A Theoretical Study of the Binding of Polychlorinated Biphenyls (PCBs), Dibenzodioxins, and Dibenzofuran to Human Plasma Prealbumin

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Binding energies to human plasma prealbumin using the energy minimization program AMBER are found for a series of polychlorinated biphenyls, dibenzodioxins, and dibenzofuran. Corrections for solvation free energies of the chlorinated analogues lead to estimates of the differential free energies of complex formation. These are compared in a number of cases to known experimental log (K_{PCB}/K_{ref}) values. The theory correctly separates strong, intermediate, and nonbinders. On the basis of calculations, 2,3,7,8-tetrachlorodibenzodioxin and 2,3,7,8-tetrachlorodibenzofuran are predicted to be strong binders, 3,3′,5,5′-tetrachlorodiphenoquinone is predicted to be a weak binder, and octachlorodibenzodioxin is predicted to not bind at all. This theoretical model for prealbumin interactions may be of use in estimating the toxic potential of PCBs and related halogenated aromatic hydrocarbons of environmental importance.

The distribution of polychlorinated biphenyls (PCBs), dibenzodioxins, and dibenzofurans in the environment constitutes a potentially serious public health problem. Several accidents[1-3] have helped to focus the concern relating to these and related compounds on their molecular mechanism of toxic action. It is now known, for instance, that the compounds bind to cytosolic receptors and thereby apparently enhance the activity of certain enzymes implicated in carcinogenic behavior.[4] Recently, we have shown[5] that PCBs hydroxylated to increase solubility will quantitatively displace thyroxine ([^{125}I]thyroxine) from its complex with prealbumin, one of the major thyroxine (T_4) transport proteins. From this data we have been able to determine equilibrium constants for PCBs binding to prealbumin as well as verify the thyroxine–prealbumin association constant (8×10^7 M^{-1}). Similar behavior is also seen with solubilized nuclear receptors in rat liver tissue.[6] PCBs quantitatively displace nuclear receptor-bound thyroxine. This latter observation is intriguing since it has been suggested that the nuclear receptor for thyroxine may be an evolutionarily-close ancestor to prealbumin,[7] or perhaps even prealbumin itself.[8] We have recently proposed[9] a new cooperative receptor mechanism model for dioxin (and related compound) toxic action, possibly involving the same receptor proteins responsible for thyroid hormone action.[10] The structural requirements for binding the dioxin or Ah receptor combined with the structural requirements for binding prealbumin, a model for a nuclear protein receptor, correspond with those required to produce toxicity for this class of compounds.[11] Thus, in view of the predictive potential of the prealbumin interaction model, it was of interest to develop a theoretical model based on molecular parameters.

One modern method for getting at the details of molecular interactions for system involving proteins and nucleic acids is to utilize the combined techniques of computer graphics and energy minimization.[12] The thyroxine–prealbumin complex is especially well-tailored for this since the X-ray structure of both the bound[7,13-15] and unbound protein is known. An earlier study[16] has used the theoretical techniques discussed above to compare the relative binding of closely related thyroxine analogues to thyroxine itself. In this seminal work, Blaney et al. utilized an early version of the AMBER force field;[17] the relative binding results found for the various analogues compared encouragingly well with the experimental binding results.[18] In a followup study,[19] the energy was partitioned into various contributions for thyroxine and the thyroxine analogues. On the basis of the success of these works, we have herein performed similar computations on a number of the hydroxylated PCBs for which binding data exists[5] as well as several closely related molecules (Chart I).

Methods

Theoretical. A recent version[20] of the AMBER force field was utilized to calculate energies of the separated molecules and the bound complexes for prealbumin and a number of hydroxylated PCBs. The AMBER force field results from a straightforward formulation in which bond stretches and angle bends are treated quadratically, torsional barriers with a single trigonometric variable, van der Waals terms

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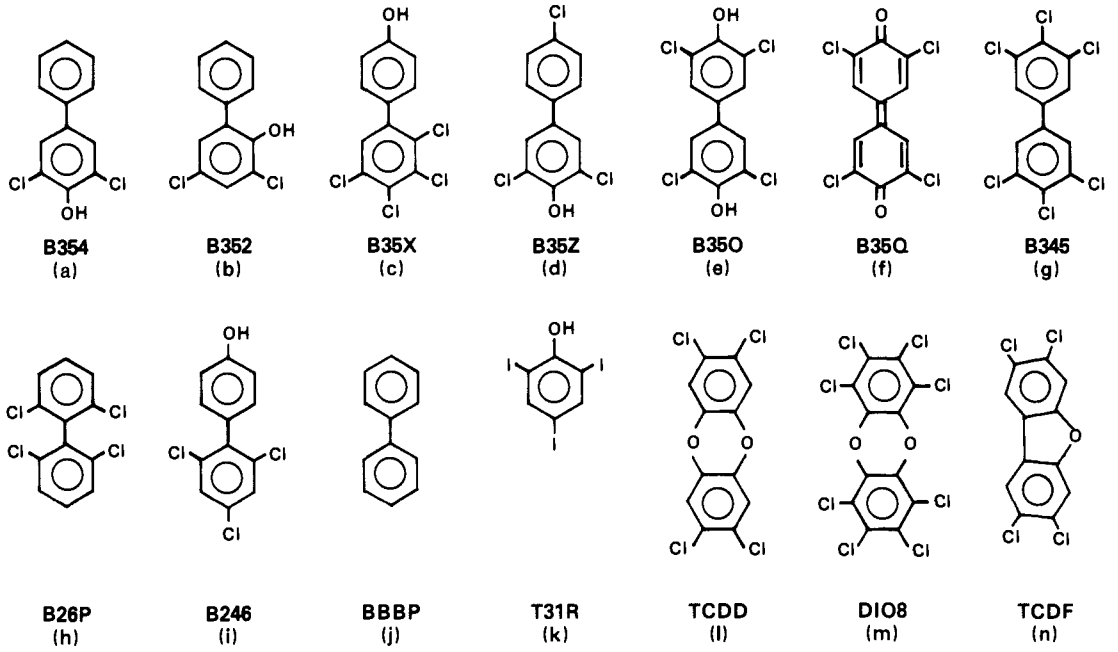
Chart I[a]



