Computer Graphics in Drug Design: Molecular Modeling of Thyroid Hormone–Prealbumin Interactions

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Computer graphics modeling of the thyroxine–prealbumin complex provides a detailed picture of the interactions between thyroxine and prealbumin. A wide variety of thyroid hormone analogue–prealbumin complexes were modeled by calculating the molecular surfaces of the analogues and the prealbumin hormone-binding site. Analogues with high binding affinity were observed to fill more of the hormone-binding site than low-affinity analogues. These surface models described many aspects of the hormone–protein interaction which were not obvious using simple wire models and led us to develop a model which accounts for thyroid hormone–prealbumin structure–activity relationships and ultimately to predict and measure the relative binding affinities of four previously untested thyroid hormone analogues to prealbumin.

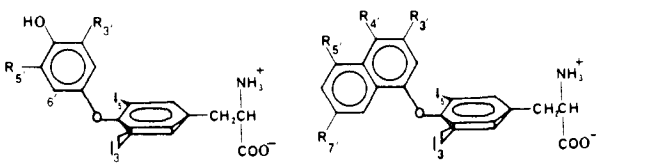
The biological response of most drugs is assumed to result from the interaction of the drug with a specific receptor. The detailed three-dimensional structures of several drug–receptor interactions have now been established by X-ray crystallography (e.g., daunomycin–DNA, methotrexate–dihydrofolate reductase, and trimethoprim–dihydrofolate reductase).[1-4] As more such structures become available, it should become possible to design highly selective drugs tailored to fit the structure of their receptor.

There are few reports in the literature of attempts to integrate structure–activity relationships and drug design with the three-dimensional structure of a macromolecule. Hansch et al.[5] and Smith et al.[6] showed in their work on papain that quantitative structure–activity relationships are especially effective in understanding enzyme–ligand interactions when combined with the X-ray crystallographically determined structure of the enzyme. Beddell et al.[7] demonstrated the potential power of the molecular modeling approach to drug design in their investigation of compounds intended to bind to the 2,3-diphosphoglycerate (DPG) site on human hemoglobin. Using wire models of the DPG-binding site, they successfully designed and predicted the relative oxygen-displacing activities of several compounds bearing little structural resemblance to DPG. They were also able to correlate the oxygen-displacing activities of these compounds with the variations in the structure of the DPG-binding site found in other hemoglobins.[8] Poe has described the use of wire molecular models to design a highly active inhibitor of bacterial dihydrofolate reductase.[9]

We are interested in using the thyroxine–prealbumin complex as a model for the interaction of thyroid hormones with the nuclear thyroid hormone receptor[10-12] and for drug–receptor interactions in general. Although prealbumin is not a true drug receptor, acting only as a transport protein for thyroxine, the interaction of thyroid hormones with prealbumin provides a convenient, well-defined, and relatively simple model of a drug–receptor interaction.[13]

First we describe the interaction, as determined by X-ray crystallography,[12-16] of the thyroid hormones with the prealbumin hormone-binding site and thereby develop a model which explains thyroid hormone–prealbumin structure–activity relationships. Next we use molecular surface models to predict the relative binding affinities of four previously untested thyroid hormone analogues to prealbumin. Finally, we test our predictions experimen-

Chart I



T_4 $R_{3'}=R_{5'}=I$ (L, DL)
1a $R_{3'}=R_{5'}=Br$ (L)
1b $R_{3'}=Br, R_{5'}=H$ (L)
2a $R_{3'}=R_{5'}=H$ (L)
1 $R_{3'}=Br, R_{4'}=OH, R_{5'}=R_{7'}=H$ (L)
2 $R_{3'}=R_{5'}=R_{7'}=H, R_{4'}=OH$ (DL)
3 $R_{3'}=R_{4'}=R_{7'}=H, R_{5'}=OH$ (DL)
4 $R_{3'}=R_{4'}=R_{5'}=H, R_{7'}=OH$ (L)

