Binding Modes of PCBs to a Degrading Enzyme: a Receptor-Mapping Study

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Abstract. The binding site of a PCB-degrading enzyme was mapped using the published data on biodegradation rates of individual PCB congeners by the *Acinetobacter* P6 strain For this purpose an approach allowing for multiple binding modes of individual congeners resulting from the symmetry of the biphenyl skeleton was used The effect of substitution patterns and conformational flexibility of individual congeners on their binding to a protein were investigated The resulting map of the binding site is described by three parameters that indicate the importance of positions 4 5′ 5 2′ in a basic substitution pattern, the first two being favourable while the other two unfavourable for binding An incorporation of conformational energy dependences of individual ligands into the model showed that ligand's conformation is either not a limiting factor for binding or that ligands bind in their relaxed conformations

Key words: PCBs — Congeners Receptor mapping — Binding energy — Rotational barrier

Introduction

Halogenated aromatic hydrocarbons such as the polychlorinated biphenyls (PCBs), dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) show a number of common physicochemical biological and toxic properties They induce cytochrome P 450 dependent monooxygenases including aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD) show the affinity for binding to cytosolic

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Ah receptor and elicit a number of common toxic responses (Bandiera et al 1982, 1983, Denomme et al 1983) Supposedly, the toxicity of halogenated aromatic hydrocarbons is mediated through their binding to the Ah receptor

Both the substitution pattern and the degree of chlorination of these compounds varies highly and so they all form large congeneric series (209 congeners for PCBs 135 for PCDFs, 75 for PCDDs) Ah (or dioxin) receptor protein appears to be quite similar to receptors for steroid hormones and retinoids, for instance, it was shown that hydroxylated PCBs quantitatively displace thyroxine from its complex with prealbumin (Rickenbacher et al 1986) This receptor may therefore play an important role in both the toxic and induction responses These are some reasons why a molecular interpretation of the relationship between Ah receptor binding and the molecular structure of the hydrocarbons appears interesting The possibility that the Ah receptor has a role in modulating thyroid hormone action is of particular interest to both endocrinologists and toxicologists In addition, all these compounds belong to important environmental pollutants and an effective way of their degradation is sought

The importance of PCBs elicited substantial interest for an elucidation of their protein binding sites Structural experimental data are not available, therefore methods of receptor mapping were used The methods deduce some details of the structure of the binding site from the binding data of a set of compounds A rectangular box model (Poland and Knutson 1982) requires a molecular structure to conform to a planar rectangle (3×10 Å) with halogen atoms in the four corners The stacking model (McKinney et al 1985) was based on the description of interaction with conveniently chosen hypothetical receptor structure, taking the receptor-molecule separation distance and dispersion forces as main descriptors The other treatment (Pedersen et al 1986) added the estimate of solvation free energies to improve the predictions In general, the agreement between the models and experimental data was not satisfactory, taking into account simple structure of PCBs This could be caused by multiple binding modes of PCBs which was not considered in the development of the models

Our aim in the following treatment was to investigate the modes of binding of polychlorinated biphenyls as well as the influence of their conformation on the binding Taking advantage of small conformational flexibility of PCBs, we investigated the relevance of the change of their conformation, which can be described analytically thanks to their single degree of freedom

Methods

This work attempts to elucidate the binding of PCB congeners to a degradation enzyme trying to utilise as much information encoded in their structure as possible The computational procedure is based on the method of *multiple binding modes*

The theoretical principle of this method was introduced and described in earlier works (Balaž et al 1994, 1995), here we only give the key equation and explain some terms (in Italics) that are necessary for the understanding of the used computational procedure Though the geometry of the receptor site is not known, the method enables to make a reasonable estimate of the site configuration using the published degradation rates (Furukawa et al 1978)

