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FORMATION OF A FLUORESCENT GLUCOCORTICOID RECEPTOR–STEROID COMPLEX IN HTC CELL CYTOSOL

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Summary—An intensely fluorescent rhodamine derivative of dexamethasone (i.e. Dex-C_2-Rho) was synthesized. Dex-C_2-Rho possessed high affinity for HTC cell glucocorticoid receptors in cell-free systems. Whole cell activity and receptor affinity of Dex-C_2-Rho were both much lower, apparently due to problems with cell permeability and/or metabolism. A specific, fluorescent receptor–steroid complex at concentrations as low as 1×10^{-9} M could readily be observed with crude HTC cell receptors after removal of the free Dex-C_2-Rho. This appears to be the first report of a fluorescent glucocorticoid receptor–steroid complex.

INTRODUCTION

Glucocorticoid receptors are obligatory components for the expression of glucocorticoid hormone action and appear to constitute an excellent model system for studying the regulation of gene expression in eukaryotes. Both processes require steroid binding to the glucocorticoid receptor before the observed biological activity can be elicited. In order to examine how steroid binding to receptors triggers the expression of biological activity, we have sought new methods for probing steroid–receptor interactions [1–5]. Our development of a radioactive affinity label ([^{3}H]dexamethasone 21-mesylate), which gives a covalent glucocorticoid receptor–steroid complex, has already facilitated studies of the homology of different forms of receptor–steroid complexes from the same and different tissues, analysis of the steroid binding site of the receptor, and partial purification of receptors [5–9]. However, the types of information provided by radioactively labeled steroids are limited. A fluorescent steroid would be a useful adjunct to probe the polarity of the steroid binding site, to examine local conformational changes of the receptor, and possibly to permit real-time visualization of the steroid in cells [10]. In this paper, we describe the synthesis of the highly fluorescent steroid Dex-C_2-Rho, its affinity for the glucocorticoid receptor in rat hepatoma tissue culture (HTC) cells, its biological activity in whole HTC cells, and its ability to form the first specific, fluorescent glucocorticoid receptor–steroid complex.

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EXPERIMENTAL

Instrumentation

Melting points were determined on a Fisher-Johns hot stage or a Thomas Hoover capillary melting-point apparatus and are corrected. Beckman 4230 grating infrared and Hewlett–Packard Model 8450A spectrophotometers were used to record i.r. and u.v. spectra, respectively. Low-resolution mass spectra were obtained on a Hitachi Perkins–Elmer RMU-6E (electron-impact (EI) mode) or Finnigan 1015D (chemical-ionization (CI) mode) spectrometer by Mr Bill Landis or Mr Noel Whittaker of the Laboratory of Chemistry, NIADDK. Analyses were performed by the Microanalytical Section of the Laboratory of Chemistry, NIADDK, Bethesda, MD.

Steroids

Dexamethasone was purchased from Sigma. [^{3}H]Dexamethasone (20 and 46 Ci/mmol) was obtained from Amersham.

N-tBOC-cysteamine

Cysteamine HCl (61.2 mg, 0.54 mmol, Aldrich) was dissolved in 50 μl of water and treated with 245 μl of triethylamine (3.3 eq.), 0.4 ml of dioxane, and 140 μl of di-*t*-butyl dicarbonate (1.1 eq., Aldrich) at room temperature. Brief mixing gave a mass of solid with evolution of gas. Addition of 2.2 ml of 0.5 N HCl followed by extraction with ethyl acetate,

which was then dried over $MgSO_4$, yielded 111 mg of the crude product (theory = 86.8 mg) as a pale yellow liquid that contained (by i.r. analysis) a small amount of the unreacted di-*t*-butyl dicarbonate.