[a] (a) B354 = 4-hydroxy-3,5-dichlorobiphenyl, (b) B352 = 2-hydroxy-3,5-dichlorobiphenyl, (c) B35X = 4′-hydroxy-2,3,4,5-tetrachlorobiphenyl, (d) B35Z = 4-hydroxy-3,5,4′-trichlorobiphenyl, (e) B350 = 4,4′-dihydroxy-3,3′,5,5′-tetrachlorobiphenyl, (f) B35Q = 3,3′,5,5′-tetrachlorodiphenoquinone, (g) B345 = 3,3′,4,4′,5,5′-hexachlorobiphenyl, (h) B26P = 2,2′,6,6′-tetrachlorobiphenyl, (i) B246 = 4′-hydroxy-2,4,6-trichlorobiphenyl, (j) BBBP = biphenyl, (k) T31R = 2,4,6-triiodophenol, (l) TCDD = 2,3,7,8 = tetrachlorodibenzo-*p*-dioxin, (m) DIO8 = octachlorodibenzo-*p*-dioxin, (n) TCDF = 2,3,7,8-tetrachlorodibenzofuran.

with a standard 6–12 potential, electrostatic terms with the normal Coulomb interaction involving partial charges found quantum mechanically and a 10–12 potential for binary hydrogen bond interactions. Cross terms (bending and stretching, etc.) such as are present in other formulations[21] are not included. Most parameters have been chosen so as to mimic energy/geometry information on small molecules.

The most recent X-ray data on prealbumin (refined to 1.8 Å[16] and S. J. Oatley, unpublished) was used to estimate the atom positions of the pocket region of the prealbumin system. The binding system exists as a tetramer composed of four identical subunits. A binding pocket consisting of two subunits exists at either end of a channel that runs through the center of the tetramer. Binding of thyroxine is anticooperative;[22] however, both binding sites are identical. We shall concern ourselves only with the complex with one thyroxine bound. As in the earlier studies, we shall restrict the number of atoms included in the energy minimization to those residues that are directly involved in the formation of the hydrophobic binding pocket. The sequence of human plasma prealbumin is given in ref 23; the residues (36 total) explicitly included are Met[13]-Val[14]-Lys[15]-Val[16]-Leu[17], Ser[52]-Gly[53]-Glu[54]-Leu[55]-His[56], Ala[108]-Ala[109]-Leu[110],Ser[117]-Thr[118]-Thr[119]-Ala[120]-Val[121] from the A chain and the corresponding amino acids from the C chain. Of this group amino acids with side chains pointing away from the pocket region are replaced with glycines (these amino acids are underlined). To reduce the change of artifactural pocket reorganization, the nitrogens of all amino acids included were constrained to the X-ray positions with a force constant of 100 kcal/(mol Å^2). The initial choice of PCB position was defined by graphically matching one of the rings of the PCB (chosen to be the ring with the largest amount of Cl substitutuon) with the position of the ring of the X-ray-refined thyroxine[16] (S. J. Oatley, unpublished) which was deepest in the binding pocket. Consistent with the earlier studies on the thyroxine/prealbumin system,[16,19] the 1–4 nonbonded and electrostatic terms in the force field were reduced by one-half; likewise, a distance-dependent dielectric constant ($\epsilon = R$) was employed, a procedure that attempts to damp out the long-range electrostatic terms and thereby partially account for solvent and counterions. A conjugate-gradient minimizer was used to compute energies until no lower energy was found; typical values for the root-mean-square (rms) gradient at minimization was 0.07 kcal/(mol Å).

Special treatment for the PCB analogue was necessary. The PCBs themselves involve a rather unique bond connecting the two rings. While the connecting atoms are each part of aromatic systems, and thus are formally sp^2-aromatic atom types, the bond between the rings is apparently only slightly shortened (1.51 Å) from that expected for C–C single bonds. Our previous quantum chemical studies[24] on ortho- and nonortho-substituted PCBs indicated that (a) nonortho-substituted PCBs have a minimum energy for a interplane dihedral angle of about 40° with rather small rotational barriers at 0° and 90° and (b) ortho-substituted compounds have large barriers at 0° and 180° with very small barriers at 90°. These calculations have been substantiated by a recent photoelectron study[25] and by a recent X-ray study on 2,4,5,2′,4′,5′-hexachlorobiphenyl.[26] Thus the nonortho-substituted PCBs can be expected to be able to attain almost any dihedral in the field of a