The *binding site* is described in terms of several *binding regions* which come into contact with one or more *binding points* of individual molecules The decision of what represents the binding points need not always be a simple task because it is not always clear what structural features are essential on binding However, the choice is quite straightforward in case of PCB molecules where differences on binding can only arise from different chlorine substitution patterns of individual congeners Therefore, binding points are represented by chlorines substituted in different positions on a biphenyl skeleton The construction of the binding site constituted from binding regions shall be described later Once the binding points and binding regions are chosen, we can define *binding mode* as a specific alignment of binding points in binding regions The conceptual difference between multiple binding modes method and other receptor mapping methods resides in the fact that the former allows simultaneous realisation of several binding modes for every individual compound The resulting association constant of binding for a compound reflects all its binding modes This is stated in the basic equation that actually represents the core of the multiple binding modes method and has the following form (Balaž et al 1995)

$$K_i = \sum_j \exp\left(-CE_{ij} + \sum_k \nu_{ijk}\,\Delta G_k\right) \tag{1}$$

K_i stands for the association constant of ligand binding to the receptor The first summation in Eq 1 adds contributions of individual binding modes (subscript j) of the i-th compound to the overall binding Each compound in a series can have different number of binding modes The second summation consists of partial binding energies which arise from contacts of individual binding regions and corresponding binding points Parameter ΔG_k characterises the binding properties of k th binding region which can be empty or occupied by one or even more binding points The presence or absence of binding of the j-th binding point to the k-th region is indicated by an independent variable ν_{ijk} (0 or 1) Its values are precomputed for all compounds based on their binding modes and change only when the proposal of the binding site changes Thus the second summation gives the total binding energy for i th congener in its j-th binding mode if the molecule is conformationally rigid or in energetically relaxed conformation However, molecules having internal degrees of freedom can adopt other than their stand-alone minimum energy on binding In

that case the binding affinity is decreased by the internal energy associated with conformation taken by the bound molecule This fact is taken into account by the first term in the parentheses where C is unknown proportionality constant and E_{ij} is the conformational energy of i-th congener in its j-th mode The conformation of bound congeners in each binding mode is roughly the same and is fully described by the only parameter dihedral angle φ between the two phenyl rings

To incorporate the influence of conformation into our model equation (Eq 1) a continuous functional dependence of the internal conformational energy on the dihedral angle φ would be most convenient For this purpose the values of internal energy were fitted to equation

$$E(\varphi) = [B_1 + (B_2 - B_1)|\sin(\varphi/2)|]|\cos(\varphi)|^{\varepsilon} \qquad (2)$$

The only optimised variable is the exponent ε The barriers B_1 and B_2 are the calculated values for the dihedral angle φ equal to 0° and 180° respectively

After substitution of Eq 2 into Eq 1, we will get the equation that is used to fit the experimental data by nonlinear regression analysis The optimised parameters are the contributions of individual binding regions ΔG_k to the experimental binding energy and the proportionality constant C The mapping procedure is started with the model of the binding site comprising all possible binding regions The binding site corresponding to the experimental data is sought by deletion and joining of the initial binding regions in a systematic way As the actual number of all possible configurations is huge we follow only those configurations that correlate best on a given level of simplification, because they most probably give birth to sites with good correlations Thus we gradually simplify the site representation unless its prediction ability drops below some chosen limit The optimum binding site is given as a minimum set of binding regions providing an acceptable fit to the experimental data

Results

The receptor mapping procedure considering multiple binding modes was applied to the published data on degradation rates of PCB congeners by the strain *Acinetobacter* P6 (Furukawa et al 1978) Structure of the congeners and their first order elimination rate constants are given in Table 1 Standard numbering of the chlorine positions on both phenyl rings is shown in Fig 1

The values of internal energy of the studied PCB congeners were calculated by semi-empirical AM1 method with full optimisation of all variables except the dihedral angle fixed in 30° increments in the range 0 360° The quality of the fit of the calculated values to Eq 2 was excellent, as indicated by the lowest value of the correlation coefficient $r = 0.999$ and the highest standard deviation $s =$

Table 1. Numbering substitution patterns the elimination rate constants and the parameters from Eq 2 for a series of PCB congeners