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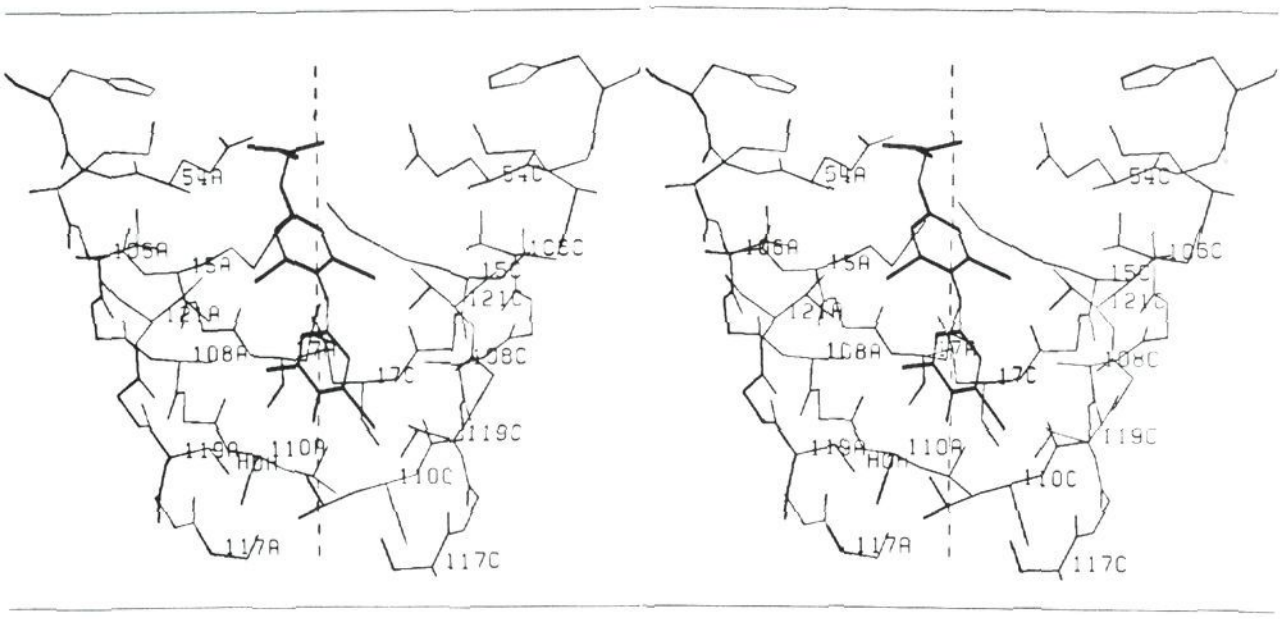

Figure 1. L-Thyroxine in the prealbumin hormone-binding site. The dashed line indicates the twofold symmetry axis. Labeled residues include Lys-15, Leu-17, Glu-54, Thr-106, Ala-108, Leu-110, Ser-117, Thr-119, Val-121, and the bound water (HOH).