N-tBOC-Dexamethasone 21-S-ethyl-2'-amine

Dexamethasone 21-mesylate (83.3 mg, 0.177 mmol) [11] was dissolved at room temperature under argon in 2.2 ml of acetone (dried over 4A molecular sieves), cooled to 0°C, and treated with a 0°C solution of the above crude *N-t*BOC-cysteamine (~87 mg, ~0.54 mmol) and 75.1 μl of triethylamine (0.54 mmol) in 1 ml of dried acetone. After 1 h at 0°C, an additional 175 μl of triethylamine (1.26 mmol) was added and the temperature raised to room temperature for 1 h. Addition of 3.0 ml of 0.5 N HCl, followed by removal of some acetone under reduced pressure, addition to 40 ml of water, and extraction with methylene chloride, which was then dried over $MgSO_4$, yielded 204 mg of a light yellow oil. Dissolution of the oil in $CHCl_3$ followed by addition of petroleum ether afforded a granular off-white solid. This solid was dissolved in $CHCl_3$ and purified by preparative TLC (4% MeOH in $CHCl_3$ on a 2000 μ silica gel plate; extraction with ethyl acetate) to give an oil. Trituration with ethyl acetate–petroleum ether afforded 96.7 mg (98.8% yield) of TLC pure material (m.p. 170°C). Recrystallization from ethyl acetate afforded the analytically pure material: m.p. 168–170°C. Anal. calcd for $C_{29}H_{42}O_6FNS$: C, 63.13%; H, 7.67%; N 2.54%. Found: C, 62.95%, H 7.83, N, 2.22%. Mass spectral peaks were observed at *m/e*: 552 (MH^+, 75%), 496 (M-55; 100%), 452 (M-99, 35%), 434 (20%), 377 (40%), in chemical ionization mode with ammonia. i.r. (KBr): 3450, 3400, 1690 (broad), 1665, 1625, 1607, 1509, 1169 and 891 cm^{-1}.

Dex-C_2-Rho (III)

*N-t*BOC-dexamethasone 21-*S*-ethyl-2'-amine (56.0 mg, 0.101 mmol) was treated with 650 μl of 1.5 M HCl in glacial acetic acid at room temperature to give an immediate reaction with evolution of gas. The solution was cooled in ice and treated with diethyl ether to give, with scratching, the amine salt as a white powder. The amine salt was dissolved in 900 μl of dimethylformamide (Note: all further operations performed with minimum light) and added to a solution of ~45 mg (0.101 mmol) of tetramethyl rhodamine isothiocyanate (Research Organics, Inc.), that had been partially purified by TLC (16% MeOH–$CHCl_3$ on silica gel, extracted with 1:1 $CHCl_3$–MeOH), in 300 μl of dimethylformamide and 108 μl of triethylamine (0.775 mmol). After 15 min, the reaction solution was cooled in ice, treated with 8 ml of cold water, adjusted to pH ~5 with 0.5 M HCl and centrifuged to give a clear, light red supernatant which was discarded. The pellet was treated with water and extracted with $CHCl_3$ to give, after being dried over $MgSO_4$, a dark red oil comprised of two fluorescent components, as assayed by TLC (20% MeOH in $CHCl_3$ on silica gel). The two fluorescent components were almost completely separated by preparative TLC (20% MeOH in $CHCl_3$ on silica gel; elution with 1:1, MeOH–$CHCl_3$) to give 4.45 mg (12% yield) of the mostly upper R_f component and 6.12 mg (17% yield) of the mostly lower R_f component. In each product, the two fluorescent components comprised all of the fluorescent material and $\gtrsim$95% of the total material. Concentrations of the Dex-C_2-Rho isomers were based on the observed extinction coefficients at $\lambda_{max} = 540$ (abs EtOH): $\varepsilon = 55{,}000$ for the lower R_f isomer, $\varepsilon = 57{,}100$ for the upper R_f isomer. Molecular weights were determined by Drs Henry M. Fales and Ron Macfarlane by Cf^{252} plasma desorption mass spectra [12]: Calcd $MH^+ = 895.38$ (no ^{13}C included). Observed for lower R_f isomer = 895.40, for upper R_f isomer = 895.54.

Cells

Details of the growth of HTC cells in spinner cultures with Swim's S-77 medium [13] or in monolayer cultures with Richter's IMEM with zinc [14] or Swim's S-77 media, all supplemented with 0.03% glutamine and 5% fetal calf serum with or without 5% newborn calf serum (K. C. Biological), and the storage of frozen HTC cells [15] have appeared elsewhere.