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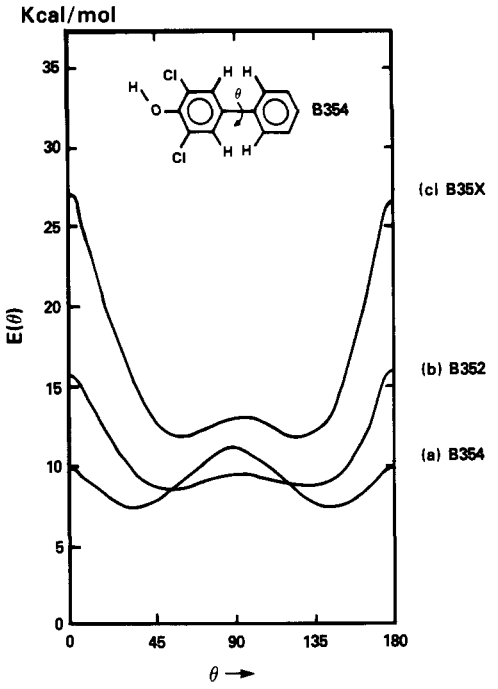

Figure 1. Potential energy curves computed with AMBER for (a) B354 (a nonortho PCB), (b) B352 (an ortho-hydroxylated PCB), and (c) B35X (an ortho-chlorinated PCB). The energy was minimized at all points along the rotation with the angle held fixed.

protein because of the small barriers to internal rotation. On the other hand, the ortho-substituted class of PCBs cannot attain conformations approaching planarity. To accomodate these observations, the PCBs were constructed with the PREP option of the AMBER package[17] with explicit hydrogens in the ortho positions and the force field was extended to include rotational barrier terms that would reasonably simulate the quantum calculations. In addition, the ring–ring bond distance was constrained to 1.50 Å with a large force constant. Figure 1 shows typical rotational energy curves for the class of compounds for which calculations were performed.

It was necessary to make an assignment of partial charges for the PCB compounds. Andrea et al.[27] performed CNDO calculations to act as a guide for the assignment of charges. The partial charges developed were R–C(0.14)–O(–0.4)–H(+0.4); R′–C(+0.26)–O(–0.24)–C(+0.12)–R″ with the –C(0.12) carbon in the ring with the phenol group and I (–0.07). The remaining atoms on the diphenyl ether rings were chosen to have zero charge. From the dipole moments of iodobenzene (1.70 D) and chlorobenzene (1.69 D), and the C_{ar}–I bond/C_{ar}–Cl bond distance ratios (2.05/1.70) an effective charge of –0.083 is estimated for Cl based on a choice of –0.07 for I. STO-3G ab initio calculation using GAUSSIAN 82[28] suggest a similar charge for Cl. For the PCBs of this study, a partial charge of –0.083 was adopted for all Cl, with the distribution of charge chosen to be similar to the earlier thyroxine analogue study. Thus carbons attached to chlorine are not explicitly charged.

Since experimental equilibrium constants exist for the hydroxylated PCBs from which formation free energies can be computed, it is then of interest to approximate an

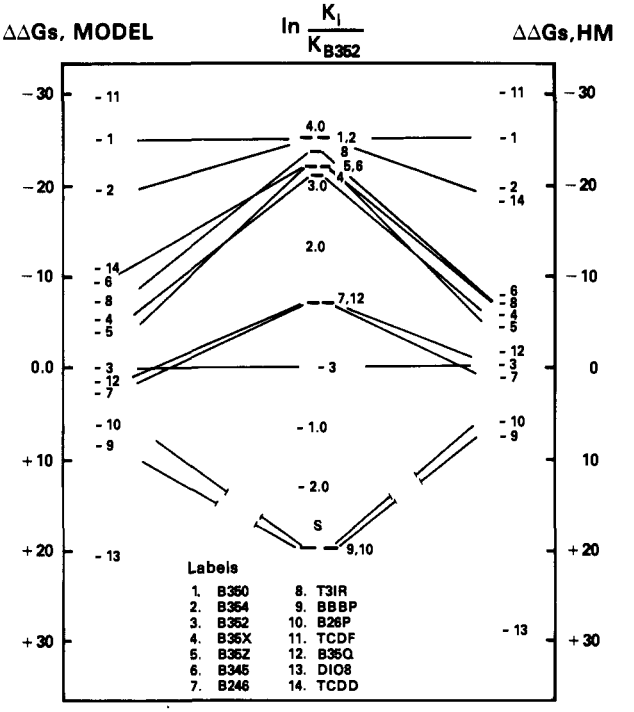

Figure 2. Comparison of the experimental values in (K_i/K_{B352}) which is proportional to a differential free energy with corresponding theoretical values $\Delta\Delta G^{g\to s}$ computed with the use of the solvation $\Delta G^{g\to s}$ found from either MODEL or from ref 31. The theoretical values for TCDF, B35Q, DIO8, and TCDD represent predictions.

equivalent quantity from the calculations. Blaney et al.[16] developed an expression for the prealbumin/thyroxine binding for the relative free energy of complex formation on the basis of a thermodynamic cycle for the formation reaction in solution and in the gas phase:

$$\Delta\Delta G_s \cong \Delta\Delta E_g - \Delta\Delta G^{g\to s}$$

In this expression ΔG_s is the free energy for the complex formation in solution, ΔE_g is the formation internal energy (i.e., $E_{complex} - E_{protein} - E_{PCB}$) in the gas phase and $\Delta G^{g\to s}$ is the solvation free energy of the PCB. The ΔΔ symbology is used to indicate that a difference is taken between the appropriate energy of any of the PCBs and a reference compound from the class. To arrive at the approximation for $\Delta\Delta G_s$, it was necessary to assume that the solvation free energy for the bound protein was independent of analogue; this is reasonable since the PCB is quite buried in the pocket. Also, the relative enthalpic and entropic contributions to the gas-phase complex formation reaction were neglected; these terms should roughly cancel for the analogue and reference if the compounds are sufficiently similar.