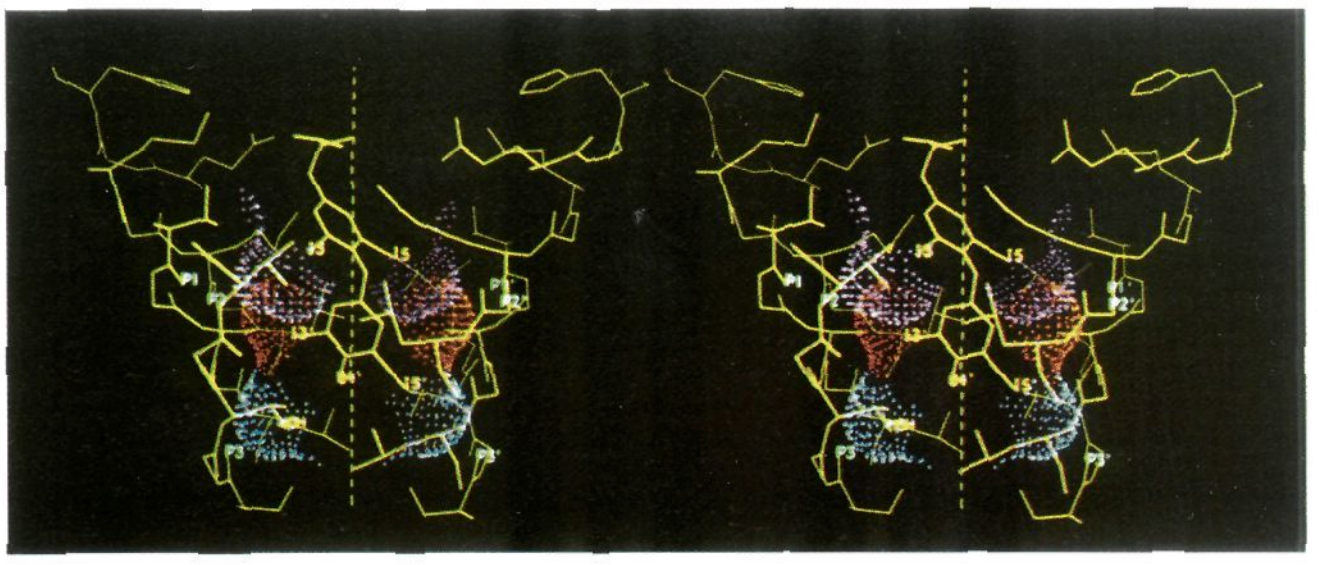

Figure 2. The three pairs of symmetry-equivalent pockets in the hormone-binding site. A (top): Schematic drawing of the thyroxine–prealbumin interaction. B (bottom): L-thyroxine, the bound water molecule, the binding site, and the molecular surfaces of the pockets P_1 and $P_{1'}$ (magenta), P_2 and $P_{2'}$ (red), and P_3 and $P_{3'}$ (blue). The dashed line indicates the twofold symmetry axis.

tally by determining the binding affinities of the four compounds.

Molecular Modeling

X-ray Crystallographic Background. X-ray crystallographic analysis of the prealbumin–thyroid hormone complex was carried out at the Laboratory of Molecular Biophysics, University of Oxford. The atomic structure of the human serum prealbumin molecule has been determined at 1.8-Å resolution[12,13] and refined by difference-gradient and least-squares methods to a crystallographic residual ($\sum||F_{obsd}| - |F_{calcd}||/\sum|F_{obsd}|$) of 0.19.[17] This has provided a very detailed and accurate description of the atomic structure of the protein and, in particular, of its thyroid hormone-binding site.

The binding of L-thyroxine to prealbumin has also been investigated at 1.8-Å resolution.[14-16] The initial difference electron density map was dominated by the features corresponding to the electron-dense iodine atoms, although

