Thermodynamics of Triiodothyronine Nuclear Receptor Interaction

ROLE OF HYDROPHOBICITY IN TRIIODOTHYRONINE BINDING TO ITS RECEPTOR*

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We have investigated the thermodynamic properties of triiodothyronine (T₃)-solubilized hepatic nuclear receptor interaction. These studies revealed that the equilibrium constant was markedly dependent on temperature and van't Hoff plots revealed a significant deviation from linearity. Both enthalpy and entropy changes decreased with increasing temperature such that at 37 °C both were negative. (ΔH = −27.1 kcal/mol; ΔS = −45.1 cal/mol-deg; and the heat capacity ΔCₚ (25 °C), −759 cal/mol-deg). Several T₃ analogues revealed similar thermodynamic characteristics. Our finding that the T₃-receptor interaction is characterized by a negative heat capacity is compatible with the previous proposal of hydrophobic bonding by Jorgensen. Several T₃ analogues revealed similar thermodynamic characteristics. Our finding that the T₃-receptor interaction is characterized by a negative heat capacity is compatible with the previous proposal of hydrophobic bonding by Jorgensen. Several T₃ analogues revealed similar thermodynamic characteristics.}

The characteristics of triiodothyronine-nuclear receptor interaction have been primarily investigated by the study of the inter-relationship of the structure of T₃ analogues, the strength of receptor binding, and the biological response induced by the analogue (1, 2). Several studies suggest that although hydrophobicity of the T₃ molecule plays an important role in the binding process, steric hindrance of binding introduced by some of the modifications of the T₃ structure, prevents the establishment of a simple direct relationship between hydrophobicity of specific analogues and their ability to bind to the receptor (2-4). Silva et al. (5) also have provided chromatographic evidence which supports the hypothesis that the binding of T₃ alters the conformation of the receptor.

Another potential approach to the investigation of the characteristics of T₃-solubilized hepatic nuclear receptor interaction is evaluation of the thermodynamic properties of the reaction. For example, hydrophobic binding is characterized by the finding that enthalpy change is dependent on temperature and a negative heat capacity (6). In other instances, thermodynamic considerations may be of value in assessing the effect of ligand binding on the conformation of the receptor (7). Thus, agonists-receptor interactions are associated with a conformational change in the receptor, and in some cases, characterized by entropy changes more negative than antagonist-receptor interaction (7-9).

In this report, we provide evidence that the enthalpy change of T₃-receptor interaction is dependent upon temperature and that the reaction is characterized by a negative heat capacity change. Consonant with the interpretation of similar findings for other ligand receptor interactions, we infer that hydrophobicity of the T₃ molecule plays an important role in the binding process. Furthermore, we demonstrate that at 37 °C the binding of T₃ to receptor is associated with a negative entropy change, a finding distinctly different from observations on T₃-non-receptor protein reactions. We speculate that the difference in entropy change between receptor and non-receptor T₃-binding proteins may reflect a specific T₃-induced conformational change in the receptor.

EXPERIMENTAL PROCEDURES

Treatment of Animals and Preparation of Nuclei—Male Sprague-Dawley rats weighing 150 to 200 g, obtained from Taconic Farms, Germantown, NY, were surgically thyroidecetomized by the supplier and given 100 μCi of 125I after 1 week of a low iodine diet. Animals were considered to be adequately hypothyroid only after growth retardation could be demonstrated, some 3 to 4 weeks after 131'I administration. Hepatic nuclei were obtained by centrifugation through 2.4 M sucrose as previously described (10). The nuclei were washed with 0.14 M NaCl, 3 mM MgCl₂ (1 to 2 ml/g of tissue) and harvested by centrifugation at 1,000 × g for 10 min. Recovery of nuclei as judged by measurement of DNA ranged from 60% to 70%.

The protein/DNA ratio was approximately 2.5.

Solubilization of Nuclear Receptor—Solubilized nuclear receptors were prepared by the technique of Silva et al. (5). The nuclear pellet was resuspended in TEM (30 mM Tris, 2 mM EDTA, 5 mM mercaptoethanol, and 10% (v/v) glycerol, pH 6.0 at 25 °C, 0.4 M NaCl, and 5 mM MgCl₂) by mechanical stirring with a Vortex mixer for 1 min. The suspension was kept at 4 °C for 45 min and blended on a Vortex mixer for 15 s at 5-min intervals. The volume of extraction buffer was 4 ml/g of liver homogenate. The suspension was centrifuged at 20,000 × g for 20 min at 4 °C. The supernatant was used as the solubilized nuclear receptor.