Whereas $\Delta\Delta E_g$ can be estimated from the molecular mechanics procedure, $\Delta\Delta G^{g\to s}$ cannot. In their work on thyroxine analogues, Blaney et al. approximated this term by assuming that the amino acid terminus led to the dominant contribution to the solvation. Experimental solvation free energies determined by Wolfenden et al.[29] for acetic acid and methylamine were used to estimate the differential solvation of the analogues. For the compounds for which we have experimental binding constants, some have two hydroxyls, some have one, and a few have none. Thus, it can be expected that a substantial difference in solvation free energy might result over the range of these

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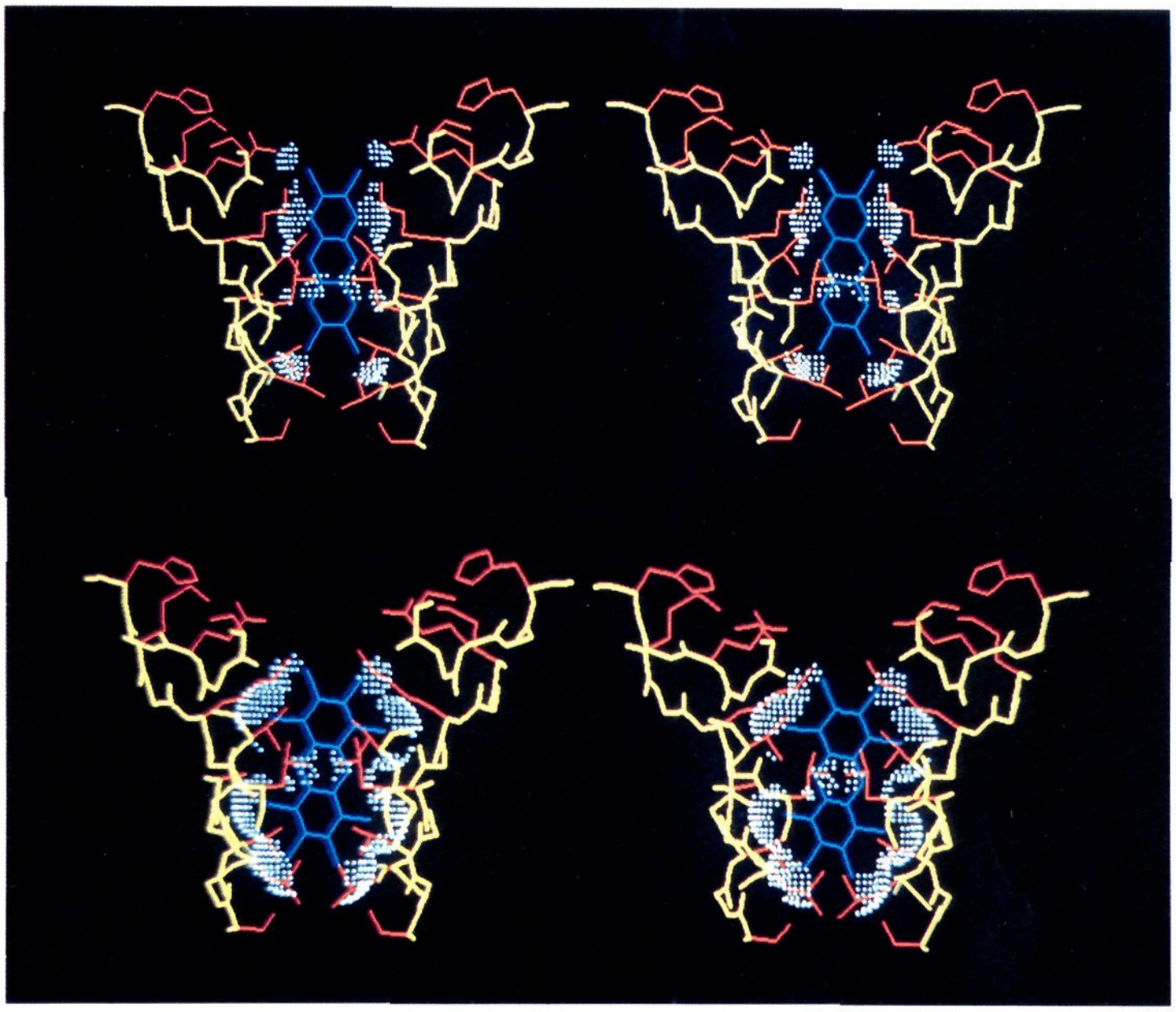

Figure 3. Energy-built models were examined by using a Silicon Graphics Iris 1400. The stereopair energy-built complex of TCDD (top) and DIO8 (bottom) are shown. The TCDD (or DIO8) structure is shown in blue, and the amino acid side chain and backbone structure of the binding pocket are shown in red and yellow, respectively. The crystallographically well-defined water molecules are shown in green. The white dots indicate the areas of "contact" between the ligand and the protein as determined by the overlap of the van der Waals spheres.

compounds, and consequently, the differential analogue solvation term cannot be neglected.

