Structurally Specific Binding of Halogenated Biphenyls to Thyroxine Transport Protein

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Prealbumin is a major thyroxine binding protein in blood that has been well studied crystallographically and has also been proposed as a model for the thyroxine nuclear receptor in tissue. The high-affinity T_4 binding site in prealbumin gave a linear plot on Scatchard analysis. The interactions of selected polychlorinated biphenyls (PCBs) with prealbumin have been studied with use of computer graphics and predictions made regarding relative binding affinities for such structures. These modeling predictions were tested by using competitive binding experiments involving selected PCBs and hydroxylated derivatives as soluble structural probes. The results are in excellent agreement with the modeling predictions and demonstrated that these compounds can be highly effective (3–8 times better than thyroxine itself) competitive binding ligands for thyroxine specific binding sites in prealbumin. Laterally (3,3′,5,5′-) substituted PCBs show the highest binding activity and further substitution on nonlateral (2,2′,6,6′-) positions lowers binding activity. Lateral chlorine substitution was common to all PCBs studied that showed high binding affinities. The binding model may also suggest a preference for a linear and symmetrical molecular shape. These structural requirements for binding are substantially consistent with the structure–toxicity relationship for closely related compounds of environmental interest. These specific binding interactions are likely to modulate the distribution of certain PCBs and related compounds and alter hormone–protein interactions that are responsible for the maintenance of normal thyroid status. Since prealbumin is also a model for the putative thyroxine nuclear receptor in tissue, our hypothesis that high toxicity of certain halogenated aromatic hydrocarbons is at least in part due to their thyromimetic properties is further supported.

The chlorinated dioxins, furans, biphenyls, naphthalenes, and benzenes, collectively termed the halogenated aromatic hydrocarbons are among the most important environmental contaminants because of their widespread occurrence and persistence in the environment and concentration in the food chain.[1] There is serious concern about their entering the human food chain and becoming concentrated in the human body such as has been clearly demonstrated[2] for the polychlorinated biphenyls (PCBs). The toxic potency of these compounds varies considerably and is remarkably dependent on the number and positions of halogen atoms in their molecular structure. Qualitative structure requirements for high toxicity include planarity or coplanarity of structure in a shape approximating a rectangle and a sufficient degree of halogenation concentrated about lateral positions of the molecule.[3] Halogenation, for example, in nonlateral positions invariably decreases the biological and toxic potency.

Thyroid hormones (thyroxine, T_4, and 3,5,3′-triiodothyronine, T_3) are known[4] to have profound effects on growth, differentiation, and development of tissues. The metabolism of carbohydrates, proteins, and lipids is influenced by the thyroid gland. Since some signs of dioxin and related compound toxicity are common to those seen in thyroid dysfunction, the possibility exists for these responses to be mediated by thyroid hormone binding proteins and associated changes in serum thyroid hormone concentrations. A characteristic feature of the literature on thyroid effects of these compounds is that the decrease in serum T_4 concentration in rats is reproducible from one study to the next, but the direction of the change in serum T_3 concentration is highly variable. In general, thyroid goiters showing high ^{131}I uptake associated with increased serum levels of thyrotropin (TSH), decreased serum levels of total and free T_4, and variable serum levels of T_3 have been observed[5,6] in rats exposed to PCBs and polybrominated biphenyl (PBBs) mixtures. In the case of PBBs, it has been suggested that the major functional and morphological alterations in the thyroid gland of rats are caused by those PBB congeners that are toxic and can compete with dioxin for binding to its receptor. Enhanced biliary excretion of T_4 is due in part to the induction of hepatic microsomal UDP-glucuronyltransferase activity[6] and probably also to decreased serum protein binding of T_4.[7] It is also of interest that thyroxine binding prealbumin (TBPA) binds retinol binding protein[8] and that the concentrations of vitamin A[9] and TBPA[10] are affected by these toxic halogenated aromatic compounds. Thus, study of the structurally specific binding of PCBs to serum binding proteins specific for T_4 was of interest.

These toxic compounds are also inducers of cytochrome P-448 mediated mixed-function oxidase enzyme systems. The toxicity and induction responses are both thought to involve initial binding of the hydrocarbons to the same cytosolic receptor (dioxin or Ah receptor associated with the *Ah* locus, the structural gene for this protein), but the subsequent events are not completely understood.[11] A key enzyme induced is aryl hydrocarbon hydroxylase (AHH) and good correlations between potency to induce AHH activity and toxicity have been found for certain compound

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0022-2623/86/1829-0641$01.50/0

classes. However, this parallelism is distorted by certain exceptions.[3] There is growing evidence that the induction response may be a characteristic response for certain animal species not implicated directly in the mechanism of toxicity. Binding the *Ah* receptor may be a necessary but not sufficient condition for toxicity. Thus, we seek additional protein binding models that can account for the structure–toxicity relationships. Recently we suggested that TBPA could be used as a model for studying the effects of varying kinds, numbers, and positions of halogens in these toxic compounds and when combined with the structural requirements for binding the Ah receptor could account for the structure–toxicity relationships.[12] However, these initial studies involving interactions of dioxin and furan systems with TBPA were seriously limited by solubility problems that limited our ability to develop a complete structure–binding relationship.

In this work, we describe a more complete investigation involving halogenated biphenyl interactions with TBPA. The biphenyls were chosen for study because of our background and experience in understanding both the chemistry and biology of their interactions.[13-15] In addition, a number of specific isomers and homologues that includes a number of closely related hydroxylated derivatives that have more desirable solubility properties for study under aqueous conditions were available from synthesis or commercial sources. In this biphenyl series, it was also possible to examine the effect on binding of a basically linear shaped molecule (relative to the nonlinear diphenyl ether system characteristic of thyroid hormones) and chlorine substitution in lateral (3,3',5,5'-) and nonlateral (2,2',6,6'-) positions (or both) of the molecule. Thus, the compounds studied in this work primarily represent soluble structural probes for the structural requirements known[3] to be important in the toxicity of these compounds.