Quantitation of 125I: Binding to Nuclear Extracts—A modification of the techniques described by Torresani and DeGroot (11) was used. Equal volumes (0.5 ml) of the nuclear extract containing 125I were mixed at 5-min intervals with 0.2 to 10 × 10⁻¹⁰ M TEM, 0.4 M NaCl containing 160 mg of AG 1-X8 resin (300 to 400 mesh)/ml were mixed at 5-min intervals for a total of 20 min at 4 °C. At the end of 20 min, the resin was removed by centrifugation at 1,800 × g for 5 min and an aliquot of the supernatant was counted in an Auto-Gamma scintillation spectrometer. "Nonspecific" binding was determined by the addition of 10⁻⁷ M T₃ to the nuclear extract containing 125I. The nonspecific binding varied from 1.5% to 4.7%, was proportional to the concentration of the nuclear extract, and was subtracted from all data. The resin removed 99% of 125I from TEM, 0.4 M NaCl within 20 min.

Estimation of Rate and Equilibrium Constants—The association

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3 The abbreviation used is: T₃, triiodothyronine.
The equilibrium constant (\(K_\text{a}\)) for \(T_3\) was determined by the method of Scatchard (12) using \([\text{T}^{25}\text{I}]T_3\) concentrations from 0.2 to 10 \(\times\) 10\(^{-10}\) M and receptor concentrations of approximately 1 to 2 \(\times\) 10\(^{-9}\) M. For the study of analogues, a fixed concentration of \([\text{T}^{25}\text{I}]T_3\) (0.2 \(\times\) 10\(^{-10}\) M) was incubated with increasing concentration of unlabeled ligand and the \(K_\text{a}\) was determined by the method of Scatchard.

The dissociation rate constant was determined by pseudo-first order kinetic analysis with addition of excess unlabeled \(T_3\) (10\(^{-10}\) M) following attainment of a plateau value of \([\text{T}^{25}\text{I}]T_3\) binding (15 min at 37 \(^\circ\)C and 48 h at 0 \(^\circ\)C) and according to the linearized first order rate equation:

\[
\ln \frac{[T_3,R]}{[T_3,R]_0} = -k_\text{-d} t
\]

where \([T_3,R]\) is the concentration of \([\text{T}^{25}\text{I}]T_3\) bound at time, \(t\), following addition of excess unlabeled \(T_3\) and \([T_3,R]_0\) is the concentration of \([\text{T}^{25}\text{I}]T_3\) bound prior to addition of excess unlabeled \(T_3\). The association rate constant was determined from tracer kinetic equations such that

\[
y = \frac{k_\text{a}[R]}{k_\text{a}[R] + k_\text{-s}} \left[ 1 - e^{-k_\text{-d}[R] + k_\text{-s} t} \right]
\]

where \(y\) is the fraction of \(T_3\) bound to specific binding site at time, \(k_\text{a}\) is the total receptor concentration as determined by Scatchard analysis; and \(k_\text{a}\) and \(k_\text{-s}\) the association and dissociation rate constants. Equation 2 may be rearranged to

\[
\ln \frac{Y_{\text{max}} - Y_i}{Y_{\text{max}}} = -\Delta t
\]

where \(Y_{\text{max}} = k_\text{a}[R]/\lambda\) and \(\lambda = k_\text{a}[R] + k_\text{-s}\). The value of \(\lambda\) was determined by linear regression analysis and \(k_\text{a}\) = \((Y_{\text{max}})/[R]\). Regression coefficient \(r^2\) for the linear regression determination of \(\lambda\) were always greater than 0.97.

Receptor stability was assessed by preincubation of the receptor at 30 \(^\circ\)C with various concentrations of \([\text{T}^{25}\text{I}]T_3\) for 40 min followed by a 48-h incubation at 0 \(^\circ\)C. The association constant (\(K_\text{a}\)) and the receptor concentration \([R]\) of the preincubated receptor preparation were 81% and 96% of the values observed in a receptor preparation not preincubated at 30 \(^\circ\)C. In addition, the plateau of binding remained stable (within 10% of the maximum value obtained for a given temperature) for a period of time equal to twice the time required to first achieve plateau binding. Thus, within the time frame of the experiments performed at higher temperatures (30 min at 30 \(^\circ\)C), there appeared to be only minimal changes in the ability of the receptor to bind \(T_3\).