A possible way out of the solvation dilemma was suggested recently by Frömmel.[30] A linear relationship between solvation energies for side chains of the common amino acids as measured by Wolfenden et al.[29] and the accessible polar surface area (APSA) of the side chain was tested and found to be successful in accounting for the variation in solvation. The molecular mechanics program MODEL 1.3 (kindly given to us by C. Still, Columbia University) estimates polar accessible surface area and suggests the relationship $\Delta G^{g\rightarrow s} \cong -0.075 \times$ APSA kcal/mol. To test the applicability of this equation, we first minimized the energy of a number of amino acid side chain analogues using MODEL and computed the APSA and thus the solvation free energy. The actual computation of the surface area was modified (by T.A.D.) so as to spread points randomly over solvated van der Waals spheres; enough points were included to reach convergence. These solvation free energies were then plotted against the values measured by Wolfenden et al. (after correcting for ionization fraction). Since the result is a reasonably linear relationship, ($\Delta G^{g\rightarrow s}$(ref 29) = $0.905\Delta G^{g\rightarrow s}$ (MODEL) − 0.232 kcal/mol, correlation coefficient = 0.951), this procedure was then used to compute solvation free energies for the PCBs.

Another approach to solvation was suggested earlier by Hine and Mookerjee.[31] From the measurement of vapor pressures for a broad class of organic molecules (some with chlorine substituents) an empirical least-squares procedure from which bond contributions to the solvation free energy (or log [vapor]/[solution]) were computed. We have used this method to compute differential solvation free energies by summing the appropriate bond contributions for our class of PCBs. The two methods give surprisingly similar results.

Experimental Section

The competition binding data (PCBs displace [^{125}I]thyroxine) of Rickenbacher et al.[5] was treated with a single binding site model:

$$T_4 + P = T_4{:}P \qquad K_{T_4}$$

$$PCB + P = PCB{:}P \qquad K_{PCB}$$

$$P_T = P + T_4{:}P + PCB{:}P \qquad \text{(mass balance of protein)}$$

In these equations, P is the unbound protein, P_T is the total protein, and the K's are association binding constants. These equations can be solved to find an equation for T_4:P

$$T_4{:}P = \frac{T_4 \times P_T \times K_{T_4}}{1 + T_4 \times K_{T_4} + PCB \times K_{PCB}}$$

The binding assay is such that T_4:P, T_4, P_T, and PCB are measured or can be estimated; therefore, K_{T_4} and K_{PCB} can be determined by nonlinear least squares. For the latter purpose we used the SAS[32] program DUD (Doesn't Use Derivatives) adapted for the VAX-11/780. The equilibrium constants for the com-

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Table I. Experimental Binding Constants for T_4 and Competitors (Ref 5)[a] (See Chart I for Compound Labels)

compd	K_{T4} ($\times 10^7$ M^{-1})	K_{PCB} ($\times 10^7$ M^{-1})	K_{PCB}/K_{352}
B350	7.62	101	45.5
B354	6.60	100	45.0
B352	6.00	2.22	1.00
B35X	9.93	53.9	24.3
B35Z	8.27	61.0	27.5
B345	5.03	61.6	27.7
B246	8.19	6.46	2.91
T31R	7.39	75.0	33.8
BBBP		~no binding	~<0.1
B26P		~no binding	~<0.1
B35Q	8.60	6.49	2.92

[a] The association constants were obtained from the competitive binding curves based on the gel filtration assay. Equilibrium dialysis and fluorescence quenching assays give very similar results for thyroid hormones (see Nilsson, S. F.; Peterson, P. A. *J. Biol. Chem.* **1971**, *246*, 6098).

Table II. Energies Computed from AMBER[a] (E_{POCKET} = −462.2 kcal/mol; See Chart I for Compound Labels)

compd	$E_{complex}$	E_{indiv}	ΔE_g	$\Delta\Delta E_g$
B352	−460.5	8.54	−6.83	0.0
T31R	−468.0	7.98	−13.78	−6.95
TCDF	−458.1	36.61	−32.61	−25.78
BBBP	−449.5	8.40	+4.30	11.13
B35Q	−458.6	11.28	−7.78	−0.95
B26P	−448.9	11.15	+2.15	8.98
DIO8	−435.1	11.70	+15.3	22.13
B246	−457.4	10.82	−6.32	0.51
B350	−491.2	6.25	−35.25	−28.42
B35X	−464.3	10.93	−13.03	−6.2
B35Z	−467.7	7.03	−12.53	−5.7
B354	−479.6	7.34	−24.64	−19.81
TCDD	−471.2	8.93	−18.03	−11.2
B345	−463.1	8.845	−9.75	−2.92

[a] The initial conformations of B35Z, B350, B345, and BBBP are the same as B354 (Chart I); for B352 and B35X (see Chart I). B26P and B246 have their two rings at 90° initially. B35Q, T31R, TCDD, DIO8, and TCDF are all initially planar. Upon minimization in the pocket, all molecules essentially retain their initial conformations except for DIO8, which has the two planes somewhat twisted in the final minimized conformation.

pounds studied here are given in Table I. The thyroxine binding constant K_{T_4} is computed for each competing compound and compares favorably with that determined from an independent Scatchard analysis experiment: $(K_{T_4})_{av}$ from the displacement study = 7.37×10^7 M^{-1}, K_{T_4} Scatchard analysis = 8.0×10^7 M^{-1}.[5]