No	IUPAC number	Substitution	$-\log(k^{el})$	B_1 (kJ/mol)	B_2 (kJ/mol)	ε
1	8	2,4′	0 008	29 7	29 7	9 33
2	14	3 5	0 016	0 0	0 0	0 00
3	5	2 3	0 032	29 7	29 7	9 33
4	30	2 4 6	0 036	66 9	66 9	8 04
5	12	3 4	0 055	0 0	0 0	0 00
6	26	2,3′,5	0 083	29 7	29 7	9 33
7	28	2 4 4′	0 095	29 7	29 7	9 33
8	33	2 3′ 4′	0 112	29 7	29 7	9 33
9	29	2 4,5	0 188	29 7	29 7	9 33
10	21	2,3,4	0 194	29 7	29 7	9 33
11	31	2 4′ 5	0 216	29 7	29 7	9 33
12	15	4,4′	0 298	0 0	0 0	0 00
13	61	2 3 4 5	0 420	29 7	29 7	9 33
14	11	3 3′	0 432	0 0	0 0	0 00
15	4	2 2′	0 553	60 2	81 9	8 41
16	40	2,2′,3,3′	0 836	60 2	81 9	8 41
17	18	2 2′,5	0 991	60 2	81 9	8 41
18	10	2 6	1 086	66 9	66 9	8 04
19	52	2 2′ 5,5′	1 155	60 2	81 9	8 41

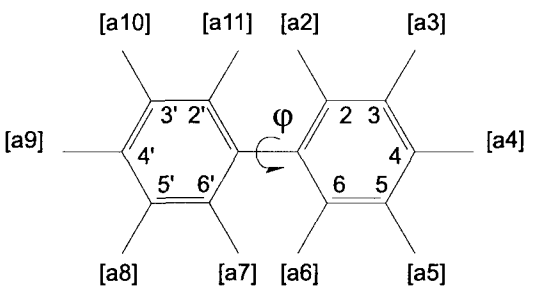

Figure 1. PCB molecule and the designation of substitution positions Dihedral angle φ represents the only degree of freedom The designation of all binding regions forming the initial binding site is shown in square brackets

0 565 for the 13 fitted points For the rotation around the single bond connecting two phenyl rings, presence of ortho chlorines is decisive the occurrence of further chlorines causing only minor differences Among further chlorines, those in positions

Table 2. The classification of PCB congeners to six groups according to the number and positions of the ortho chlorines and conformational energy profiles. Each group is characterised by three constants B_1, B_2, ε. The studied PCB congeners (numbering in Table 1) are assigned to the groups according to their structure

Group	PCB congeners	B_1 (kJ/mol)	B_2 (kJ/mol)	ε	Description of the group
(1)	2 5 12 14	0 0	0 0	0 00	no ortho-Cl
(2)	1 3 6 11,13	29 7	29 7	9 33	one ortho-Cl
(3)	4 18	66 9	66 9	8 04	two ortho-Cl on the same ring
(4)	15 17 19	60 2	81 9	8 41	two ortho-Cl on different rings
(5)		111 6	111 6	7 56	three ortho-Cl
(6)		151 3	151 3	6 95	four ortho-Cl

vicinal to the ortho chlorines will cause an increase in the rotation barrier. This so-called buttressing effect is small in comparison with the total height of the rotation barriers (Andersson et al. 1997) and was not considered. According to the number and positions of the ortho chlorines, all PCB congeners were classified into six groups characterised by coefficients B_1, B_2 and ε. The description of these groups is given in Table 2 and the corresponding functional dependencies are plotted in Fig. 2. The values of B_1 and B_2 are identical in all cases except group 4 (two ortho chlorines on different rings). The values of the coefficients B_1, B_2 and ε are also summarised in Table 1 for each studied congener.

The dependencies of the internal energy on the dihedral angle exhibit significant maxima at 0° and 180° and broad flat minima for the angles in the range 60–120° for all groups except group 1 where however only the variation of about 8 kJ/mol is observed. Therefore, for group 1 comprising the congeners without ortho chlorines the internal energy is taken as being zero for any value of the dihedral angle.

The initial configuration of the binding site is proposed in such a way that it can accommodate all positions of chlorine substitution. Therefore, initial binding site is created from 10 binding regions [a2] to [a11] as shown in Fig. 1. Obviously, each PCB congener can be embedded into this site in four possible ways. The four embeddings are generated from the initial orientation simply by applying symmetrical flipping around the two principal axes of the molecule and, in fact, represent four possible binding modes. Some of these modes may be identical depending on the site configuration and substitution pattern of a given congener. In these cases, the number of binding modes drops below four.