This study would extend work generating reasonable structural models based on known structures for TBPA interactions,[12,16] which can then provide experimentally testable predictions of the activity of new analogues. TBPA is a major thyroxine binding protein in the blood,[17] and it shows in addition to the thyroxine binding sites a site that is complementary to the DNA double helix, indicating a possible relationship to the thyroxine nuclear receptor.[18] Prealbumin is a tetramer consisting of four identical subunits that are oriented to form a central channel containing the two identical T_4 binding sites. The molecule has twofold symmetry, and the binding site is lined primarily with hydrophobic amino acid side chains that form polarizable pockets for halogen interactions.[16]

It was important to our studies to investigate the potential of the presence of PCBs and related compounds in blood to alter hormone–protein interactions that are responsible for the maintenance of normal thyroid status.[17] In addition, since TBPA is a model for the thyroxine nuclear receptor,[18] we could further test our hypothesis that the high tixocity of certain halogenated aromatic hydrocarbons is in part due to their thyromimetic properties in which interactions with the nuclear receptors may play a key role.[19]

Chart I

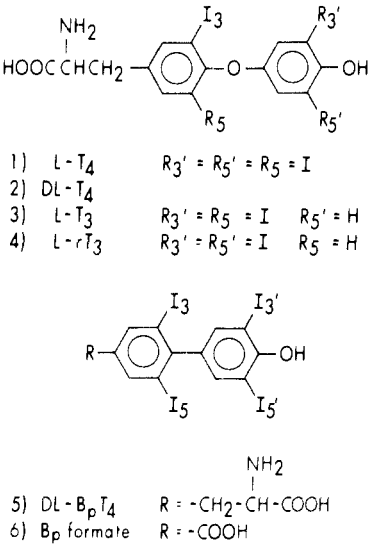

Chart II

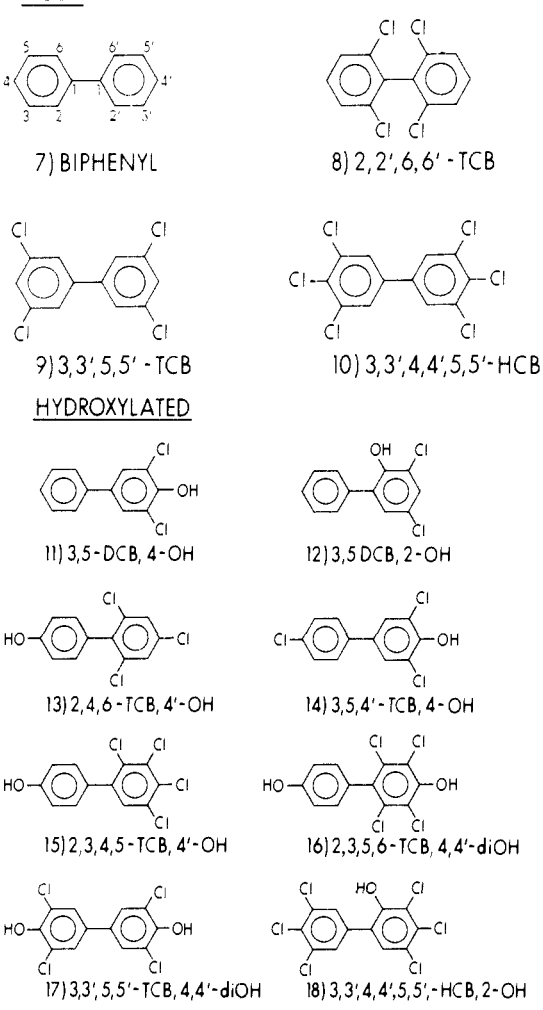

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Table I. Interatomic Contacts <4 Å of 3,3′,4,4′,5,5′-Hexachlorobiphenyl in the T_4-Specific Binding Site of TBPA

binding atom	amino acid	contacts (<4Å)	binding atom	amino acid	contacts (<4 Å)
Cl-3′[a] (≡ Cl-5′)	CB,[b] Ala-108	3.89	C2 (≡ C6)	CG, Lys-15	3.61
	C, Ala-108	4.00			
	N, Ala-109	3.70			
	CA, Ala-109	3.93	C3 (≡ C5)	CG, Lys-15	3.58
	C, Ala-109	3.31		CD, Lys-15	3.94
	O, Ala-109	3.50		CE, Lys-15	3.62
	N, Leu-110	3.28			
	CA, Leu-110	3.64	Cl-3 (≡ Cl-5)	CB, Lys-15	3.13
	CB, Leu-110	3.26		CG, Lys-15	2.51
				CD, Lys-15	2.52
C3′ (≡ C5′)	CB, Ala-108	3.92		CE, Lys-15	2.65
				NZ, Lys-15	3.26
C2′ (≡ C6′)	CB, Ala-108	3.62			
	CD1, Leu-17	3.88			

[a] See numbering system in Chart II; equivalent atoms in parentheses make identical contacts with other side of binding site (residue numbers >500). [b] Atom contacted in given amino acid residue; A, B, G, D, E, and Z denote α, β, γ, δ, ϵ, and ζ position of atom in side chain.

Our approach to this problem was first to devise simple structural models for PCB interactions with TBPA that would provide experimentally testable predictions using competitive binding assays. At the same time, the resulting structure–binding relationship could be compared in a general way with known structure–toxicity relationships for these compounds.[3] This type of approach was possible since TBPA has been well defined crystallographically[20] and the important conformationally properties of the PCBs have been investigated previously by using both X-ray crystallographic[14,21] and quantum chemical methods.[22] Initially, structurally well-defined thyroid hormone analogues were compared to two closely related iodinated biphenyl analogues (Chart I). This was followed by study of a number of hydroxylated PCBs and several PCBs that had sufficient solubility in the assay system for direct study (see Chart II).