Results

Once the PCBs force field parameters (rotational barriers, partial charges, etc.) were defined, their individual energies were minimized. These are tabulated in Table II. With the aid of computer graphics (a Silicon Graphics Iris 1400 workstation) these molecules were then placed in the prealbumin pocket so as to locate the most chlorinated ring at the X-ray location of the phenolic ring of thyroxine. All initial positions were chosen to maximize the overlap of the orientation of the "inner ring" iodines on thyroxine with the corresponding chlorines on the PCBs and analogues. These starting coordinates are available upon request. The complex was then minimized until no lower energy was found. Several orientations (rotations along the long axis) for the ortho-substituted PCB B352 (see Chart I for label definitions) and the nonortho-substituted PCB B354 were investigated, with the lowest energy orientation being used in subsequent calculations. The nonbonded terms (excepting H bonds) were evaluated without a cutoff for interaction to avoid complications in correcting for split dipoles or discontinuous forces brought about by an abrupt cutoff.

Table III. Solvation Free Energies Computed from MODEL (See Text) and Ref 31 (HM) (See Chart I for Compound Labels)

compd	$\Delta G^{g\to s}_{MODEL}$	$\Delta G^{g\to s}_{HM}$	$\Delta\Delta G^{g\to s}_{MODEL}$	$\Delta\Delta G^{g\to s}_{HM}$
B352	−2.54	−6.83	0.0	0.0
T31R	−2.57	−6.77	−0.03	+0.10
TCDF	−1.51	−1.47	1.03	5.36
BBBP	0.0	−2.99	+2.54	3.84
B35Q	−5.64	−7.64	−3.1	−0.81
B26P	0.0	−3.37	+2.54	3.46
DIO8	−1.28	+0.33	1.26	−6.50
B246	−4.34	−6.93	−1.8	−0.10
B350	−5.75	−9.58	−3.21	−2.75
B35X	−3.74	−7.02	−1.2	−0.19
B35Z	−3.33	−6.93	−0.79	−0.10
B354	−3.17	−6.83	−0.63	0.0
TCDD	−2.19	+0.55	0.35	7.38
B345	0.0	−2.47	+0.35	4.36

Chlorinated dibenzodioxins and chlorinated dibenzofurans are very similar to PCBs in their spatial extent. Although binding measurements are not available (because of solubility limitations in the binding assay), we have built the tetrachloro-substituted analogues (TCDD and TCDF) that are expected to act most nearly like the laterally substituted PCBs with the PREP option of AMBER, used charge distributions based on the earlier choices for PCBs and thyroxine, and locked the appropriate dihedral angles (for TCDF) to ensure planar structures when minimized. In addition, a fully chlorinated dioxin was included in the set to test earlier binding measurements[33] with the dioxin or Ah receptor which suggests that reduced binding is seen for all except the TCDD isomer.

The minimized energies for these molecules are given in Table II; these results then constitute predictions of the relative binding of these molecules compared to PCBs. In addition, one of the PCBs, the 3,5,3′,5′-tetrachloro-4,4′-dihydroxybiphenyl (B350) analogue, which was found to be a very strong binder, in a later set of experiments on binding to a nuclear extract preparation,[6] was seen to undergo such strong binding that a set of experiments were designed to look at the properties of this molecule. Consequently, it was discovered that this molecule is susceptible to oxidation to the corresponding diphenoquinone structure (the phenolic OH's replaced by = O) with the Cl remaining intact. A search of the chemical literature did not reveal a known structure for this compound; therefore, we performed ab initio (STO-3G) geometry optimization calculations,[34] varying enough parameters to predict that (a) the molecule is planar with a formal double bond separating the rings, (b) the ring double and single bonds are relatively lengthened and shortened, respectively, compared to formal double and single bonds. This structure was then used as a basis with which to construct a molecule using PREP, which was then energy minimized in the prealbumin pocket with AMBER. The energy result is shown in Table II. Finally, as a "blind" test of the entire procedure, the molecule 2,4,6-triiodophenol (TIP) was studied in the competitive binding assay. Simultaneously its energy (after using PREP to construct the molecule) was minimized in the prealbumin pocket. The charge of the iodine (−0.07) in the 4-position was balanced with 0.035 unit of charge on each of the adjacent carbons (the 3- and 5-positions). The remainder of the molecule reflected the

(32) SAS User's Guide: Statistics (SAS Institute, Box 8000, Cary, NC 27511); Chapter 2.

(33) Poland, A.; Glover, E.; Kende, A. S. *J. Biol. Chem.* **1976**, *251*(16), 4936.

(34) McKinney, J. D.; Pedersen, L., unpublished calculations.

Table IV. Final Free Energies for Complex Formation Using MODEL and Ref 31 (HM) for Solvation Corrections (See Chart I for Compound Labels)

compd	$\Delta\Delta G_{s,MODEL}$	$\Delta\Delta G_{s,HM}$	compd	$\Delta\Delta G_{s,MODEL}$	$\Delta\Delta G_{s,HM}$
B352	0.0	0.0	B246	2.31	+0.61
T31R	-6.92	-7.05	B350	-25.21	-25.67
TCDF	-26.81	-31.14	B35X	-5.0	-6.01
BBBP	8.59	7.29	B35Z	-4.91	-5.80
B35Q	2.15	-0.14	B354	-19.18	-19.81
B26P	6.44	5.52	TCDD	-10.85	-18.58
DIO8	20.87	28.63	B345	-10.10	-7.28

standard charge distribution for the phenolic ring of thyroxine. The energies for this molecule are shown in Table II.