Assays

Whole cell induction of tyrosine aminotransferase (TAT) was performed by incubating duplicate monolayer cultures of HTC cells at 37°C with fresh media containing 1% EtOH without or with various steroids as described in the legends. After 14–16 h of incubation, the cells were harvested, ruptured, and the specific activity of TAT was determined [14, 15].

Whole cell competition receptor binding assays were conducted with suspension cultures (1 ml of 5×10^6 cells/ml) [2] in fresh tissue culture media containing ~1×10^{-8} M [^{3}H]dexamethasone, 1% EtOH, and various concentrations of non-labeled competing steroids. After incubation at 37°C for 30 min, the cells were washed with phosphate buffered saline at 22°C and processed as previously described [16] for determination of the total cell protein and the specifically bound [^{3}H]dexamethasone. The non-specific binding was equal to the binding of 1×10^{-8} M [^{3}H]dexamethasone in the presence of 100-fold excess of [^{1}H]dexamethasone.

Cell-free competition binding assays to receptors were conducted at 0°C in Buffer A (20 mM Tricine [*N-tris*-(hydroxymethyl)methylglycine], 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.8 at 0°C) ± 10 mM Na_2MoO_4 as previously described [16]. Briefly, solutions of crude HTC cell receptors (200,000 ***g*** supernatant of whole cell homogenate) were incubated with

$\sim 7.5 \times 10^{-9}$ M [^{3}H]dexamethasone for the indicated times (final volume = 250 μl; protein concentration $\simeq$ 5 mg/ml; EtOH concentration = 2.0% [up to 2% EtOH has no effect on this assay (data not shown and 8)]. Free [^{3}H] steroid was removed by the addition of 25 μl of a 10% solution of dextran-coated charcoal in Buffer A followed by centrifugation. The amount of specifically bound [^{3}H]steroid in each sample was determined by subtracting the non-specific binding of [^{3}H]dexamethasone in the presence of 400–600-fold excess non-radioactive dexamethasone.

RESULTS

Selection of fluorescent steroid

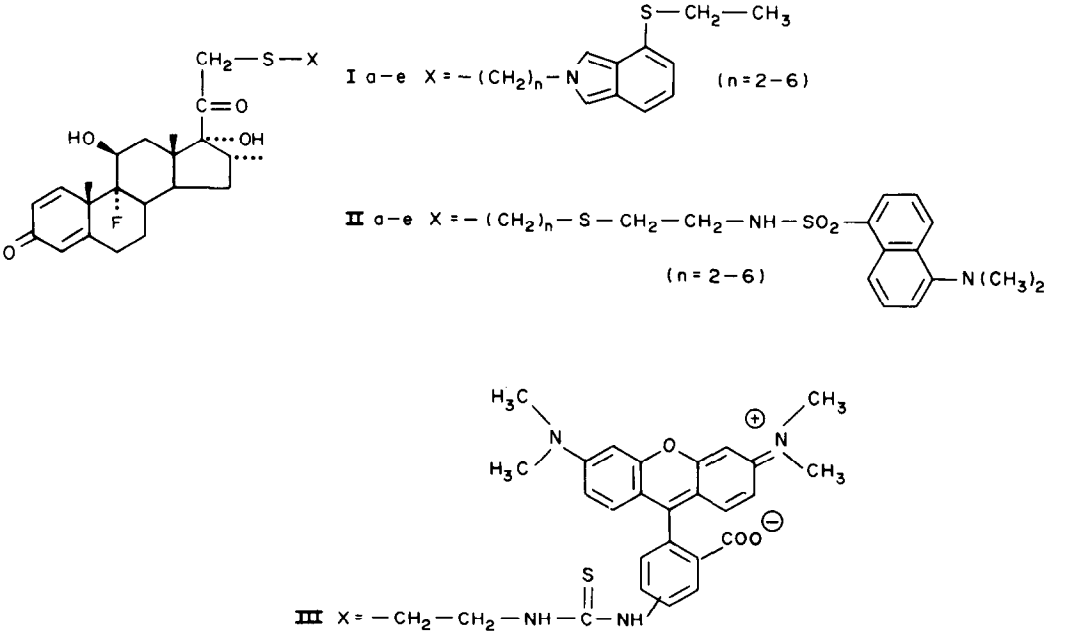
The most useful fluorescent steroids would have a high extinction coefficient (ε) at wavelengths (λ) > 340 nm (which is the λ_{max} of NADH and NADPH), a good separation between the excitation and emission λ_{max}'s, a high quantum yield of fluorescence, and a high affinity for glucocorticoid receptors. Several derivatives of dexamethasone which contain fluorescent isoindole (1,17) (**I** a–e), dansyl (**II** a–e), and tetramethyl rhodamine (**III**) groups attached to the C-21 position by varying length polymethylene chains have been screened to determine their relative affinity for cell-free HTC cell receptors. We found that, within each series of derivatives **I** and **II**, the affinity of the steroid for receptors decreased with increasing length of the polymethylene spacer unit (i.e. $n = 2 \rightarrow 6$) and that Dex-C_2-Rho (**III**) was the most promising fluorescent steroid (data not shown). The properties of Dex-C_2-Rho (**III**) were therefore more fully investigated.