Assuming Michaelis-Menten kinetics, identical maximal rate constants V_{max} for all congeners, and low PCB concentrations, the association constant K is pro-

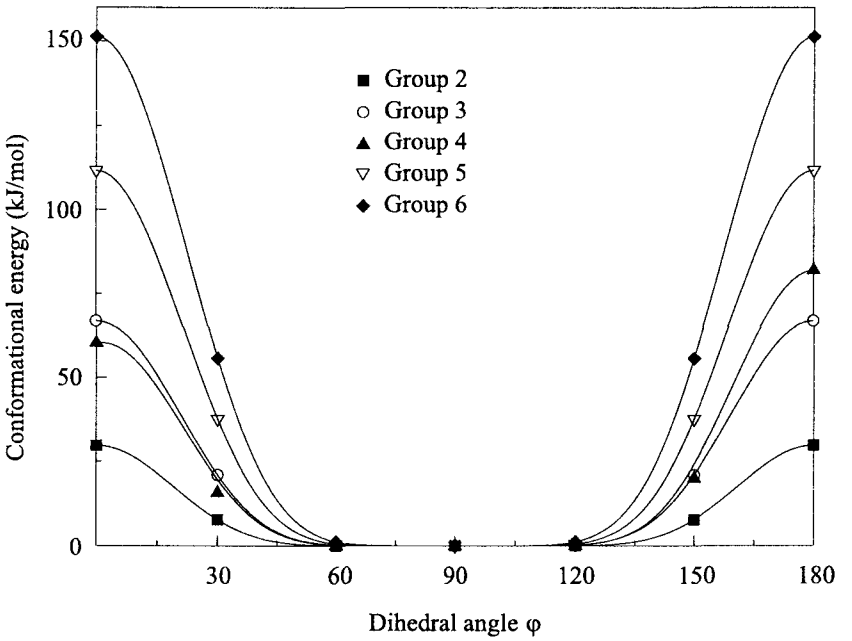

Figure 2. Conformational energy as a function of dihedral angle φ for five different groups The first group is not shown because it is practically zero over the whole range of dihedral angle Only the first half of the whole range of dihedral angle is shown as the second half is the mirror image with respect to symmetry axis located at 180°

portional to the first order elimination rate constant k^{el} Thus conversion of the experimental rate constants to a quasi-free-energy scale expressed by K is simply:

$$K = k^{el}/V_{\max} \tag{3}$$

Eq. 1 can be therefore rewritten:

$$k_i^{el} = \sum_j \exp\left(-CE_{ij} + \sum_k \nu_{ijk}\Delta G_k\right) \tag{4}$$

Proportionality of the association constant and the elimination constant allows to use the latter directly in the receptor mapping procedure. The value of the maximal rate constant is hidden in the values of the adjustable parameters ΔG_k. For the interpretation of the results, only the relative magnitudes of adjustable parameters and their signs are important, not their absolute values One of the adjustable parameters ΔG_k is always present regardless of the specific congener used. This

parameter characterises the binding of biphenyl skeleton present in every molecule and can be separated from both sums as C_1 when Eq 4 is changed to a logarithmic form Then, the final model equation that is used for the calculation is

$$\log k_i^{cl} = C_1 + \log \sum_{j=1}^{nbm} \exp\left(-CE_i(\varphi) + \sum_{k=1}^{nbr} \nu_{ijk}\Delta G_k\right) \qquad (5)$$

with E_i given by Eq 2 In Eq 5, nbm_i is number of binding modes for the i-th congener nbr is number of binding regions of the binding site and C_1 is one of adjustable parameters representing the global constant of the model Parameters ΔG_k represent the most interesting outcome of the calculation as they characterise the interaction Coefficients B_{1i}, B_{2i} and ε_i are assigned to individual congeners according to the group they belong to Their values are summarised in Table 2 and shown in Table 1 for completeness The torsional angle is also one of the adjustable parameters in the model