(17) Oatley, S. J., unpublished results

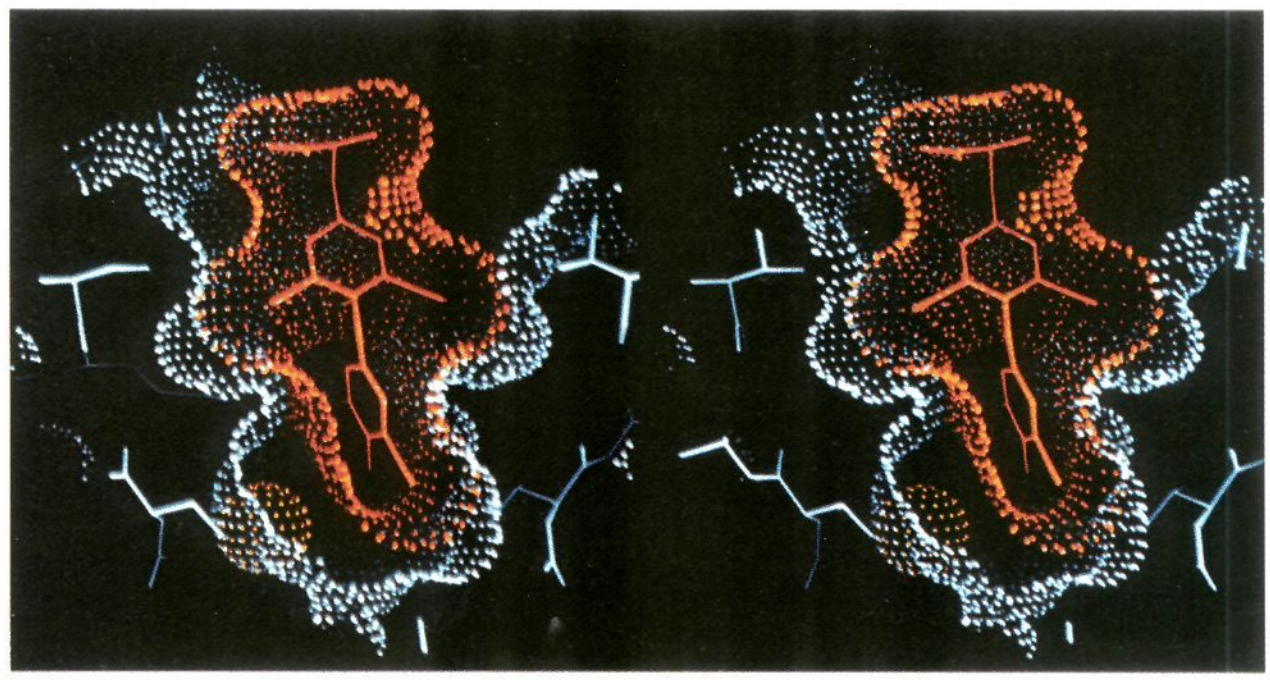

Figure 3. Cross-section of the thyroxine-prealbumin binding surfaces. The red lines and dots represent the bonds and molecular surface of thyroxine and the blue represents the bonds and molecular surface of the hormone-binding site. The yellow sphere represents the molecular surface of the water molecule (bound in pocket P_3).

extensive small conformational changes in the protein were also evident. A preliminary interpretation of this map was obtained by adjusting the torsion angles of a thyroxine molecule (derived from ref 18) so that its iodine atoms best fitted their corresponding electron-density features.[14,15] These hormone coordinates, and the refined coordinates of native prealbumin, were those used in the modeling described in this paper.

Hormone-Binding Site. Prealbumin is a tetramer consisting of four identical subunits, here labeled A, B, C, and D, which are oriented to form a central channel containing two thyroxine (T_4) binding sites. The symmetry of the molecule requires not only that the two sites are identical, but also that each site itself has twofold symmetry.[12] L-Thyroxine (Chart I) is oriented along this twofold axis with its phenolic hydroxyl group buried deep within the binding channel and its carboxyl and amino groups ion-paired with the Lys-15 and Glu-54 residues at the mouth of the binding channel (Figure 1).

The hormone-binding site contains six pockets capable of binding an iodine substituent, of which four are occupied by the iodines of thyroxine.[14] One of the remaining pockets is occupied by a crystallographically well-defined water molecule, leaving one empty pocket. Figure 2A is a schematic drawing of the thyroxine–prealbumin interaction showing the three pairs of symmetry-equivalent pockets P_1 and $P_{1'}$, P_2 and $P_{2'}$, and P_3 and $P_{3'}$; Figure 2B shows the molecular surfaces of the six pockets in the hormone-binding site. The inner-ring iodines (I_3 and I_5) bind to identical hydrophobic pockets, P_1 and $P_{1'}$, lined with the methyl groups of Leu-17, Thr-106, Ala-108, Val-121 and the polymethylene side chain of Lys-15. The 3′- and 5′-iodine atoms of the outer (phenolic) ring of thyroxine fit into pockets which are not related by symmetry. The 3′-iodine atom, proximal to the inner ring, binds to pocket P_2 defined by the carboxyl oxygen of Lys-15A, the $CHCH_3$ group from Leu-17A, the methyl group of Ala-108A, and the peptide backbone of Ala-109A. The 5′-iodine atom, distal to the inner ring, fits into pocket $P_{3'}$ formed by the methyl and carbonyl groups of Ala-108C, the backbone nitrogen and carbonyl group of Ala-109C, the backbone nitrogen and side chain of Leu-110C, and the hydroxyl groups of Ser-117C and Thr-119C. The water molecule is held by pocket P_3 by hydrogen bonds to the hydroxyl groups of Ser-117A and Thr-119A and possibly to the phenolic hydroxyl group of thyroxine. An interesting observation is the possible close contact (less than 3.2 Å) between the proximal (I3′) iodine and the backbone carbonyl oxygen of Ala-109A, which might be an important feature of the hormone–protein interaction. Close intermolecular contacts between oxygen and iodine atoms have been observed in X-ray crystallographic studies of a large number of molecules,[19] including the thyroid hormones,[20] and reflect the polarizability and charge-transfer ability of iodine.

