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Effect of molecular parameters on the binding of phenoxyacetic acid derivatives to albumins

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Abstract

The interaction of 12 phenoxyacetic acid derivatives with human and serum albumin as well as with egg albumin was studied by charge-transfer reversed-phase (RP) thin-layer chromatography (TLC) and the relative strength of interaction was calculated. Each phenoxyacetic acid derivative interacted with human and bovine serum albumins whereas no interaction was observed with egg albumin. Stepwise regression analysis proved that the lipophilicity of the derivatives exert a significant impact on their capacity to bind to serum albumins. This result supports the hypothesis that the binding of phenoxyacetic acid derivatives to albumins may involve hydrophobic forces occurring between the corresponding apolar substructures of these derivatives and the amino acid side chains. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Phenoxyalkyl acid herbicides showing auxin-like activity has been intensively used to control the growth of grass and broad-leaf weeds in many crops such as rice [1], winter wheat [2,3], bermudagrass [4], etc. The molecular basis of the mode of action of phenoxyacetic acid herbicides is not entirely understood. It was assumed that they influence proton efflux [5], and they are uncouplers of the oxidative phosphorylation and modify the structure of thylakoidal membranes. Besides their beneficial effect they show toxic side effects too. Thus, it inhibits the germination of sporangiospores of *Mucor piriformis*

and conidia of *Botrytis cinerea* and *Penicillium expansum* [6]. The fate of phenoxyalkyl acid in living organisms has been vigorously discussed. It has been often indicated that they readily bind to cytochrome P450 [7], and cytochrome P450 decomposes them [8]. The herbicides influenced not only the activity of cytochrome P450 but also other enzymes such as hydroxylase, N-demethylase [9], phenylalanine amonialyase [10], nonspecific esterase [11], and rat liver peroxisomal enzyme [12].

Human toxicity of phenoxyalkyl acid herbicides has also been reported. Thus, they may cause chronic liver injury [13], influence non-Hodgkin's lymphoma [14], soft tissue sarcoma and malignant lymphoma [15].

In the last few decades chromatographic methods have been extensively used for the study of various molecular interactions of biochemical and biophysi-

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cal importance [16]. Excellent works have been already published on the characterisation of molecular parameters [17], sterical-molar properties [18], dipole-charge features [19] and hydrophobicity balance [20] influencing molecular interactions. The application of reversed-phase thin-layer chromatography (RP-TLC) as a method for the study of molecular interactions offers considerable advantages: the method is relatively rapid, allows the simultaneous determination of more interactions on one plate, it does not need complicated instrumentation and the amount of the interacting molecules required for the investigation is relatively low. However, the RP-TLC method shows marked drawbacks too: the stoichiometry of the complex cannot be determined; and only the relative strength of the interaction can be calculated.

The objectives of the study were the assessment of the interaction of phenoxyalkyl acid herbicides with various albumins, the elucidation of the molecular substructures of phenoxyalkyl acid herbicides accounting for the interaction and the comparison of the strength of interactions.

To the best of our knowledge the binding of phenoxyalkyl acid herbicides to proteins have not been studied in detail, however, these data may help

the more profound understanding of their mode of action and toxic side effects.

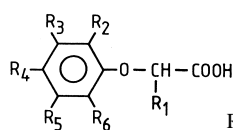
2. Experimental

Reversed-phase RP-18W/UV₂₅₄ plates (Macherey-Nagel, Dürren, Germany) were used for the determination of the relative strength of interaction without any pretreatment. Human serum albumin, bovine serum albumin and egg albumin (electrophoretic purity of each over 95%) were purchased from REANAL Fine Chemicals (Budapest, Hungary) and used as received. The chemical structures of phenoxyacetic acid congeners are compiled in Table 1. The solutes were dissolved in methanol at a concentration of 5 mg/ml, and 4 µl of the solutions were spotted separately on the plates. The mobile phases were aqueous solutions of the albumins with the concentration range of 0 to 49 mg/ml in increments of 7 mg/ml. As the molecular mass of albumins is relatively high higher concentrations cannot be employed because the mobile phase became viscous decreasing markedly the mobility of the eluent front. As the object was the study of the interaction between phenoxyacetic acid congeners

Table 1
Chemical structures of phenoxyacetic acid congeners^a

No.	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
1						
2				Cl		
3		Cl		Cl		
4		Cl	Cl	Cl		
5		Cl				Cl
6		Cl			Cl	
7			Cl	Cl		
8			Cl		Cl	
9		Cl		Cl	Cl	
10	CH ₃	CH ₃		Cl		
11	CH ₃	Cl		Cl	Cl	
12	β-Naphthoxyacetic acid					

^a General structure



R₁₋₆ = H except where stated otherwise.

and albumins and not the elucidation of the influence of albumins on their separation, the congeners were separately spotted on the plates. Development was carried out in sandwich chambers (22×22×3 cm) at room temperature, the distance of development being ca. 16 cm. After development the plates were dried at ambient temperature and the spot of phenoxyacetic acids was detected by the UV absorption spectra. Each determination was run in quadruplicate. As the reproducibility of TLC is lower than that of high-performance liquid chromatography (HPLC) the use of replications higher than three was necessary to obtain reliable results.