Results

The most favorable models for the biphenyl interactions place the biphenyl pivot bond axis coincident with that of the binding site in TBPA with the primed ring coplanar with that of the phenolic ring of T_4. In view of the highly hydrophobic/polarizable nature of the binding site, it was anticipated that the van der Waals/hydrophobic interactions would be dominant in controlling the binding strength of these biphenyl compounds. In this simple modeling exercise, a correspondence between the binding efficacy of each biphenyl system and the total number of contacts less than 4 Å between the biphenyl molecule and the protein[23] was assumed. The importance of meta chlorine substituents is clearly revealed in examining the model-built structures (Figures 1b and 2b) and comparing the number of contacts less than 4 Å listed in Tables I and II. Cl-3 (or Cl-5) substitution does lead to some close contacts with the Lys-15 residue, but this flexible side chain assumes a different conformation on hormone binding and a similar behavior would be expected in this case also to relieve these short contacts. Similar (but somewhat shorter) contacts are expected in the model-built structure with meta iodine substitution (see Table III). The coplanar meta-substituted biphenyl conformation was studied since planarity was known to be important for high toxicity.[3] The modeling was not particularly revealing with regard to the preferred conformation for binding. This problem is under separate study, using energy minimization procedures. The copolanar meta-substituted biphenyl system has many more favorable contacts than the ortho-substituted isomer lacking meta substitution. Chlorines at the 4,4′-positions had no contacts less than 4 Å. Since the hydroxyl group is similar in size to chlorine and most of the hydroxyl groups introduced into the PCB molecules to increase solubility in the assay are located in the para positions, they would not be expected to signif-

Table II. Interatomic Contacts <4 Å of 2,2′,4,4′,6,6′-Hexachlorobiphenyl in the T_4-Specific Binding Site of TBPA

binding atom	amino acid	contact (<4 Å)
C3′[a] (≡ C5)	CB,[b] Ala-108	3.92
C2′ (≡ C6′)	CB, Ala-108	3.62
	CD1, Leu-17	3.88
Cl-2′ (≡ Cl-6′)	CG, Lys-15	3.43
	CG, Leu-17	3.75
	CD1, Leu-17	3.81
	CB, Ala-108	3.17
Cl-2[c] (≡ Cl-6)	CB, Ala-108	3.20
Cl-6[c] (≡ Cl-2)	CD1, Leu-17	3.32

[a] See numbering system in Chart II; equivalent atoms in parentheses make identical contacts with other side of binding site (residue numbers >500). [b] Atom contacted in given amino acid residue; A, B, G, D, E, and Z denote α, β, γ, δ, ϵ, and ζ position of atom in side chain. [c] These contacts are those observed with the subunit 1-127; thus Cl-2 contacts CB Ala-108 and CD1 Leu-517, while Cl-6 contacts CB Ala-608 and CD1 Leu-17.

Table III. Contacts of Meta Iodines of Biphenyl System Calculated from PCB Model (Coordinates for 2,2′,4,4′,6,6′-HCB)

meta iodide	amino acid	contacts (<4 Å)
I3′[a] (≡ I5′)	C,[b] Ala-108	3.88
	CB, Ala-108	3.97
	CA, Ala-109	3.66
	N, Ala-109	3.51
	C, Ala-109	3.00
	O, Ala-109	3.25
	CA, Leu-110	3.37
	N, Leu-110	2.97
	CB, Leu-110	3.07
I3 (≡ I5)	CB, Ala-108	3.04
I5 (≡ I3)	CD1, Leu-17	3.22

[a] See numbering system in Chart I; equivalent atoms in parentheses make identical contacts with other side of binding site (residue numbers >500). [b] Atom contacted in given amino acid residue; A, B, G, D, E, and Z denote α, β, γ, δ, ϵ, and ζ position of atom in side chain. [c] These contacts are those observed with the subunit 1-127; I-3 contacts CB Ala-108 and CD1 Leu-517, while I-5 contacts CB Ala-608 and CD1 Leu-17.

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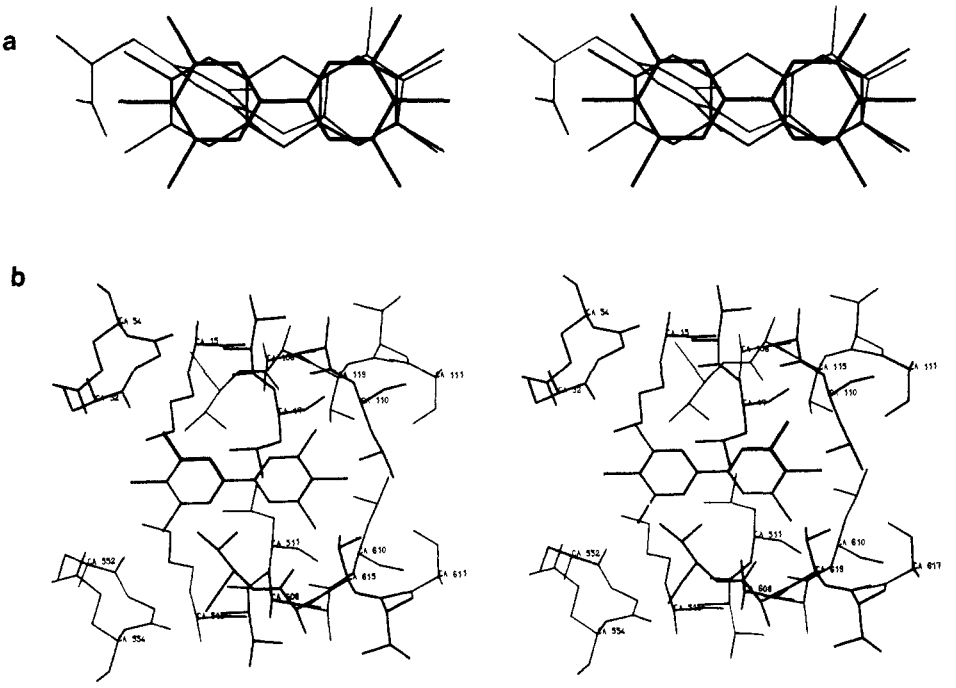

Figure 1. Stereopair superposition of (a) "bound" 3,3′,4,4′,5,5′-HCB (heavy line) and T_4 (light line) along with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD medium line) and (b) model-built structure of 3,3′,4,4′,5,5′-HCB in the hormone binding channel of prealbumin.