The details of the hormone–protein interactions will be clarified as improved X-ray maps become available and as the conformational response of the protein to hormone binding is understood in greater detail.[16]

Computational Methods. The thyroxine–prealbumin complex was modeled by calculating the molecular surfaces[21] for the prealbumin hormone-binding site and thyroxine using the molecular surface program MS;[22] these molecular surface models were displayed using an Evans and Sutherland Color Picture System 2.[23] Models of the interaction of the other thyroid hormone analogues with prealbumin were constructed by modifying the coordinates of the bound thyroxine by the addition or substitution of the appropriate functional groups using standard geometries. Each analogue was assumed to bind in the same orientation and conformation as thyroxine; no attempt was made to alter torsional angles of the analogue or the protein.

Thyroid Hormone–Prealbumin Structure–Activity Relationships. The topography and volume of the pockets in the hormone-binding site are readily observed with the molecular surface model. By assigning a different color to the surface of each atom type, the hydrophilic and hydrophobic areas of the binding site are immediately recognizable.

Figure 3 shows a cross section of the thyroxine–prealbumin binding surfaces. I_3 and I_5 of thyroxine are held

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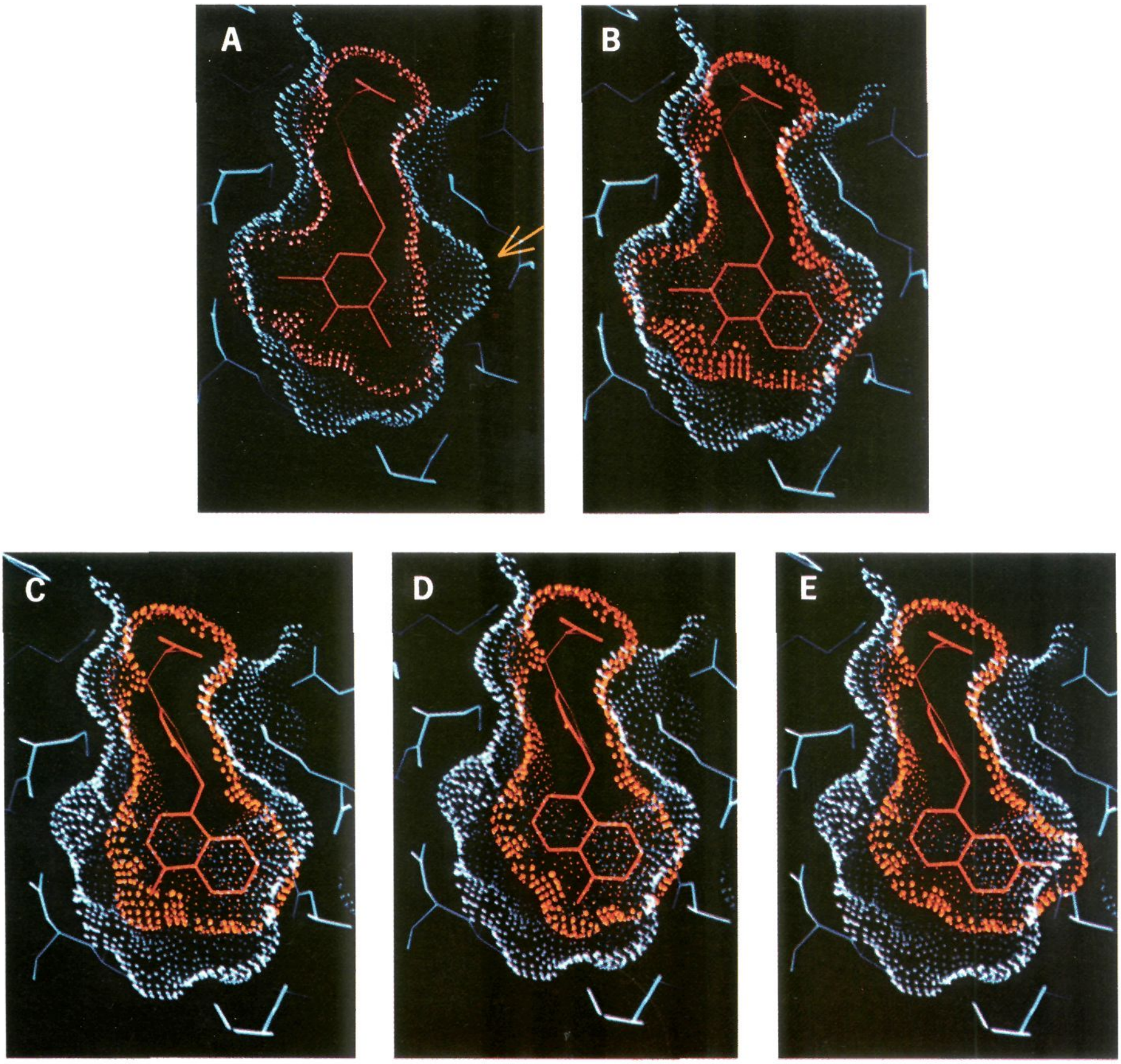