Cell-free affinity of Dex-C_2-Rho for HTC cell glucocorticoid receptors

In a standard cell-free competition assay using a 3 h incubation time, the Rodbard-corrected [18] relative affinity of Dex-C_2-Rho for HTC receptors was 7.3 ± 5.0 (SD, $n = 8$) times less than that of dexamethasone (Fig. 1). The relative affinity decreased by 44% when the incubation time of the competition assay was extended to 24 h (Fig. 1) in order to more closely approximate equilibrium conditions [8]. This decrease in relative affinity indicates that Dex-C_2-Rho has a faster rate of dissociation than dexamethasone from the receptor–steroid complex [8]. It should be pointed out, however, that the relative affinity values were very dependent on the solvent used to dissolve the Dex-C_2-Rho. Stock solutions prepared with 10% EtOH in Medium A and then diluted 1:10 in the assay solution consistently gave values for the relative affinity of Dex-C_2-Rho that were 2.9 ± 1.0 (SD, $n = 5$) lower than those values obtained for 100% EtOH solutions that were diluted 1:100 directly into the assay solution. Since the final EtOH concentration in both assays was the same (i.e. 1%), this behavior presumably reflected the limited solubility of Dex-C_2-Rho in aqueous solutions, even at concentrations $\leq 10^{-6}$ M.

Whole cell biological activity of Dex-C_2-Rho

The induction of tyrosine aminotransferase (TAT) in HTC cells is a convenient assay procedure for determining the glucocorticoid activity of synthetic steroids [8, 15,16]. In this assay system, Dex-C_2-Rho was found to be a weak glucocorticoid (Fig. 2).



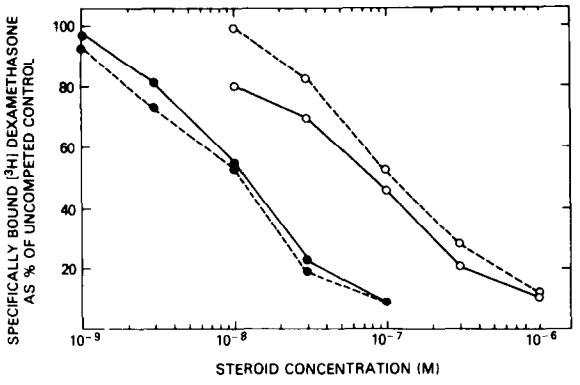


Fig. 1. Cell-free competition of [^{3}H]dexamethasone binding to glucocorticoid receptors by dexamethasone and Dex-C_2-Rho. Duplicate solutions of crude HTC cell receptors in Medium A were incubated at 0°C for 3 h (——) or for 24 h (---) with 8.0×10^{-9} M [^{3}H]dexamethasone and various concentrations of dexamethasone (●) or Dex-C_2-Rho (○) (final protein concentration = 6.2 mg/ml, final EtOH concentration = 2%). The average specifically bound [^{3}H]dexamethasone in the presence of various concentrations of non-radioactive, competing steroid was then determined as described in Experimental and plotted as % of control binding without competitor.