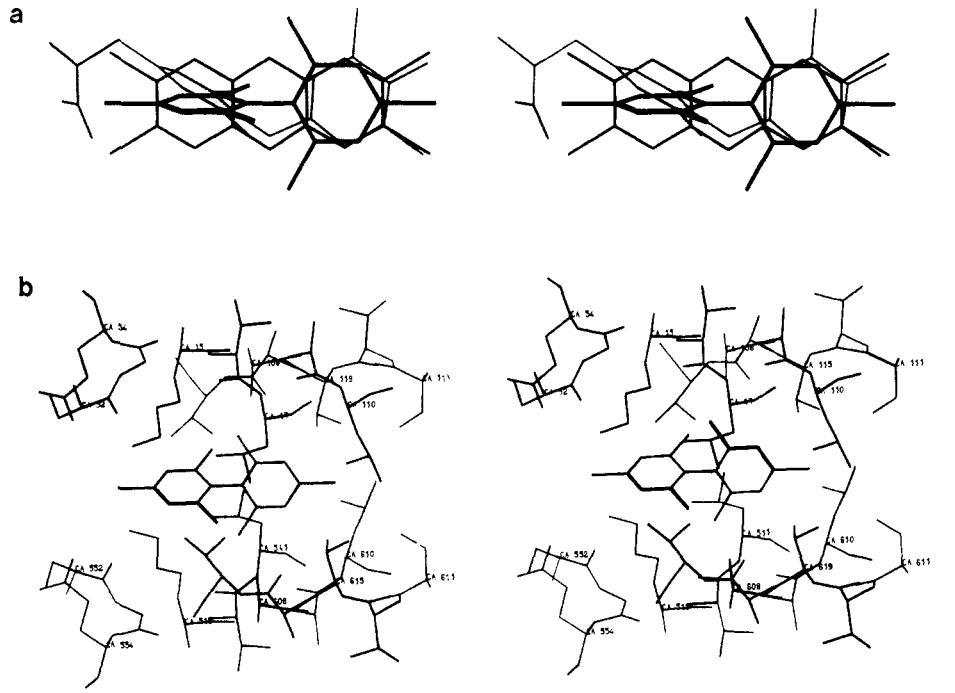

Figure 2. Stereopair superposition of (a) "bound" 2,2′,4,4′,6,6′-HCB (heavy line) and T_4 (light line) along with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, medium line) and (b) model-built structure of 2,2′,4,4′,6,6′-HCB in the hormone binding channel of prealbumin.

icantly alter the binding picture in the absence of electrostatic and hydrogen-bonding effects. However, in this modeling work we have not considered the interaction with the crystallographically well-defined water molecule that occupies one of the six pockets identified[16] in the hormone-binding site. Thus, hydrogen bonding could be a contributing factor in determining the overall binding potency of some of our test compounds. For comparison with our previous work[12] the superposition structure for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is also included in Figures 1a and 2a.

Binding of T_4 to specific binding sites of TBPA was tested by Scatchard analysis (Figure 3). The linearity of this plot suggests normal behavior, and the resulting affinity for the high affinity binding site was $K_a = 8.6 \times 10^7$ M^{-1} in good agreement with findings of Somack[24] ($K_a = 7.6 \times 10^7$ M^{-1}) using the same gel filtration method and Andrea[25] ($K_a = 3.5 \times 10^7$ M^{-1}) and Pages[26] ($K_a = 1.3 \times 10^7$ M^{-1}) using equilibrium dialysis. Nonspecific binding,

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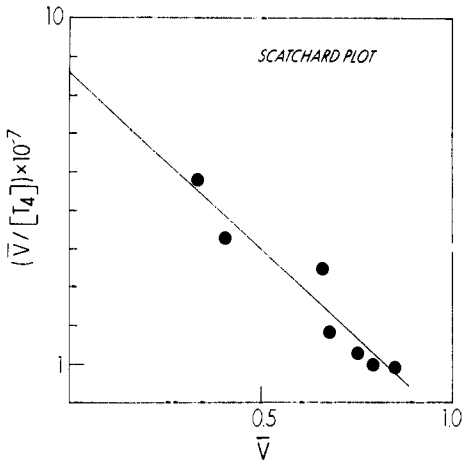

Figure 3. Scatchard plot for T_4 binding to the high affinity binding site of TBPA. $\bar{v}$ is the ratio of the molar concentration of bound T_4 to TBPA. The curve was fitted by linear regression (r = 0.96). The binding affinity (K_a) is 8×10^7 M^{-1}.

Table IV. Binding Affinities of Iodinated Test Compounds to Prealbumin

compd	concn (M) at 50% total binding	binding affinity[a]
L-T_4 (1)[b]	1.0×10^{-8}	100
L-T_3 (3)	6.0×10^{-7}	2
r-T_3 (4)	4.4×10^{-8}	23
DL-T_4 (2)	1.6×10^{-8}	63
DL-BpT_4 (5)	3.0×10^{-9}	331
Bpformate (6)	8.7×10^{-10}	1149

[a] The relative binding affinities of the compounds were determined from the competition binding assays and are expressed relative to L-T_4 (assigned a value of 100). [b] Number in Chart I.

measured by adding excess unlabeled T_4, was less than 6% and was neglected. The competitive binding curves of thyroxine and related compounds are shown in Figure 4.