The initial binding site comprises all 10 binding regions as shown in Fig 1 and accommodates every possible chlorine position We performed a number of computations reducing the initial proposal of the binding site and trying to find the optimal value of the dihedral angle φ The conformational energy term, i e the product of constant C and φ-dependent energy function showed the tendency to be negligibly small compared to the summation over the index k in Eq 5 (specifically, for the final model it represented in average about 6% of the k-summation i e it was more than one order of magnitude smaller) The product is small when either proportionality constant C or conformational energy term is small (or, both factors are small) If the constant C is very small it scales down the magnitude of conformational energy irrespective of differences in conformational energy profiles of individual ligands In other words, the ligand s conformation is not important on binding due to much larger binding energy compared to the unfavourable conformational energy forced on binding Alternatively if conformational energy term E_i is small the compounds adopt the conformation characterised by an interval of φ values where the energy is in its broad minimum i e somewhere between 60° and 120° The two possible interpretations were supported by calculations that we performed with scaling parameter C (1st case) or dihedral angle φ (2nd case) held at constant values during optimisation In the first case, parameter C was fixed at the conveniently chosen value of 0 1 because such value brings both terms in parenthesis (Eq 5) to a comparable magnitude (E_i varies from 0 to 80 kJ/mol, k-summation is usually within the range −5 to 5) Optimised dihedral angle always converged to the region of conformational energy flat minimum In the second case we successively fixed φ at different values from interval (0, 180°) This resulted in $C \cong 0\ 005$ for those values of φ that were outside the region of flat $E_i(\varphi)$ minimum With respect to these findings the φ-dependence was dropped in early stages from

the search for the final representation of the binding site The search for the minimum number of adjustable parameters yet providing sufficiently precise description of the receptor site yielded four parameters The resulting binding regions and their values are summarised in Table 3 The predictions of biological activity and proportions of individual binding modes towards the overall binding based on the values of parameters from Table 3 are given in Table 4

Table 3. The resulting values of ΔG_k parameters obtained from nonlinear regression analysis of the degradation rate constants according to Eq 5 The structure of the binding site is given in the first column Statistical indices are given below the Table and in the third column (standard deviations for parameters)

Binding region	ΔG_k	Standard deviation
[a2a9a3a10a7]	−0 542	0 203
[a4a8]	1 438	0 256
[a5a11]	−1 933	0 359
[C_1]	−0 584	0 155

$n = 19$ $r = 0\ 915$ $s = 0\ 173$ $F = 25\ 584$

Discussion

Thanks to only one type of interaction occurring in binding of PCBs to a degrading enzyme broadly assumed to be of hydrophobic type, every region is described by the single parameter ΔG_k Apparently the differences in binding strength are only due to different number of chlorines and their substitution patterns No fragmentation of molecules and partitioning of interaction energy into its hydrophobicity, polarity and charge components is needed as is common in receptor mapping methods These features together with a small conformational flexibility of the PCBs make them especially suitable for the analysis using multiple binding modes method On the other hand, the same reasons may cause difficulties when one tries to use the classical Hansch approach Such type of study (Parsons et al 1992) did not succeed to find any quantitative explanation for exactly the same experimental degradation data with the help of usual hydrophobic and electronic parameters (octanol-water partition coefficients, Hammet, inductive and hydrophobic substituent constants)

Our treatment of PCB-binding indicates that only four congeners (Table 4 Nos 2, 4 12 and 16) were bound in predominantly one binding mode that was chosen variously from four given possibilities The presence of several modes of binding might serve as a possible explanation why the previous quantitative studies

Table 1 Comparison of the experimental and calculated values of the elimination rate constants of PCBs and contributions of individual binding modes to the overall binding affinity (in bold, if over 10%) Numbering and structure of the ligands correspond to Table 1 The first binding mode represents orientation of the molecule corresponding to numbering in Fig 1, the second mode arose by flipping around the x-symmetry axis, the third mode around y-symmetry axis and the fourth mode around both of them