Dex-C_2-Rho at $\sim 10^{-5}$ M induced TAT to levels that were only $59 \pm 22\%$ (SD, $n = 4$) of the full induction seen by the potent glucocorticoid dexamethasone. Therefore the concentration of Dex-C_2-Rho required for half-maximal induction of TAT was $\geq 2.4 \pm 1.2 \times 10^{-6}$ M (SD, $n = 3$; equals ≥ 130 times the concentration of dexamethasone required in the same experiments for half-maximal induction of TAT), depending on whether or not the induction at 10^{-5} M Dex-C_2-Rho represents a plateau value. In a whole cell competition assay, the affinity of Dex-C_2-Rho for whole cell receptors was found to be 330 ± 170 ($n = 2$) times less than that of dexamethasone which is in reasonable agreement with the biological activity data. It thus appears that Dex-C_2-Rho has a high affinity for cell-free receptors but that permeability problems and/or metabolism drastically reduce the amount of Dex-C_2-Rho that can reach the receptor in whole cells. Alternatively, a single metabolite of Dex-C_2-Rho with a much reduced affinity for receptors could be the biologically active steroid.

Fluorescent properties of Dex-C_2-Rho in solution

The excitation and emission λ_{max} of Dex-C_2-Rho were relatively insensitive to changes in solvent polarity (Fig. 3a). The fluorescent intensity was much more responsive to changes in polarity (Fig. 3b). In very non-polar solutions (e.g. chloroform and isooctane), Dex-C_2-Rho was essentially non-fluorescent (data not shown), presumably due to the intramolecular attack of the carboxylate group of **III** to give the 5-membered lactone with a concomitant loss of several resonance structures [19]. The fluorescence intensity of Dex-C_2-Rho increased slightly ($\sim 40\%$) with decreasing temperature but the absolute amount varied with solvent composition. The problem of Dex-C_2-Rho solubility in aqueous solutions (see above, but also detected by fluorescence measurements) was greatly reduced by the presence of cytosolic proteins.

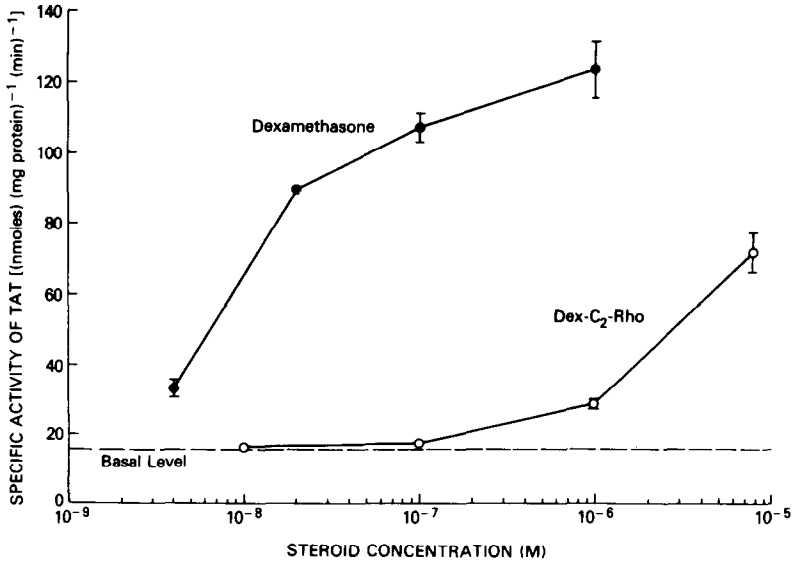


Fig. 2. Induction of TAT by Dex-C_2-Rho in whole HTC cells. Duplicate monolayer cultures of HTC cells in IMEM (plus 5% fetal calf serum) were incubated with various concentrations of dexamethasone (●) or Dex-C_2-Rho (○) for 14 h at 37°C. The specific activity of TAT in cell extracts (see Experimental) was plotted against the concentration of steroid. The range of each duplicate determination is shown by error bars when it exceeds the area of the data point. The basal level of TAT activity is indicated by the broken line.