The relative binding potencies are given in Table IV. It can be seen that the binding potency of T_3 is distinctly lower than that of T_4. In contrast, by simply moving one iodine atom from the ortho position of the inner ring to the meta position of the outer ring (r-T_3), the binding potency reaches a value much closer to L-T_4 or DL-T_4. This indicates the importance of the meta positions of the outer ring, which is consistent with similar findings of Blaney et al.[16] Further interesting observations are the very high binding potencies of the two iodinated biphenyls. They show an even higher affinity to TBPA than T_4. Whereas the DL-amino acid biphenyl **5** is directly comparable to DL-T_4, the possibly lower desolvation energy of the carboxybiphenyl **6** might contribute to its higher binding affinity. But it is evident that the linear biphenyl system is favored over the normal diphenyl ether bridged system and that the ether bridge is not strictly required for binding to TBPA.

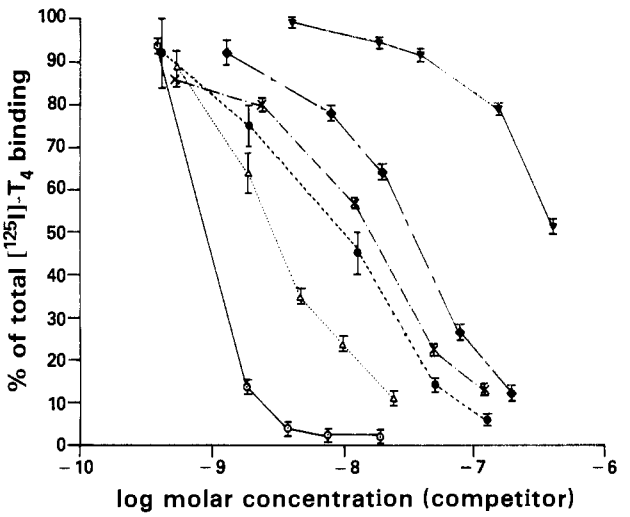

Figure 4. Competitive binding curves of the iodinated compounds: (1) L-T_4 (●), (2) L-T_3 (▼), (3) r-T_3 (♦), (4) DL-T_4 (×), (5) DL-BPT_4 (△), (6) Bp-formate (⊙). Every point represents the average of a duplicate (see also Chart I).

Consistent with this observation, the PCBs also showed high binding affinities, and meta chlorine substituents were a strong determinant of high binding activity as well. In Table V the relative binding potencies of PCBs with varying degrees of chlorination and hydroxylation are given along with the degrees of meta, ortho, and para substitution. It can be seen that meta substitution is common to all the PCBs that showed high binding affinity. Figure 5 shows most of the PCB binding curves. Further analysis of these data support the following conclusions.

Ortho chlorines do not increase binding activity but may even lower it somewhat. Compound **8** with four ortho chlorines shows absolutely no binding in the concentration range tested, whereas with compound **16** (two chlorines in ortho position) and with compound **15** (one chlorine in ortho position) binding was achieved but in combination with meta chlorine substitution. These results suggest that ortho chlorines may lead to some bad contacts with the protein binding surface, resulting in repulsion or affect binding by altering the ionization potential of the phenolic hydroxyl[27] group or by altering the polarizability properties

Table V. Binding Affinities of Chlorinated Test Compounds to Prealbumin

compound	no. in Chart II	concn (M) at 50% total binding	binding affinity[a]	no. of meta Cl substit	no. of ortho substit		no of para substit	
					Cl	OH	Cl	OH
biphenyl	**7**	$>>10^{-7}$[b]	<<5	0	0	0	0	0
2,2′,6,6′-Cl_4	**8**	$>>10^{-7}$[b]	<<5	0	4	0	0	0
3,5-Cl_2, 2-OH	**12**	3.5×10^{-8}	28	2	0	1	0	0
2,4,6-Cl_3, 4′-OH	**13**	2.2×10^{-8}	46	0	2	0	1	1
3,3′,4,4′,5,5′-Cl_6, 2-OH	**18**	4.8×10^{-9}	208	4	0	1	2	0
2,3,4,5-Cl_4, 4′-OH	**15**	3.5×10^{-9}	286	2	1	0	1	1
3,3′,4,4′,5,5′-Cl_6	**10**	3.2×10^{-9}	313	4	0	0	2	0
2,3,5,6-Cl_4, 4,4′-$(OH)_2$	**16**	2.5×10^{-9}	398	2	2	0	0	2
3,3′,5,5′-Cl_4	**9**	2.4×10^{-9}	411	4	0	0	0	0
3,5,4′-Cl_3, 4-OH	**14**	2.0×10^{-9}	501	2	0	0	1	1
3,3′,5,5′-Cl_4, 4,4′-$(OH)_2$	**17**	1.5×10^{-9}	661	4	0	0	0	2
3,5-Cl_2, 4-OH	**11**	1.2×10^{-9}	851	2	0	0	0	1

[a] The relative binding affinities of the compounds were determined from the competition binding assays and are expressed relative to L-T_4 (assigned a value of 100). [b] Highest concentration tested.