					Proportions of individual modes			
No	Substitution	y^{obs}	y^{cal}	$y^{obs} - y^{cal}$	mode 1	mode 2	mode 3	mode 4
1	2,4'	−0 008	0 016	−0 024	0 085	**0.146**	**0.153**	**0.616**
2	3 5	−0 016	−0 180	0 164	0 033		**0.967**	
3	2 3	−0 032	−0 104	0 072	**0.112**	0 048	0 028	**0.812**
4	2,4,6	−0 036	−0 186	0 150	**0.980**		0 020	
5	3,4	−0 055	−0 053	−0 002	**0.721**	**0.179**	0 100	
6	2,3',5	−0 083	−0 128	0 045	0 017	**0.859**	**0.124**	
7	2,4 4'	−0 095	0 042	−0 137	**0.337**	**0.579**	0 084	
8	2,3',4'	−0 112	−0 107	−0 005	0 066	**0.816**	**0.118**	
9	2,4,5	−0 188	−0 107	−0 081	**0.118**	**0.816**		0 066
10	2,3,4	−0 194	−0 265	0 071	**0.684**	**0.292**	0 023	
11	2,4',5	−0 216	0 058	−0 274	0 011	0 077	**0.586**	**0.325**
12	4,4'	−0 298	−0 195	−0 103	**1.000**			
13	2,3,4,5	−0 420	−0 441	0 021	**0.148**	**0.255**		**0.597**
14	3,3'	−0 432	−0 607	0 175	**0.357**	**0.643**		
15	2,2'	−0 553	−0 761	0 208	**0.126**	**0.874**		
16	2,2' 3,3'	−0 836	−1 001	0 165	0 074	**0.926**		
17	2 2' 5	−0 991	−0 736	−0 255	0 017	**0.480**	**0.503**	
18	2,6	−1 086	−0 761	−0 325	**0.874**		**0.126**	
19	2,2',5,5'	−1 155	−1 189	0 034	**0.207**	**0.793**		

(McKinney et al 1985; Pedersen et al. 1986) provided not too persuasive results in spite of limited conformational flexibility, low variation in substituents, and comparative inertness of PCBs The final model contains just three parameters describing the binding site and one parameter representing the global constant. This is quite a substantial reduction from the initial 10 parameters. Comparing relative magnitudes of parameters in Table 3 reveals the importance of regions [a4], [a8], [a5] and [a11], the first two taking the role of joined attractive region while the last two are repulsive. The presence of ortho-chlorines tends to diminish the overall activity although not necessarily by preventing the PCBs from assuming coplanar conformation

While some researchers lay stress on substitution on both para and at least two meta positions (Bandiera et al 1983; Denomme et al 1983), others ascribe high biological activity to PCBs with laterally (3,3',5,5'-) substituted chlorines giving no

relevance to para substitution and activity lowering effect to substitution in non-lateral positions (2,2′,6,6′) (Pedersen et al 1986, Rickenbacher et al 1986) These qualitative criteria are in fact incorporated in the binding site suggested by our results The interpretation of the binding site model, supported by quantitative values of parameters is compared to the previous views as follows para positions increase the binding strength (positive value for [a4a8] parameter), ortho positions always lower the activity (negative value for binding region [a5a11], any of 2,2′,6,6′ positions can be repulsed by this region due to multiplicity of binding modes), substitution in 3,3′ 5,5′ may increase or decrease binding affinity (interplay of regions [a4a8] and [a5a11]) depending on the specific substitution pattern In general, the dominant modes of all congeners have at least one chlorine in a favourable [a4a8] region with the exception of congener Nos 15 and 18 which are free of any meta and para chlorines and congener No 19 where the advantage of one meta substituent binding is overwhelmed by two strong unfavourabe interactions

As the data were published in the form of the elimination rate constants and not directly as the binding constants, more sophisticated processing of input experimental data may be needed A treatment considering membrane accumulation and the distribution of PCB molecules in the bacterial cells could provide better input values for the consequent multiple binding modes analysis In comparison to our previous study (Balaž et al 1994) no exclusion of experimental data points was needed thanks to the improved procedure of the binding site generation

The used method of receptor mapping could be expected to work best in situations where one type of interaction is dominating and the studied molecules are not very flexible and contain some kind of symmetry so that the simple procedure for generating binding modes can be used In such cases, quite a detailed information can be obtained especially with regard to geometry of binding and relative importance of individual binding modes The theoretical model applied in this work may be of use for estimating the efficiency of the biodegradation of PCBs

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