**RESULTS**

The equilibrium constant (Fig. 1) (van't Hoff plot) as well as the Gibbs free energy, \(\Delta G^0\), \(\Delta G^0 = -RT\ln K_\text{a}\) (Fig. 2) demonstrate a nonlinear dependency on the inverse of temperature, and temperature, respectively. One explanation of this finding is that the enthalpy (\(\Delta H^0\)) and entropy (\(\Delta S^0\)) of the reaction are dependent upon temperature. The enthalpy change at any temperature is provided by the instantaneous slope of the van't Hoff plot and was approximated by the procedure of polynomial expansion as described by Osborne et al. (13) using the following equation:

\[
\Delta G^0 = -RT\ln K_\text{a} = \alpha_1 + \alpha_2 T + \alpha_3 T^2
\]

The best fit analysis of the experimental data is represented by the solid line in Fig. 2 and the values for \(\alpha_1\), \(\alpha_2\), and \(\alpha_3\) were 119.28, -0.89376, and 0.001507, respectively. The regression coefficient equaled 0.975. From the polynomial expansion of Equation 1, it follows that:

\[
\Delta H^0 = \delta(\Delta G^0)/\delta(1/T) = \alpha_1 - \alpha_2 T^2
\]

\[
\Delta S^0 = \delta\Delta G^0/\delta T = -\alpha_2 - 2\alpha_3 T
\]

\[
\Delta C_p^0 = \delta\Delta H^0/\delta T = -2\alpha_3 T
\]

where \(\Delta C_p^0\) is the heat capacity change or the dependency of enthalpy on temperature.

The influence of temperature on \(\Delta H^0\) and \(\Delta S^0\) is shown in Fig. 3. The slopes of \(\Delta H^0\) and \(\Delta S^0\) as a function of temperature are negative. The negative slope of \(\Delta H^0\) indicates that the heat capacity change is also negative. It should be pointed out that the value of \(\Delta C_p^0\) represents a second derivative of the initial data and is therefore imprecise. The approximate value of \(\Delta C_p^0\) at 25 \(^\circ\)C is in -600 to -900 cal/mol deg.

Since the above analysis examines only the thermodynamic properties of the equilibrium reaction, it was of interest to examine the relation between temperature and the association (\(k_\text{a}\)) and dissociation (\(k_\text{-s}\)) rate constants. This analysis permitted an evaluation of the thermodynamic properties of the formation of the activated complex \(T_3R^*\):

\[
T_3 + R \rightleftharpoons T_3R^* \rightleftharpoons T_3R
\]

where the association \(\Delta H^0\) and \(\Delta S^0\) reflect the process of step 1 whereas the dissociation \(\Delta H^0\) and \(\Delta S^0\) reflect the process of step 2. Figs. 4 and 5 show the Arrhenius plots for the associ-
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The effect of temperature on enthalpy ($\Delta H^e$) and entropy ($\Delta S^e$) for $T_3$ receptor interaction calculated from the data given in Fig. 2 and Equations 5 and 6.

**FIG. 3.** The effect of temperature on enthalpy ($\Delta H^e$) and entropy ($\Delta S^e$) for $T_3$ receptor interaction calculated from the data given in Fig. 2 and Equations 5 and 6.

**TABLE I**
Energy, enthalpy, and entropy of activation for association and dissociation of $T_3$ with receptor

<table>
<thead>
<tr>
<th>Process</th>
<th>$E_a$ (kcal/mol)</th>
<th>$\Delta H^1$ (kcal/mol)</th>
<th>$\Delta S^1$ (cal/mol.deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association</td>
<td>16.8</td>
<td>16.1</td>
<td>24.8</td>
</tr>
<tr>
<td>Dissociation</td>
<td>30.8</td>
<td>30.2</td>
<td>29.4</td>
</tr>
</tbody>
</table>

**FIG. 4.** Arrhenius plot for the association reaction of $T_3$ and receptor. The different symbols (C, ○) represent two separate experiments and the line represents the best fit according to linear regression analysis.