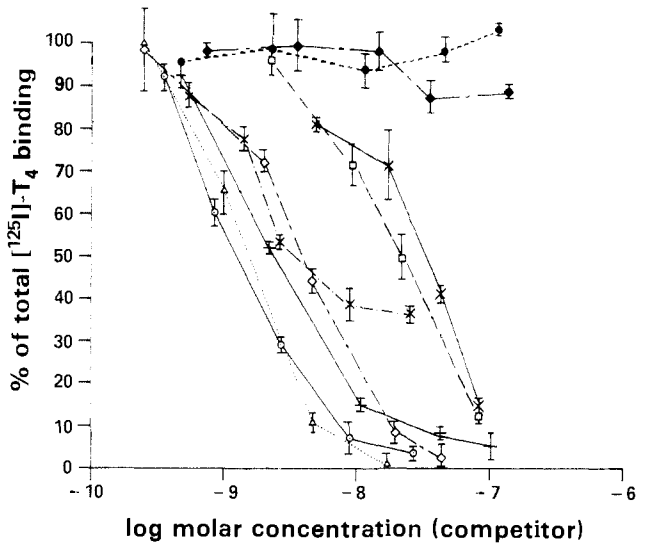

Figure 5. Competitive binding curves of the PCBs: (7) biphenyl (♦), (8) 2,2′,6,6′-Cl_4 (●), (9) 3,3′,5,5′-Cl_4 (+), (10) 3,3′,4,4′,5,5′-Cl_6 (×···×), (11) 3,5-Cl_2, 4-OH (⊙), (12) 3,5-Cl_2, 2-OH (× –), (13) 2,4,6-Cl_3, 4′-OH (□) (15) 2,3,4,5-Cl_4, 4′-OH (◇), (17) 3,3′,5,5′-Cl_4, 4,4′-OH (△). Every point represents the average of a duplicate. Binding curves for compounds 14, 16, and 18 are not shown (see also Chart II).

of the molecule by affecting the conformational state.[28]

An ortho-hydroxyl group might be expected to produce similar effects on binding on the basis of steric considerations but could increase binding due to solubility factors. However, the ortho-substituted compounds were in general poorer binders than the non-ortho-substituted counterparts, suggesting a basic stereochemical difference. Thus greater conformational flexibility and accessibility of the coplanar state may be important in explaining the binding behavior of the non-ortho-substituted compounds, and this condition necessarily confers a more rectangular shape on the molecule. A more symmetrical biphenyl molecular structure may be important in matching the twofold symmetry of the protein molecule. Furthermore, since meta-unsubstituted biphenyl shows no binding activity in the concentration range tested, meta chlorines are necessarily required for high binding affinity but do not always guarantee it.

The binding curve of compound **10** has a different shape compared to the others. This is most probably due to the low solubility of this compound, because the hydroxyl (4,4′) substituted counterpart (compound **17**) shows a "normal" binding curve. It is possible that compound **10** would have a much higher binding activity without the solubility limitation. It was not possible to demonstrate binding for the 3,3′,4,4′-tetrachlorobiphenyl apparently as a result of its poor solubility and lack of two meta chlorines on one ring (data not shown). However, solubility or desolvation energy differences do not account qualitatively for the different binding potencies because the degrees of chlorination and hydroxylation are equally distributed between strongly binding (non-ortho-substituted) and the more weakly binding (ortho-substituted) compounds. Compounds with the highest binding potencies have at least two meta chlorines, and the introduction of ortho chlorine or hydroxyl groups lowers binding. However, it is interesting to note that the the three strongest binders contain a para hydroxy substituent flanked by two meta chlorine substitutes analogous to the diiodophenolic ring system in T_4. This suggests an additional role of the para hydroxy group in hydrogen bonding interactions possibly with the water molecule held in the binding site.[16]

Discussion

The structure–binding relationship for these PCBs and TBPA is in excellent agreement with the modeling predictions and indicates that these compounds can be highly effective competitive binding ligands for thyroxine-specific binding sites in prealbumin. The highly toxic 3,3′,4,4′,5,5′-hexachlorobiphenyl (**10**) in spite of its low solubility in the binding assay had about 3 times the binding affinity of thyroxine itself. In order to establish our PCB–TBPA interaction model for further investigations of this type, we are attempting to cocrystallize 3,3′,5,5′-tetrachloro-4,4′-dihydroxybiphenyl (**17**) and TBPA for X-ray study. This compound (**17**) is very nearly isosteric and isoelectronic with the toxic 3,3′,4,4′,5,5′-hexachlorobiphenyl (**10**). Other work will attempt to estimate binding enthalpies by using molecular mechanics energy minimization procedures.[29]

Our finding that laterally (3,3′,5,5′-) substituted PCBs show the highest binding activity and that further substitution in nonlateral (2,2′,6,6′-) positions may decrease binding is consistent with the structure–toxicity relationship not only for the PCBs but for related compounds as well.[3] Furthermore, lateral chlorine substitution was always required for high affinity binding, but does not guarantee it, especially when ortho substituents are also present. Ortho substitution in the biphenyl system is generally associated with significantly reduced conformational freedom[22] and blocks the potential for coplanarity of the two phenyl rings that could affect binding.[28] Therefore, the binding model can reasonably account for the requirement of lateral halogens and possibly a need to maintain a (or near) symmetrical molecular shape. For example, 3,3′,5,5′-tetrachlorobiphenyl (**9**), a compound that can be nearly isosteric with TCDD (compare Figure 1a), binds with 4 times the affinity of T_4. Because of the solubility limitation, it was not possible to demonstrate binding for the toxic 3,3′,4,4′-tetrachlorobiphenyl. We believe the additional meta chlorines in the 3,3′,4,4′,5,5′-hexachlorobiphenyl increase binding affinity sufficiently to overcome the solubility limitation. However, 3,3′,4,4′-tetrachlorobiphenyl is significantly less toxic[30] than 3,3′,4,4′,5,5′-hexachlorobiphenyl and some of the toxic effects of the tetra congener could be due to meta hydroxylation to toxic metabolites.[31] The major metabolite of 3,3′,4,4′-tetrachlorobiphenyl is in fact the result of meta hydroxylation.[31] Our results emphasize the importance of the lateral halogens (or possibly similar sized groups) in filling the binding pockets normally occupied by the diiodophenolic ring of the thyroxine molecule. The *o*-diiodophenolic structure is very important in hormone binding to all three transport proteins, viz., TBPA, thyroxine-binding globulin (TBG) and albumin.[32] The lat-

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erally substituted PCBs appear to closely match the structure and chemistry of the diiodophenolic ring system of T_4 and thus may effectively bind all three transport proteins.