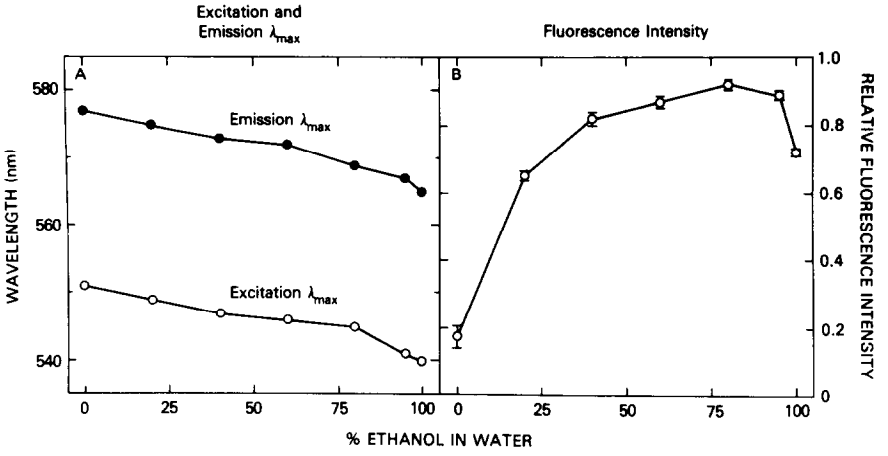


Fig. 3. Effect of solvent polarity on fluorescence properties of Dex-C_2-Rho. (A) The excitation spectra (excitation slit width = 1 nm, emission slit width = 9 nm, emission λ varied from 565 to 575 nm) and emission spectra (emission slit width = 3 nm, excitation slit with = 3 nm, excitation λ varied from 540 to 549 nm) were determined for 8.0×10^{-7} M Dex-C_2-Rho in solutions of 0–100% EtOH in water. (B) The relative fluorescence intensity for 6.6×10^{-7} M Dex-C_2-Rho (emission slit width = 6 nm, excitation slit width = 1.5 nm, excitation λ = 540 nm) was determined at r.t. in solutions of 0–100% EtOH in water.

Detection of fluorescent Dex-C_2-Rho receptor–steroid complexes

Initial experiments in which the free and receptor-bound Dex-C_2-Rho were not separated failed to reveal a specific, fluorescent receptor–steroid complex. In view of the relatively small differences in fluorescence emission λ_{max} and intensity of free Dex-C_2-Rho with most changes of solvent polarity (Fig. 3), this result was not unexpected. However, upon removal of free Dex-C_2-Rho either by Sephadex G-25 chromatography or by absorption with dextran-coated charcoal, stable macromolecularly associated, Dex-C_2-Rho fluorescence was readily observed (Fig. 4). That portion of the total Dex-C_2-Rho fluorescence in Fig. 4 that is specifically inhibited by excess dexamethasone qualifies as being due to fluorescent receptor—Dex-C_2-Rho complexes, especially since Dex-C_2-Rho efficiently competes for [^{3}H]dexamethasone binding to receptors (Fig. 1). In experiments using $3.5–8.0 \times 10^{-8}$ M Dex-C_2-Rho $\pm$ 100-fold excess dexamethasone, the total Dex-C_2-Rho fluorescence (=uncompeted fluorescence) at λ = 576 nm was 2.4 ± 0.5 (SD, $n = 15$) times the fluorescence seen in solutions that were co-incubated with excess dexamethasone (=competed fluorescence).