Figure 4. Cross-sections of the thyroid hormone analogue–prealbumin binding surfaces; oriented as in Figure 2: (A) L-thyroxine, the arrow indicates the empty pocket ($P_{2'}$), (B) 1, (C) **2**, (D) **3**, (E) **4**.

by symmetry-identical pockets (P_1, $P_{1'}$) lined with the side chains of hydrophobic residues (Ala, Leu, Val) and the polymethylene portion of Lys-15. The amino group of Lys-15 may form a dipolar association with the polarizable iodine atoms, thus explaining the higher binding affinities conferred by halogen substituents at the 3- and 5-positions relative to alkyl groups of the same size.[24] All four iodines of thyroxine match the surfaces of their pockets very closely. Replacement of an iodine by a smaller substituent, such as bromine or chlorine, results in a loss of complementarity with the pocket; therefore, iodinated analogues would be expected to have a higher binding affinity to prealbumin than brominated or chlorinated analogues, as has been demonstrated experimentally.[24] The outer-ring pockets (P_2, $P_{2'}$, P_3, $P_{3'}$) are much more polar and polarizable than the inner-ring pocket (P_1, $P_{1'}$); therefore, the interaction between an iodine and the outer-ring pockets should be enhanced relative to the inner-ring pockets. In fact, recent work has shown that the outer-ring iodines ($I_{3'}$, $I_{5'}$) contribute more to binding to prealbumin than the inner-ring iodines.[24] Halogen substituents on the outer ring were observed to contribute much more to binding than alkyl groups of the same size, a result of the polarity and polarizability of the outer-ring pockets.

Modeling a wide variety of thyroid hormone analogues led to the following generalization: analogues with high binding affinity were observed to occupy at least three of the four outer-ring pockets (P_2, $P_{2'}$, P_3, and $P_{3'}$); the more empty space in the binding site, the weaker the analogue. Presumably, increased surface complementarity leads to stronger van der Waals attraction between the hormone and the protein, resulting in higher binding affinity.

Prediction of Binding Affinities of Thyroid Hormone Analogues to Prealbumin. The molecular surface modeling pointed out many relationships between the bound hormone and the protein which were not obvious using simple wire models. In particular, we realized that

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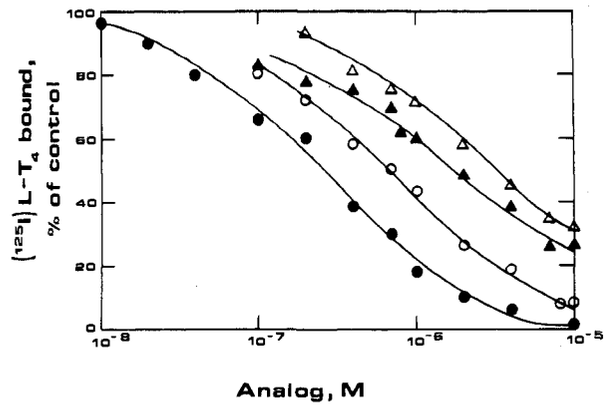

Figure 5. Competitive binding curves for 1–4 against L-[^{125}I]-thyroxine. 100% = cpm with no unlabeled analogue added. Each point represents the mean of two to three determinations: (●) 1, (▲) 2, (○) 3, (△) 4.

an empty pocket ($P_{2'}$) in the hormone-binding site (Figures 2A,B and 4A) could be filled by the addition of a substituent at the 6′ position of thyroid hormone analogues. This led us to expect that the addition of an appropriate group at the 6′ position should result in increased binding affinity to prealbumin relative to the 6′-unsubstituted compound. Analogues of this type had not been tested previously with prealbumin.