The thermodynamic parameters for formation of the activated complex for the association ($T_3 + R \rightarrow T_3R^*$) and dissociation ($T_3R \rightarrow T_3 + R^*$) of $T_3$ with its receptor at 37°C. Values are derived from linear regression analysis of data shown in Figs. 4 and 5. $\Delta H^1 = E_a - RT$ and $\Delta S^1 = R\ln (A h/k T - 2.718)$ where $R$ is the gas constant, $h$ is Planck constant, $A$ is pre-exponential factor of the Arrhenius equation, and $k$ is Boltzmann constant.

**FIG. 5.** Arrhenius plot for the dissociation reaction of $T_3$ and receptor. The different symbols (C, ○) represent two separate experiments and the line represents the best fit according to linear regression analysis.

The activated complex) for both the forward and reverse reactions were determined (Table I). Since the van’t Hoff plot for $T_3$ binding was curvilinear, it was anticipated that at least one of the Arrhenius plots would be curvilinear, however, both plots appear linear. The difference between the anticipated and the actual plots may arise from the relatively large magnitude of change of the individual rate constants and experimental variation in the rate constants. Therefore, the values of $\Delta H^f$ and $\Delta S^f$ are approximate. It appears that $\Delta S^f$ and $\Delta H^f$ for the association reaction (step 1) and dissociation reaction (step 2) are positive at 37°C, suggesting an entropy-driven reaction in formation of the activated complex from either direction and is consistent with a hydrophobically controlled process.

Comparison of the $K_d$ determined by the kinetic data shown in Figs. 4 and 5 with the $K_d$ determined by equilibrium techniques (Fig. 1) revealed that the $K_d$ by kinetic data generally exceeded the equilibrium $K_d$ by a factor of 1.2 to 4. However, the van’t Hoff plot using the $K_d$ determined by kinetic data suggested a curvilinear relation between $K_d$ and $1/T$. Calculation of $\Delta H^f$, $\Delta S^f$, and $\Delta C^o_p$ using Equations 4 through 7 from the $K_d$ determined by kinetic data resulted in values of $-32.8$ kcal/mol, $-63.8$ cal/mol.deg, and, $-940$ cal/mol.deg. Thus, despite the apparent differences between the $K_d$ values by the two techniques, similar thermodynamic characteristics are revealed.

The thermodynamic properties of several $T_3$ analogues were examined to better define the role of ring substitution. Similar to the observations of Welland et al. (7) for $\beta$-adrenergic agonists, we have found that the binding of $T_3$, an agonist of the receptor, to the receptor at 37°C is dominated by negative enthalpy changes and that the negative entropy changes are unfavorable to binding. Of interest are the thermodynamic properties of several analogues of $T_3$ which displayed a wide range of affinities for the receptor (Table II). Each analogue was characterized by a negative $\Delta C^o_p$ and $\Delta S^o$.

**DISCUSSION**

The interaction of $T_3$ with its receptor initiates a sequence of events which culminate in the induction of a biological process. This sequence of events includes at least two processes: ligand binding and alteration of the receptor. The evaluation of the thermodynamic properties of $T_3$ receptor interaction clearly indicates that $T_3$ binding with its receptor is a hydrophobically controlled reaction.

Several hormone receptor reactions have thermodynamic properties characteristic of hydrophobically controlled reactions. Wolff et al. (18) for corticosterone receptor binding and Waelbroeck et al. (19) for insulin lymphocyte receptor inter-
action have demonstrated a negative heat capacity change for these reactions which are consistent with the overall hydrophobicity of the ligands. Similar observations have been made for a number of other ligand-binding protein interactions (20-21). The data presented clearly demonstrate the thermodynamic property of a negative heat capacity characteristic of hydrophobic binding. Although the heat capacity is a relatively inaccurate determination, in all experiments the ΔCp° was negative. The negative heat capacity as well as the positive ΔS° for the formation of activated complex provides evidence which supports the hypothesis that hydrophobicity plays an important role in this reaction.