The fact that the emission λ_{max} of the specifically bound, the non-specifically bound, and the free Dex-C_2-Rho were all the same (i.e. ~576 nm; Fig. 4a and data not shown) indicates that the rhodamine group is freely accessible to solvent, even when Dex-C_2-Rho is bound to the receptor. On the strength of this observation, it was possible to add known amounts of Dex-C_2-Rho to construct linear plots of relative fluorescence vs steroid concentration for each cytosol solution. From these plots, it was determined in two experiments that 1.2 to 1.7×10^{-9} M fluorescent receptor–steroid complexes could readily be detected (uncompeted fluorescence/competed fluorescence = 2.7; initial concentration of Dex-C_2-Rho = 5.3 to 6.1×10^{-8} M; the amount of receptor present in 3 ml of eluant from the Sephadex G-25 column was derived from $\sim 3 \times 10^7$ cells). Furthermore, in those experiments employing 6×10^{-8} M Dex-C_2-Rho, $70 \pm 34\%$ (SD, $n = 6$) of the available receptors (as quantitated from the binding of 5×10^{-8} M [^{3}H]dexamethasone) were detected as fluorescent receptor–steroid complexes. This percent of receptor occupancy is consistent with the calculated K_d of about 3×10^{-8} M for Dex-C_2-Rho.*

Activation of the Dex-C_2-Rho receptor–steroid complexes by salt and heat (0.15 M NaCl/30 min/20°C) [20], followed by removal of free steroid by Sephadex G-25 chromatography, caused a dramatic decrease in the observed specific fluorescence signal (uncompeted fluorescence/competed fluorescence = 1.22 ± 0.05 [$n = 2$]) with no noticeable change in the emission λ_{max} (Fig. 4b). This decrease in fluorescence signal is probably due to dissociation of the receptor–steroid complex during activation. Alternatively activation may cause changes in receptor conformation that reduce the fluorescence intensity.

*The relative K_d of Dex-C_2-Rho after 24 h cell-free competition assays with HTC cell receptors was about 10 times less (see above) than that of dexamethasone ($= 3.2 \times 10^{-9}$ M [8]).

DISCUSSION

We have presented evidence that Dex-C_2-Rho (**III**) is capable of forming a fluorescent glucocorticoid

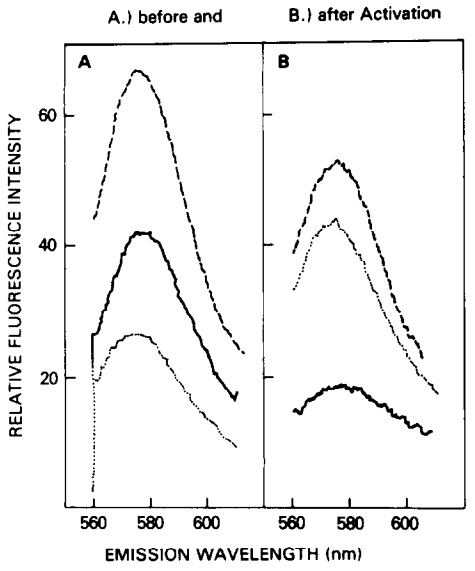


Fig. 4. Fluorescence emission spectrum of specific HTC cell glucocorticoid receptor–Dex-C_2-Rho complex (A) before and (B) after activation. HTC cell cytosol (1.6 ml at 25.4 mg/ml) was treated with Medium A $\pm$ Na_2MoO_4, and Dex-C_2-Rho $\pm$ dexamethasone to give 1.7 ml of solution at 0°C containing 10 mM Na_2MoO_4, 1.77% EtOH, and 6.0×10^{-8} M Dex-C_2-Rho $\pm$ 1.0×10^{-5} M dexamethasone (no Na_2MoO_4 in solution to be activated). After incubation at 0°C for 3 h (for activated cytosol, the last 30 min was at 20°C with 0.15 M NaCl), each sample was chromatographed over a PD-10 G-25 column (Pharmacia) at 0°C that had been equilibrated with Medium A containing 10 mM Na_2MoO_4. The first 3.2 ml was discarded; the next 3.0 ml fraction (F3) contained most of the fluorescent material. The fluorescence emission spectra (excitation slit width = 3 nm, excitation = 530 nm, emission slit width = 10 nm, sample sensitivity = 30/0 for unactivated cytosol and 30/3 for activated cytosol) were recorded at 0°C for the F3 fractions of cytosol treated with Dex-C_2-Rho (---) and of cytosol treated with Dex-C_2-Rho plus excess dexamethasone (····). The specific fluorescence spectrum (——) was obtained by subtracting the spectrum of the solution that had been treated with Dex-C_2-Rho + dexamethasone (and which was stored in the memory of a Perkin–Elmer Differential Corrected Spectra Unit (DCSU)) from a real time spectrum of the solution that had been treated only with Dex-C_2-Rho.