We modeled the compounds shown in Chart I in the prealbumin hormone-binding site; the α-naphthyl analogues, originally synthesized for evaluation of their in vivo thyromimetic activity,[25,26] were chosen because of their lack of conformational mobility. The additional ring attached at the 5′- and 6′-positions forces the outer ring to be essentially perpendicular to the inner ring and also forces the diphenyl ether moiety to be conformationally rigid,[27] which greatly simplifies the modeling of these analogues.

Based on observation of their molecular surface models in the hormone-binding site, we predicted analogue **1** to have a significantly higher binding affinity than either **1a** or **1b**, since 1 is the only analogue which can simultaneously fill all the available pockets in the binding site. Similarly, we expected analogue **2** to have a higher binding affinity than **2a**.

Inspection of the models for the interaction of analogues 1–4 with prealbumin (Figure 4B–E) revealed that analogue 1 matched the surface of the binding site very closely with no bad contacts (i.e., contacts closer than van der Waals). Compounds 2–4 all lacked the bromine substituent so that one pocket was left empty in the binding site. The phenolic hydroxyl groups on 2 and 3 appeared to be equally well accomodated by the binding site, but the hydroxyl group on 4 was observed to "collide" with the surface of the binding site. Although prealbumin is certainly not static and rigid as we have modeled it here and may actually be quite flexible, it would require a large conformational change in the hormone-binding site (with a concomitant increase in free energy) to accept the hydroxyl group on 4 if the rest of the analogue is constrained to bind in the same orientation as thyroxine (as modeled here). Such a conformational change would require a distortion of the peptide backbone; the steric collision cannot be relieved by simply rotating the prealbumin amino acid side chains or changing the diphenyl ether torsion angles of 4. Alternatively, readjustment of the entire hormone analogue 4 in the binding site may occur to relieve the bad contact, but this would cause less favorable contacts for the rest of the analogue (particularly I_3 and I_5) with the protein. Therefore, we predicted the relative order of binding affinities for the α-naphthyl analogues as follows: **1 > 2 ≈ 3 > 4**.

Table I. Binding Affinities of Thyroid Hormone Analogues to Prealbumin

no.	% $K_{a(T4)}$[a]
1	28.7 (0.3)
1a	8.6 (0.3)[b]
1b	1.6 (0.1)[b]
2	1.6 (0.1)
2a	0.3 (0.03)[b]
3	6.3 (0.5)
4	0.7 (0.1)

[a] % $K_{a(T4)}$ is the ratio (×100) of the apparent association constant of the analogue to that of DL-thyroxine (1-3) or L-thyroxine (1a, 1b, 2a, 4). The numbers in parentheses indicate the standard deviation for each value. [b] Data from Andrea et al.[24]

Experimental Section

Thyroid Hormone Analogues and Protein. L-Thyroxine was purchased from Sigma Chemical Co. L-[^{125}I]Thyroxine (1200 μCi/μg) was obtained from New England Nuclear Corp. The α-naphthyl analogues were prepared previously in this laboratory.[25,26] Attempts to prepare the 3′-iodo analogue of 1 were unsuccessful.

Human serum prealbumin was obtained from Dr. R. C. Hevey, Behring Diagnostics, Somerville, NJ. The protein behaved as a homogenous preparation after NaDodSO$_4$ gel electrophoresis and had an immunological purity of 98%.

Competition Binding Assays. The gel-filtration procedure described by Somack et al.[28] was used to measure the binding affinities of the thyroid hormone analogues to prealbumin. In this assay, analogues are tested for their ability to displace L-[^{125}I]thyroxine from the high-affinity prealbumin binding site. Stock solutions of analogues were prepared and stored in methanol and diluted to their final concentration in the assay buffer. The assay mixture contained 10^{-8} M prealbumin, 1.25×10^{-8} M L-[^{125}I]thyroxine, unlabeled analogue (10^{-5} to 10^{-8} M), and 0.1 M Tris-Cl (pH 8.0) containing 0.1 M NaCl and 1 mM EDTA in a volume of 0.5 mL. After incubation for 30 min at 25 °C, the assay mixture was chilled to 0 °C, and protein-bound L-[^{125}I]thyroxine was isolated by gel filtration on Sephadex G-25 (medium) columns (bed volume = 2.0 mL) equilibrated with Tris buffer at 0–4 °C. A 0.4-mL aliquot of the assay mixture was applied to the column, and the column was rinsed with 0.4 mL of buffer. The protein fraction was eluted with an additional 0.8 mL of buffer. The amount of ^{125}I was then determined with a Searle Auto-Gamma spectrometer (efficiency = 74%). Free hormone binds tightly to the gel matrix and does not elute in the volumes used.[28] Since the half-life for dissociation of the thyroxine–prealbumin complex at 4 °C measured by this technique was 13 min,[28] there was minimal dissociation of bound L-[^{125}I]thyroxine during the less than 2 min required for gel filtration. Doubling the elution time had no significant effect on the K_a values for the analogues tested.