Halogenated biphenyls in blood are not thought to be bound to specific sites on blood proteins but rather are believed to be associated with hydrophobic sites in plasma proteins or the cellular component of blood. This generalization was based on distribution and localization studies in blood components, using a limited number of individual PCB congeners.[33] However, in the correlation derived from this work between fraction found in lipoprotein component of blood vs. fraction in hexane, it was necessary to exclude the 3,3′,4,4′- and 3,3′,5,5′-tetrachlorobiphenyl isomers in order to get a good correlation. This is the PCB type that this work predicts would show specific binding behavior and is also the one of greater toxicological importance.

On the basis of our results, it is likely that binding proteins specific for T_4 could play a significant role in modulating the distribution and toxicity of certain PCBs in the body. There is evidence[34] that T_4 has intrinsic hormonal activity and is not merely the prohormone of T_3. It has been proposed[35] that the nuclear thyroid hormone receptor is composed of a holoreceptor with high affinity for T_3 and a core subunit showing similar binding properties to TBPA, but this remains to be established.[36] However, evidence that the expression of thyroid hormone activity is mediated by more than one binding protein (or different forms of the same protein) in the nucleus with different binding properties is increasing.[35,37] In preliminary studies with the solubilized nuclear receptor for thyroxine in rat liver, we have indeed found a qualitatively similar binding profile to TBPA on binding these chlorinated aromatic hydrocarbons.[38] The high binding affinities and structural specificities demonstrated for PCBs and related compounds in these binding studies with thyroxine binding proteins are remarkable and likely to be physiologically relevant. Effects of PCBs and related compounds on serum binding proteins and thyroid status have been previously noted.[39-40] The ability of chlorinated insecticides of the DDT type to produce similar effects was recognized over 15 years ago.[41] Further studies are needed to delineate the role of thyroxine specific binding proteins in mediating the goitrogenic and other toxic effects of PCBs and related compounds.

There are likely to be many halogenated biphenyls found in commercial mixtures and animal tissue[42] that can bind the thyroxine specific binding proteins in blood and perhaps alter normal hormone–protein interactions involved in maintenance of thyroid status as proposed for the related organochlorine insecticides.[43] The non-ortho-substituted PCBs with potential for coplanarity[22] could produce significant toxicity through concentration in the nucleus where they could function as potent and persistent thyroxine agonists. Since the toxic compounds reduce the circulating levels of T_4 and cannot replace T_4 in terms of its amino acid and T_3 precursor functions, a complex overlay of hyper- and hypothyroid effects could be produced that may result in toxicity. Further research is in progress to assess the thyromimetic action of these compounds at the biochemical level.

Experimental Section

Materials. L-Thyroxine (T_4), DL-thyroxine (DL-T_4), 3,5,3′-triiodothyronine (T_3), and 3,3′,5′-triiodothyronine (r-T_3) were purchased from Sigma Chemical Co. (Milwaukee, WI). The iodinated biphenyl analogues (DL-BpT_4 and Bpformate) were kindly provided by Dr. M. Bolger, School of Pharmacy, University of Southern California, Los Angeles. [^{125}I]-T_4 (L) with a specific activity of 1250 μCi/μg and a radiochemical purity of >99% was purchased from New England Nuclear Corp. Hydroxylated PCBs were obtained from Ultra Scientific (Hope, RI) with the exception of structures **16** and **18**, which were synthesized previously in our laboratory by adaptation of methods in the PCB synthetic literature.[44] The remaining PCBs used were available from our previous work.[45]

Human prealbumin (80% pure) was obtained from Calbiochem Behring Corp. (La Jolla, CA) and further purified by preparative gel electrophoresis to homogeneity. Sephadex G-25, particle size 50–150 μm, was purchased from Sigma Chemical Co. (St. Louis, MO). Sephadex was deaerated by boiling for at least 1 h in buffer. For gel filtration minicolumns (Pasteur pipet, 2-mL bed volume) were used. The buffer used in all experiments was 0.1 M imidazole adjusted to pH 7.4 with glacial acetic acid.

Molecular Modeling. All the model building was carried out on a Silicon Graphics Iris 1200 at the University of California, San Diego. The basis of the prealbumin modeling is the same as described in our previous work.[12] Two symmetrically substituted hexachlorobiphenyl (HCB) isomers (3,3′,4,4′,5,5′- and 2,2′,4,4′,6,6′-) were selected for modeling study since they represented the extremes in conformational properties and would yield contact information for all possible chlorine-substituted positions on the biphenyl nucleus. The torsional relationship in the 3,3′,4,4′,5,5′ isomer was assumed to be 0° (this conformation has a small barrier (~3.5 kcal/mol) relative to the minimum at ~42°) and in the 2,2′,4,4′,6,6′ isomer 78° (this orientation of the rings also approximated that in T_4); these values are consistent with our previous quantum chemical results.[22] The coordinates of the two biphenyl systems were otherwise based on standard geometries or previous crystallographic measurements.[14,21] We chose to use a simple model in which the interaction of the biphenyl was modeled so that its symmetry matched that of the TBPA binding site. The twofold axis along its length is coincident with that of the binding site and the primed phenyl ring plane almost coplanar with that of the phenolic ring of T_4, so that the meta substituents are positioned close in space to the 3′- and 5′-iodines of the hormone. Figure 1a shows the superposition of "bound" 3,3′,4,4′,5,5′-HCB (heavy line) and T_4 (light line) along with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, medium line) from our previous work.[12] Figure 2a similarly shows the superposition of "bound" 2,2′,4,4′,6,6′-HCB with T_4 and TCDD. Figures 1b and 2b show the model-built structures of the two biphenyl systems in the hormone binding channel of TBPA (viewed down

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the crystal y axis). The list of contacts are given in Tables I and II. Table III lists meta contacts for an iodinated biphenyl system (numbered as in Chart I) calculated on the basis of the coordinates for the 2,2',4,4',6,6'-HCB.