The free energies of solvation for the chlorinated analogues that are necessary for completion of the solvation term $\Delta\Delta G_{g\rightarrow s}$ (see the Methods section) calculation were computed by using two different techniques, the first a computer graphics technique using the program MODEL 1.3, the second a bond-contribution approach due to Hine and Mookerjee[31] (discussed in the Methods section), and the results are shown in Table III.

Discussion

The differential free energies in the solution phase for all molecules minimized in the pocket were then computed from the approximation for $\Delta\Delta G_s$ by using both the Still and Hine techniques to estimate $\Delta\Delta G^{g\rightarrow s}$ (see the Methods section) for the chlorinated analogues. The computed free energies are tabulated in Table IV.

Whereas the differential free energies for the displacement experiments (computed from the equilibrium constants) spans only several kcal/mol, the free energies computed from the AMBER procedure corrected for analogue solvation show differences of about 30 kcal/mol for the experimentally studied group. Thus, our computations are not quantitative in terms of absolute free energy differences. However, when comparison is made as to which are strong, intermediate, or nonbinders, a different story emerges. Figure 2 shows the correlation of the experimental log (K_i/K_{352}) (which is proportional to experimental free energies for complex formation) and the computed $\Delta\Delta G_s$ for all molecules studied. Generalizations that follow are as follows: experimental nonbinders (log $(K_i/K_{352}) <$ -2.0) are also predicted to be nonbinders, strong experimental binders (log $(K_i/K_{352}) > 2.0$) are predicted to be strong, and intermediate binders are predicted to be intermediate. The two molecules that show no experimental binding turn out to be the two molecules with the largest positive free energies. The "blind" study molecule, triiodophenol, showed an experimental binding approximately at the mean of the molecules studied; the theoretically computed free energy concurred with the observation. Both TCDD and TCDF are predicted to be strong binders (somewhat more so if the Hine solvation method is used) whereas the octachlorinated dibenzodioxin has the largest positive free energy of any molecule studied and therefore is predicted to be a nonbinder. These predictions are consistent with earlier experimental findings with polar dioxin and furan derivatives in competition studies with [^{125}I]thyroxine for prealbumin.[9] The equation relating experimental and calculated free energies is ΔG_s(exptl) = $0.0686\Delta G_s$(calcd, MODEL) - 1.011, correlation coefficient = 0.761, n = 9 (Table I); ΔG_s(exptl) = $0.0695\Delta G_s$(calcd, HM) - 0.9721, correlation coefficient = 0.741, n = 9 (Table I).

In earlier experimental[35] and theoretical studies[36] of the binding of PCBs to cytosol receptors it had been observed that ortho-substituted PCBs bound less well than nonortho-substituted PCBs given the same degree of chlorine substitution. We observe the same phenomenon in this study: compounds B246, B352, and B26P all exhibit low or no binding; the theory concurs on this point. The ortho-substituted compound that does show intermediate binding (B35X), and is predicted to show intermediate binding from the theory, also has four chlorines for increased hydrophobic interactions with the pocket. Thus, provided there is a sufficient degree of chlorine substitution covering the lateral positions, (3,3',5,5') there does not appear to be a dominant need for a coplanar condition in producing good binding.

As to the question of which method is superior for solvation, we find that both methods result in the same qualitative correlations discussed above. It would appear, however, that the oxygen in molecules with an ether linkage between aromatic systems has unusual polar properties (is actually hydrophobic) in the Hine method, where it is counted as a polar atom in the MODEL procedure. The result of this difference is to predict somewhat stronger binding for TCDD and TCDF with the Hine method. This question is worth further study.

It is of interest to examine the energy decomposition of the binding energies ΔE_g (see the Methods section) in an attempt to find the major contributions to the binding energy. A reasonable rationale might be to expect the van der Waals and electrostatic terms in the potential energy expression to be the dominant factors. The equation can be derived relating ΔE_{exact} (the computed binding energy) with the sum of the change in the van der Waals and electrostatic energies ($\Delta E_{nonbond}$) neglecting the 1-4 interactions for these two and also neglecting changes in bond stretches, angle bends, torsion, and H bonding. ΔE_{exact} is a linear function of $\Delta E_{nonbond}$ (ΔE_{exact} = $0.952\Delta E_{nonbond}$ + 4.71 kcal/mol, correlation coefficient = 0.9854). A similar relationship exists for ΔE_{es}, the change in electrostatic energy on binding, which likewise obeys a linear equation (ΔE_{exact} = ΔE_{es} - 19.3 kcal/mol, correlation coefficient = 0.904); this suggests that the large spread of computed free energies is due mostly to the change in the electrostatic part of the potential. The van der Waals terms show a spread of about 10 kcal/mol (average = -24 kcal/mol) with no apparent trend over the class of molecules studied. Thus, one would conclude that the dominant term responsible for the separation in the binding energies is due to the electrostatic energy changes with some fine tuning from the van der Waals term.