The binding affinities of **1a**, **1b**, and **2a** were determined by Andrea et al.[24] and are listed in Table I. The competitive binding curves for the analogues tested in this study are shown in Figure 5. Binding affinities (K_a) were determined by the method of Oppenheimer et al.[29] by fitting the data of Figure 5 to the following equation:

$$\text{free/bound} = (1/K_{a(T4)}[P]) + (K_{a(A)}(\text{concn of analogue added})/K_{a(T4)}[P])$$

Where $K_{a(T4)}$ is the equilibrium association constant for L-thy-

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roxine, $K_{a(A)}$ is the equilibrium association constant for the unlabeled analogue, and [P] is the concentration of prealbumin. We determined the K_a of L-thyroxine and DL-thyroxine to be 2.9×10^7 M^{-1} and 1.7×10^7 M^{-1}, respectively. Andrea et al.[24] reported values for L-thyroxine and D-thyroxine of 3.5×10^7 M^{-1} and 1.3×10^6 M^{-1}, respectively.

Results

The apparent K_a values for the analogues tested in this study are listed in Table I. The experimentally determined binding affinities of **1**, **1a**, and **1b** (also **2** and **2a**) are in the predicted order and show that the addition of the ring at the 5′- and 6′-positions makes a strongly positive contribution to binding to prealbumin. This demonstrates the ability of the pocket in the hormone-binding site to accept a 6′-substituent, as suggested by the computer graphics modeling.

The binding affinities observed for **1**–**4** are in the predicted order except for the difference in the binding affinities for **2** and **3**. Although casual inspection of the molecular surface models for **2** and **3** indicates that they should fit equally well into the binding site and thus have similar binding affinities, closer examination of the models shows that the hydroxyl group from **3** can interact more strongly with the binding site than the hydroxyl group from **2**. The hydroxyl group on **3** fits more tightly into its pocket ($P_{3'}$) and comes within hydrogen-bond distance of the hydroxyl groups of Ser-117C and Thr-119C. No such interactions are available for the phenolic hydroxyl group on **2**; it is not located deep enough within its pocket (P_3) to contact the protein surface and can only bind indirectly through the intervening water molecule to the Ser-117A and Thr-119A hydroxyl groups. This explains why **3** has a greater binding affinity than **2** and shows that the detailed atomic environment surrounding a group must be considered in addition to the "quality" of fit of the group into the binding site.

The difference in binding affinity between **2** and **4** is small, indicating only a slight difference in the free energy of binding of these analogues to prealbumin. Although binding of **4** must be accompanied by a conformational change in the binding site and/or a different orientation of the analogue in the binding channel than with **2**, the 7′-OH of **4** is within hydrogen-bonding distance of a carbonyl oxygen on the peptide backbone. The poor steric fit of **4** into the binding site is apparently compensated by the formation of this hydrogen bond, resulting in a binding affinity which is only slightly lower than that of **2**.

Conclusions

This work demonstrates the usefulness of the molecular modeling approach as a predictive tool in drug design. The display of both the molecular surface and the usual skeletal model proved extremely powerful and clearly indicated the potential significance of an empty pocket in the hormone binding site as being ideally positioned to accept a 6′-substituent on the outer ring of thyroid hormones. This led us to model, predict, and measure the binding affinity to prealbumin of the previously untested α-naphthyl thyroid hormone analogues. The recognition and subsequent exploitation of such empty pockets in the molecular surface of a ligand-binding site provides an attractive route for the design of new, more tightly bound ligands. These results account for thyroid hormone–prealbumin structure–activity relationships and demonstrate that these models can successfully be used to design and predict qualitatively the binding affinities of new compounds.

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