receptor–steroid complex. Other fluorescent steroid derivatives with affinity for glucocorticoid receptors have been described [21] but, to our knowledge, the present study represents the first observation of a fluorescent glucocorticoid receptor–steroid complex. Four lines of evidence indicate that the Dex-C_2-Rho forms a receptor–steroid complex in the steroid binding site of the receptor. First, Dex-C_2-Rho is a potent competitor of [^{3}H]dexamethasone binding to HTC cell receptors with a calculated K_d of $\sim 3 \times 10^{-8}$ M. Second, the bulk of the macromolecularly bound fluorescence seen upon incubation of HTC cell receptors with 2–8 $\times 10^{-8}$ M Dex-C_2-Rho is inhibited by dexamethasone. Third, the amount of available glucocorticoid receptors that were detected as a fluorescent complex complex with 6×10^{-8} M Dex-C_2-Rho is in good agreement with the expected occupancy by a steroid with a K_d of $\sim 3 \times 10^{-8}$ M. Fourth, the decrease in specific, macromolecularly bound Dex-C_2-Rho fluorescence after incubation under conditions of receptor–steroid complex activation is consistent with the apparent rapid rate of Dex-C_2-Rho dissociation from receptors and subsequent inactivation of steroid-free receptors at 20°C [22]. A demonstration of whole cell glucocorticoid activity for Dex-C_2-Rho would provide additional strong evidence that Dex-C_2-Rho occupies the steroid binding site of glucocorticoid receptors. Dex-C_2-Rho did induce TAT in whole cells at concentrations comparable to those required for Dex-C_2-Rho occupancy of whole cell receptors. Unfortunately, the whole cell activity of Dex-C_2-Rho was ≥ 13 times less than that predicted on the basis of the affinity of Dex-C_2-Rho for cell-free receptors. This diminished whole cell biological and receptor binding activity of Dex-C_2-Rho is probably due to limited entry into the cell and/or metabolism. A similar high affinity for cell-free receptors, but an apparent inability to enter the target cell, has been reported for a C-20 dexamethasone derivative containing biotin at the end of 9 or 12 atom spacer arms [23].

Dex-C_2-Rho was prepared as a mixture of isomers (see structure **III**) due to the presence of isomers in the starting tetramethyl rhodamine isothiocyanate. Two fluorescent steroid–rhodamine adducts could be almost completely separated by TLC and presumably correspond to the expected two isomers. Both isomers had identical relative affinities for HTC cell receptors and identical fluorescence spectra, whether free in solution or bound to the receptor (data not shown; majority of the above experiments were performed with the lower R_f isomer). These observations are consistent with the rhodamine group being outside of the steroid binding cavity. Such a positioning of the rhodamine group is compatible with the observations that bulky [23–25] or anionic [21], groups that are connected to the C-20 or -21 carbon of glucocorticoids by lengthy spacer arms do not drastically reduce the affinity of the steroid for the receptor while steroids with anionic groups that are directly attached to C-20 or -21 have very little affinity for the receptors [23, 25–28].

Further studies of fluorescence quenching, fluorescence depolarization, and energy transfer from added long wavelength fluorophores would help to determine if the rhodamine group is indeed outside of the steroid binding cavity when Dex-C_2-Rho is bound to receptors. Similar studies should also be useful in examining possible conformational changes in the receptor Dex-C_2-Rho complex. In view of the high sensitivity of Dex-C_2-Rho in detecting receptors in crude cytosol solutions (1 nM of complexes were readily observed), Dex-C_2-Rho should prove to be a useful probe of glucocorticoid receptor structure and function.

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