The nature of the kind of binding seen in the hydrophobic prealbumin pocket might also be viewed with a simple surface area contact argument. Such a view would dictate that the key variable is the amount of "contact" between the ligand and the protein. Further, too much contact would lead to severe repulsions on the repulsive wall of the van der Waals well; too little contact would lead to diminished attractive electrostatic and van der Waals contributions. Using an algorithm written by T.A.D. for the SI Iris, we placed van der Waals spheres on all of the atoms of the ligand and counted the surface area. The same was done for the protein pocket atoms. The parts (areas) of the van der Waals spheres for the ligand that were inside of pocket spheres were then counted. An interesting trend emerges. Good binding molecules such as TCDD or B345 (a nonortho PCB) had about 14.4% and 13.3% of their total surface area as a "contact", respec-

(35) Bandiera, S.; Safe, S.; Okey, A. B. *Chem.-Biol. Interact.* **1982**, *39*, 259.
(36) McKinney, J. D.; Long, G. A.; Pedersen, L. *Quant. Struct.-Act. Relat.* **1984**, *3*, 99.

tively. Poor binding molecules such as B26P (an ortho PCB), DIO8 (the octasubstituted dioxin), and B35Q distribute into low area contact (B26P, 7.6%) or high area contact (DIO8, 22%; B35Q; 19.6%). It is interesting that the ortho-substituted compound has too little contact; its low binding energy can be attributed to the fact that it has too few attractive (electrostatic) contributions to be a strong binder. On the other hand, the octachlorinated dioxin has too much contact with its additional chlorines riding up on the repulsive wall of the pocket.

The color display of the IRIS makes it especially easy to pick out these features, for instance to display only the "excluded" contact areas. For example, the stereopair energy-built complexes of TCDD and DIO8 are shown in Figure 3. The "excluded" contact areas for TCDD clearly reveal a good quality fit and draw attention to the importance of a rectangular shape. The "excluded" contact areas for DIO8, on the other hand, reveal a much poorer quality fit with colliding surfaces and considerable deviation from a rectangular shape. It is also interesting to note that DIO8 is positioned much deeper (than TCDD) in the binding pocket.

Conclusions

There are several avenues that should be explored to extend the work presented here. In most cases, only one initial orientation was minimized; a more prudent procedure would involve minimizing from many initial orientations with a thermal weighting of the final results. A related procedure would be to follow the trajectory of the system via molecular dynamics for a time long enough to realistically sample the various local minima for the ligand in the pocket volume. Free energies could then be assessed assuming a normal coordinate analysis would yield vibrational frequencies and thus entropies. The method of accounting for the solvation of the chlorinated analogues was empirical (with both the MODEL and the Hine procedures); experimental evaluation of the solvation energies would be difficult but very useful in this case. Only a relatively small fraction of the protein was included (see the Methods section); again a better procedure would be to include the entire protein dimer that forms the pocket or, perhaps, the entire tetramer since the binding of the second thyroxine is known to be anticooperative and thus at least some dimer–dimer interaction must be involved. The partial charges on the ligand molecules were somewhat qualitatively chosen; a procedure more consistent with the amino acid choices[20] would be to perform split valence level ab initio calculations on the ligand molecules to evaluate the electrostatic potential and then fit via least squares to find a set of partial charges that would also give the computed electrostatic potential. Finally, it would be of use and interest to employ another force field—perhaps the BIOSIM force field of Hagler et al.,[21] which includes cross terms and Morse type stretching—to determine how consistent binding results are with substantially different force fields.

In summary, the computations presented here suggest that molecular mechanics can be a useful tool for qualitatively predicting the relative binding to a protein by a series of molecules that contain the potential for varied noncovalent interactions. This work also provides theoretical confirmation of our previous experimental finding[5] that lateral chlorination is important for high binding activity with prealbumin but does not always guarantee it. Since lateral chlorination and a rectangular shape are also important for high toxicity and excess chlorination and deviation from a rectangular shape as in octachlorodibenzodioxin lower or eliminate toxicity, this theoretical model may be of use for estimating the toxic potential of PCBs and related compounds.

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Registry No. TCDD, 1746-01-6; TCDF, 51207-31-9; B35Q, 27728-29-6; DIO8, 3268-87-9; B354, 1137-59-3; B352, 5335-24-0; B35X, 67651-34-7; B35Z, 4400-06-0; B350, 13049-13-3; B345, 32774-16-6; B26P, 15968-05-5; B246, 14962-28-8; BBP, 93-52-4; T31R, 609-23-4.

Potential Tumor- or Organ-Imaging Agents. 27. Polyiodinated 1,3-Disubstituted and 1,2,3-Trisubstituted Triacylglycerols[1]

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A series of glyceryl 1,3-bis- and 1,2,3-tris[ω-(3-amino-2,4,6-triiodophenyl)alkanoates] were synthesized, radioiodinated with iodine-125, and evaluated for their ability to selectively localize in the liver for potential use as hepatographic imaging agents. Of the nine target compounds synthesized and evaluated in rats, glyceryl 1,2,3-tris[3-(3-amino-2,4,6-triiodophenyl)propionate] (**5b**) displayed rapid and sustained liver specificity. This agent was found to accumulate in the liver in concentrations of 60, 75, and 86% of the administered dose at 5 min, 30 min, and 24 h, respectively. Moreover, the 24-h liver-to-blood ratio of 235 justifies further studies in higher animal species.

The liver is a common site for metastatic disease, particularly from gastrointestinal tract primary neoplasms. There is increasing evidence that early detection of liver metastases followed by prompt initiation of therapy results in an improved prognosis for survival of the patient.[2,3] X-ray computed tomography, employing water-soluble contrast agents, is currently the most accurate noninvasive radiologic procedure for visualizing hepatic masses, but

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