To further understand the relation of structure of T3 to its hydrophobic binding, the ΔCp° of several T3 analogues were examined. The substitution of iodine in the 3′ and 5′ position appears to have no major effect on the ΔCp°. Thus, it appears that the ring structures of the T3 molecule are primarily responsible for the hydrophobic binding. Jorgensen (2, 3) has suggested that the substitution of the 3′ position with a halogen or a short chain alkyl group increases the lipophilic character of the analogue and, therefore, these structures should have more negative ΔCp°. Edelhoch and Osborne (6) have reported that for each CH2-group transferred from water to a nonpolar environment, a ΔCp° change of approximately -20 cal/mol-deg occurs. Substitution of isopropyl at the 3′ position of T3 would result in an approximate ΔCp° of T3. This rather small change could not be easily detected by the approach taken in this study. The rather large ΔCp° for 3,3′-dibromo-5′-isopropylthyronine remains without explanation, but may be related to either the dibromo substitutions or to the 5′-isopropyl configuration.

Comparison of the thermodynamic properties of the T3-receptor reaction with T3 non-receptor reactions reveals that the entropy of the T3-receptor reaction is considerably more negative than the entropy values for T3 non-receptor reactions (Table III). One possible interpretation of this difference in ΔS° is derived from the following two-step model of hormone-receptor interaction (7-9). A positive entropy change observed with some antagonist binding to receptor is presumed to arise from changes in the state of water hydration of the ligand and receptor molecules. A similar change with water of hydration occurs with agonist binding; however, in addition, the agonist induces a conformational change in the receptor, resulting in an overall decrease in entropy. Since previous observations suggest that T3 induces a conformational change in the receptor (5), we infer that the difference in ΔS° between receptor and non-receptor binding of T3 may be a reflection of the T3-induced conformational change in the receptor. Consistent with this interpretation is that several analogues of T3, all biologically active proportional to their affinity for the receptor, are characterized by a negative ΔS°. We are unable to state specifically that a T3 antagonist would be characterized by a positive ΔS°, since such an analogue has not been identified.

The receptor-non-receptor protein comparison suggests a corollary to the general two-step model of hormone-receptor interaction. The receptor protein provides an important link between the cellular milieu and the cell. The receptor serves to transfer information, and this information is processed by a change in the charge or structural configuration of the receptor. Non-receptor-binding proteins do not need this information transference capability, and therefore, a change in charge or structural configuration is not a necessary process. We predict from these considerations that, in general, hormone agonist receptor protein interaction will have a more negative ΔS° than hormone agonist non-receptor protein interaction. Such a corollary may be useful in the distinction between receptor and non-receptor-binding proteins.

Strict interpretation of these data assumes that the reaction system is characterized by a simple bimolecular reversible reaction. The apparent difference between the Kₐ determined by kinetic data and the Kₐ determined by equilibrium data suggested that the reaction system may be more complex than previously thought. If this hypothesis is correct, then interpretation of the curvilinear nature of the van’t Hoff plot must be made with caution. Recent studies in our laboratory have indicated that at 0 °C, precise measurement of Kₐ by equilibrium techniques is complicated by uncertainty of free ligand concentration (22). Our studies suggested that a significant quantity of apparent free ligand was bound to inactive binding sites, resulting in a systematic underestimation of the Kₐ value. To partially obviate this problem, we determined the ratio of the association (kₐ) to the dissociation (k₋₁) rate constants as a function of temperature. Since the thermodynamic characteristics of the reaction were similar by both methods of determining the Kₐ value, the presence of possible inactive binding sites appeared not to significantly alter the relation between Kₐ (or ΔG°) and temperature. It is important to emphasize, however, that in an impure reaction system, the occurrence of confounding reactions is possible. This limitation is not unique to the T₃ receptor reaction but to all hormone receptor systems in which the receptor is not in pure form.

Despite these technical limitations, the thermodynamic data are consistent with previous knowledge of T₃ receptor interaction. The studies of Jorgensen et al. (2, 3), and the present studies support the concept that the hydrophobicity of T3 plays an important role in the binding of T3, with its
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receptor. Further, from the thermodynamic considerations the ring structure of the T₃ molecule contributes the major portion of the hydrophobicity involved in receptor binding. Finally, the difference in ∆S° between receptor and non-receptor-binding proteins may provide an important clue in distinguishing between receptor and non-receptor T₃-binding proteins.

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REFERENCES

Thermodynamics of triiodothyronine nuclear receptor interaction. Role of hydrophobicity in triiodothyronine binding to its receptor.

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