Binding Assays. The thyroxine reference standard and all analogues were dissolved in methanol in the concentration range from 0.1 to 10 μM and were stored at 4 °C. The stock solution of TBPA contained 0.127 mg/mL of the protein in imidazole acetate buffer. The stock solution was further diluted to 200 nM prior to use. No change of the binding capacity of the TBPA stock solution due to storage was observed. However, the 200 nM solution quickly lost binding capacity and had to be prepared immediately prior to use. To characterize the affinity of L-T_4 for the high affinity binding site of TBPA, Scatchard analysis was carried out. In contrast to the procedure of the competitive binding assay, the amount of [^{125}I]-L-T_4 was varied together with the increasing concentration of unlabeled T_4 (1.9–940 nM) to achieve a constant specific activity (320 pCi/nmol). The TBPA concentration was 10 nM. Incubation was carried out at 25 °C. Nonspecific binding was measured by adding excess (940 nM) unlabeled L-T_4.

A binding assay using the gel filtration procedure described by Somack et al.[24] was used to measure the ability of various halogenated biphenyls and other analogues to displace [^{125}I]-L-T_4 from the high-affinity TBPA binding site. The assay mixture contained imidazole acetate buffer, 10 nM TBPA, 0.57 nM [^{125}I]-L-T_4 with a total activity of <0.25 μCi, and 1–5 μL of unlabeled compounds in methanol, which were added with a Hamilton syringe. The final concentration of the unlabeled compounds was in the range of 10^{-10} to 10^{-7} M. The final volume of the assay mixture was 0.5 mL. The methanol concentration was held at 1% for every assay mixture. A 20% decrease of the binding capacity of TBPA was observed with this concentration. After incubation at 25 °C for 1 h the mixtures were quickly cooled to 4 °C, and a 0.4-mL portion was filtered at 4 °C on Sephadex G-25 minicolumns (bed volume ≈2 mL). With an additional volume of 1.2 mL, the protein-bound [^{125}I]-L-T_4 and analogue compound respectively were removed from the column. Slight nitrogen pressure was applied to achieve an elution time for the first fraction of 40–60 s, minimizing the dissociation of the complex. The first fraction was followed by a 1.8-mL fraction that contained free radioactive iodide. Free hormone binds tightly to the gel matrix and does not elute in the volume used. Therefore the remaining gel that was saved contained the total amount of unbound [^{125}I]-L-T_4 (third fraction). The amount of radioactive L-T_4 in the different fractions was measured with a Packard Prias Auto Gamma Counter (60% efficiency).

The amount of total [^{125}I]-L-T_4 binding (referred to as total binding) was determined by incubation with [^{125}I]-L-T_4 alone (no unlabeled competitors added) and was used as reference standard. Two standards were run with each competitor tested. Competitive binding curves were obtained by plotting the percentage of total binding against increasing concentrations of competitor. The concentration of L-T_4 where 50% of total binding was achieved gave the binding potency of L-T_4 and was assigned a value of 100. The binding potency of each analogue relative to that of thyroxine was calculated as follows:

$$\text{analogue binding potency} = \frac{\text{concn of } T_4 \text{ at 50\% total binding}}{\text{concn of analogue at 50\% total binding}} \times 100$$

Acknowledgment. We thank Drs. L. Pedersen (UNC—CH) and K. Korach for helpful comments and suggestions and Dr. T. Darden for assistance in preparing the figures.

Registry No. 1, 51-48-9; 2, 300-30-1; 3, 6893-02-3; 4, 5817-39-0; 5, 100837-36-3; 6, 91821-67-9; 7, 92-52-4; 8, 15968-05-5; 9, 33284-52-5; 10, 32774-16-6; 11, 1137-59-3; 12, 5335-24-0; 13, 14962-28-8; 14, 4400-06-0; 15, 67651-34-7; 16, 100702-98-5; 17, 13049-13-3; 18, 63527-88-8; thyroxine, 51-48-9.

Drug Design via Pharmacophore Identification. Dopaminergic Activity of 3*H*-Benz[*e*]indol-8-amines and Their Mode of Interaction with the Dopamine Receptor

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Received July 22, 1985

The design and synthesis of a series of 3*H*-benz[*e*]indol-8-amines are described. Two of the compounds are potent, orally active dopaminergic agents as established by their ability to induce contralateral turning in rats with unilateral 6-hydroxydopamine-induced lesions of the nigrostriatal pathway, to induce ambulation in rats rendered akinetic by bilateral injections of 6-hydroxydopamine into the anterolateral hypothalamus, and to antagonize reserpine-induced catalepsy in mice. The dopamine agonist activity of the 3*H*-benz[*e*]indol-8-amines establishes that a pyrrolo ring and a phenolic hydroxyl group can interact similarly with the dopamine receptor and provides evidence for the existence of a hydrogen-bond acceptor nucleus on the dopamine receptor macromolecule that is involved in the behavioral manifestations of dopamine agonists.

We demonstrated several years ago[1] that the dopamine (DA) agonist (–)-apomorphine (1) and conformer B of the DA antagonist (+)-butaclamol (2) possess a common pharmacophore, namely a phenyl ring with its centrum situated 5.1 Å from a nitrogen atom and lying ~1 Å above the plane in which the nitrogen is situated.

This pharmacophore has since been found, by modeling studies, to be present in a wide range of DA agonists and antagonists,[2] including bromocriptine. When the X-ray-derived structures of apomorphine and the bromocriptine nucleus 3 are compared via superpositioning of their common pharmacophores[3] (Figure 1), it is evident that the

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