinity constant K. The equilibrium constants for cortisol, corticosterone, 17,21-dihydroxy-pregn-4-ene-3-20-dione and desoxycorticosterone were obtained by equilibrium dialysis at 3°, 20°, 34°C (unpublished data). All experiments were carried out in triplicate at each temperature.

Determination of the dissociation rate constant k_2. The dissociation rate constant for each steroid was determined in a flowing system [8]. The flow rate dialysis cell and the buffer reservoir were maintained in a thermostatic bath or in an ice bath. The determinations were repeated three times at each temperature.

Theory

Abbreviations

P	Concentration of free C.B.G.
S	Concentration of free steroid.
PS	Concentration of C.B.G.–steroid complex.
K	Equilibrium affinity constant.
k_1	Rate constant for association.
k_2	Rate constant for dissociation.
ΔH	Enthalpy change of the reaction.
E_{dis}	Activation energy for the dissociation.
E_{as}	Activation energy for the association.
R	Universal gas constant.
T	Absolute temperature.
n	Total number of C.B.G. binding sites.

Equations.

$$P + S \overset{K}{\rightleftharpoons} PS \qquad \text{(i)}$$

$$K = \frac{k_1}{k_2} \qquad \text{(ii)}$$

$$\frac{PS}{S} = Kn - K(PS) \quad \text{(Scatchard)} \qquad \text{(iii)}$$

$$[PS]_t = [PS]_0 e^{-k_2 t} \qquad \text{(iv)}$$

$$\Delta H = - \frac{R \, d \ln K}{d \, 1/T} \qquad \text{(v)}$$

$$\frac{d \ln k_2}{d \, 1/T} = \frac{E_{dis}}{R} \quad \text{(Arrhenius)} \qquad \text{(vi)}$$

$$E_{as} = E_{dis} - \Delta H \qquad \text{(vii)}$$

The equilibrium affinity constant K is the slope of the line obtained from a Scatchard plot (iii).
The rate constant for dissociation k_2 is the slope of the line obtained from equation (iv) when the log of bound steroid is plotted against the time (in minutes).
The enthalpy change ΔH of the reaction is calculated from equation (v) given by Kerkay and Westphall[10].
The activation energy for the dissociation E_{dis} is obtained from the Arrhenius plot (equation vi).
The activation energy for the association E_{as} is the difference between the activation energy for the dissociation E_{dis} and the enthalpy change of the reaction ΔH (equation vii).

RESULTS

For each steroid tested for K and k_2 calculations, straight lines are fitted by the method of the least squares ($r \geqslant 0.98$).

Figure 1 illustrates the results of the activation energy for the dissociation of the C.B.G.–cortisol and C.B.G.–desoxycorticosterone complexes: while Table 1 gives experimental values which will be discussed in conjunction with the polarity of the steroid.

DISCUSSION

The values of equilibrium and rate constants shown in Table 1 are the results of experiment. They allow a rational approach to the study of the types of bonds and the energies involved in C.B.G.–steroids complexes.

The dissociation rate of cortisol from C.B.G. has been examined systematically by Rosner[6, 8]. Using his experimental results on the activation energy for the dissociation of this complex and Westphall's values [1] for enthalpy change, he observed that the activation energy for the association of C.B.G.–cortisol is in the region expected for a hydrogen bond.

In this respect, our object was first to confirm or challenge this observation with our own experimental line and secondly in the event of confirmation, to determine which of the cortisol hydroxyl group is responsible for the binding.

Table 2 shows the results obtained in this study compared with those of Rosner and Westphall. The

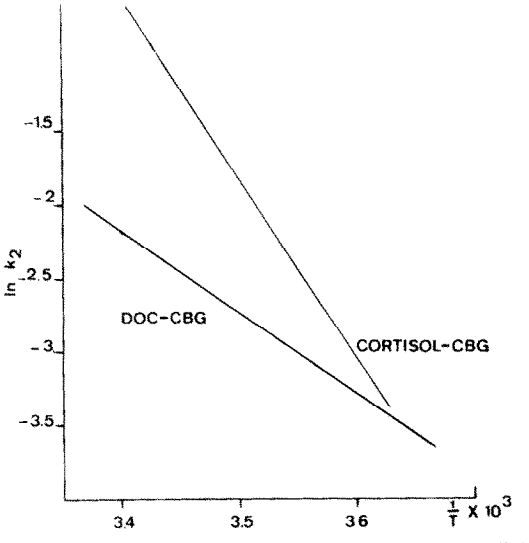

Fig. 1. Arrhénius plot of the dissociation rates (k_2) observed at different temperatures for cortisol-C.B.G. and DOC–C.B.G. bonds. The straight lines are fitted by the method of least squares.

Table 1. Binding parameters obtained for C.B.G.–steroids complexes

Steroids	E_{dis}*	ΔH*	E_{as}*
DOC 21 OH	10.69 $r = 0.988$	−7.66 $r = 0.994$	3.03
Corticosterone 21, 11 OH	13.54 $r = 0.969$	−10.78 $r = 0.97$	2.76
Cpd S 21,17 OH	16.61 $r = 0.98$	−6.63 $r = 0.966$	9.9
Cortisol 21, 11, 17 OH	21.2 $r = 0.996$	−13.19 $r = 0.982$	8.01

* = kcal M^{-1}.

Table 2. C.B.G.–Cortisol binding parameters

	E_{dis}	ΔH
This study	21.2	13.19
Rosner[6]	21.0	
Westphall[12]		15.7

main difference between their conditions and ours is that they used isolated and purified C.B.G. while our source of C.B.G. was a pool of human plasma.

Our results for the activation energy of the dissociation are in agreement with those of Rosner, while the ΔH values we found are slightly lower than Westphall references. We conclude that the practical and rapid method we describe can be of value in measuring the E_{as} values of other steroids.

We attempted to determine which of the 3 hydroxyl groups of the cortisol molecule is involved in the hydrogen bonding.

We measured the E_{dis} and ΔH and calculated E_{as} for three other steroids: 11-desoxycorticosterone with only one hydroxyl group at C-21, corticosterone with two hydroxyl groups, at C-21 and C-11β and 17-hydroxy-11-desoxycorticosterone with C-21 and C-17α hydroxyl groups.

All the experiments were made by the method used to study C.B.G.–cortisol binding parameters using a single pool of human C.B.G.

It is known that the free energy changes associated with hydrogen bond formation amount from 4 to 10 kcal M^{-1}.

From the results shown in Table 1, we conclude that:

1. The activation energy for the association of C.B.G.–11-desoxycorticosterone is weaker than the minimum value associated with a hydrogen bond. It is probably due to Van Der Waals forces. The C-21 hydroxyl group is not a hydrogen binding function.

2. The same conclusion applies to corticosterone. Its binding with C.B.G. probably results from Van Der Waals forces of the same magnitude. In the interaction C.B.G.–corticosterone, the C-11β hydroxyl group has no hydrogen binding function.

3. When a hydroxyl group is present at C-17α, with or without the presence of a second hydroxyl at C-11 the energies involved in the interaction with C.B.G. reach the value of a hydrogen bond: approximatively 10 kcal M^{-1}.

We conclude that the binding parameters of C.B.G. and corticosteroid are in accord with Westphall's deductions for albumin–steroid binding.

In the C.B.G.–corticosteroid interaction, the 17α position seems to be a "contact" point. The binding of these two molecules must be done on the alpha side of the steroid molecule: when the steroid has no hydroxyl group on this side, the interaction involves Van Der Waals forces, but if it has an hydroxyl group at the 17α position, the association of C.B.G. and the corticosteroid has an activation energy comparable with that of a hydrogen bond.

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