The R_M value characterizing the molecular hydrophobicity in RP-TLC was calculated for each drug in each eluent:

$$R_M = \log(1/R_f - 1) \quad (1)$$

When the coefficient of variation of the parallel determinations was higher than 6% the R_M value was omitted from the following calculations. The dependence of the R_M value of solutes on the concentration of albumins was calculated by

$$R_M = R_{M0} + bC \quad (2)$$

where R_M is the R_M value for a phenoxyacetic acid congener determined at given albumin concentration; R_{M0} is the R_M value extrapolated to zero albumin concentration; b is the decrease in the R_M value caused by 1 mg/ml concentration change of albumins in the eluent (related to the relative strength of interaction); and C is the concentration of albumin. Eq. (2) was applied separately for each albumin and phenoxyacetic acid congener. Eq. (2) has been previously used for the description of the dependence of the retention of anticancer drugs on the albumin concentration in the eluent [21].

In this special instance Eq. (2) has been used for the measurement of the relative strength of albumin–phenoxyacetic acid binding and not for the calculation of the first order character of the interaction.

To find the physicochemical parameters and molecular substructures of solutes significantly influencing their capacity to bind to albumins stepwise regression analysis [22] was applied. In the traditional multilinear regression analysis the presence of independent variables that exert no significant in-

fluence on the dependent variable lessens the significance level of the independent variables that significantly influence the dependent variable. To overcome this difficulty, stepwise regression analysis automatically eliminates from the selected equation the insignificant independent variables having no significant impact on the dependent variable, increasing in this manner the information power of the calculation. The relative strength of interaction (b) was the dependent variable, whereas the hydrophobicity (R_{M0}), the individual substituents, the sum of chlorine substituents and the sum of all substituents were the independent variables. The number of accepted independent variables was not limited and the acceptance limit was set to the 95% significance level. Calculations were carried out for each compound using the R_{M0} value as independent variable and congener 12 was omitted from the calculation being the substituents the independent variables.

Software of stepwise regression analysis was prepared by CompuDrug Ltd, Budapest, Hungary.

To compare the binding capacity of human and bovine serum albumin towards phenoxyacetic acid congeners, linear correlations were calculated between the corresponding relative strength of interaction.

3. Results and discussion

Egg albumin did not influence significantly the retention behaviour of phenoxyacetic acid congeners. This finding indicates that these compounds have a lower tendency to bind to egg albumin than to bind to human and bovine serum albumin and they probably will not accumulate in the protein fraction of egg. Similar results were obtained by the study of the binding of commercial pesticides to human and bovine serum albumin as well as to egg albumin [23]. The retention of each compound decreased in the presence of serum albumins the effect being higher at higher albumin concentrations. As no anomalous retention behaviour was observed it was concluded that Eq. (2) can be successfully used for the calculation of the relative strength of interaction. The parameters of Eq. (2), for human and bovine serum albumins are compiled in Tables 2 and 3, respectively. Eq. (2) fits well to the experimental

Table 2

Parameters of linear correlation between the retention of phenoxyacetic acid congeners and the concentration of human serum albumin (C , mg/ml) in the eluent^a

Compound	R_{M0}	$-b \cdot 10^3$	$s_b \cdot 10^3$	$r_{\text{calc.}}$
1	1.88	24.29	1.65	0.9977
2	0.85	12.99	0.83	0.9899
3	0.73	18.00	1.53	0.9823
4	0.58	6.41	1.02	0.9421
5	-0.05	6.45	2.07	0.8412
6	0.50	11.48	1.41	0.9645
7	0.34	9.34	1.24	0.9586
8	0.51	7.94	1.13	0.9443
9	0.49	9.86	1.16	0.9615
10	0.43	7.79	1.26	0.9300
11	0.40	8.98	1.67	0.9101
12	0.33	11.89	1.38	0.9680

$$^a R_M = R_{M0} + b \cdot C.$$

data, the significance level being over 95% in each instance (see $r_{\text{calc.}}$ values). The ratio of variance explained varied between 55.73 and 99.54%. Human and bovine serum albumins decreased the retention of phenoxyacetic acid congeners suggesting that the binding of these compounds to serum albumins enhance their hydrophilicity. This modification may result in different mobility, uptake, adsorption and decomposition rate of congeners increasing or lessening their biological efficiency. The relative strength of interaction shows a high variety. Unfortunately, charge transfer chromatography carried out on RP-TLC layers is not suitable for the

Table 3

Parameters of linear correlation between the retention of phenoxyacetic acid congeners and the concentration of bovine serum albumin (C , mg/ml) in the eluent^a

Compound	R_{M0}	$-b \cdot 10^3$	$s_b \cdot 10^3$	$r_{\text{calc.}}$
1	1.67	16.68	2.11	0.9623
2	0.81	12.89	0.43	0.9966
3	0.69	14.57	0.88	0.9892
4	0.56	7.02	1.20	0.9226
5	-0.16	4.25	1.55	0.7465
6	0.31	6.68	1.10	0.9279
7	0.16	6.00	1.68	0.8250
8	0.51	9.29	0.90	0.9727
9	0.50	11.11	0.93	0.9796
10	0.41	7.77	0.88	0.9636
11	0.36	7.99	0.89	0.9650
12	0.17	7.43	1.17	0.9326

$$^a R_M = R_{M0} + b \cdot C.$$

determination of the stoichiometry of the albumin–herbicide complex and therefore the differences in the relative strength of interaction may be due to the different stoichiometry of the complexes too. The data further suggest that the molecular structure exert a considerable impact on the binding to albumins. The relative strength of albumin–phenoxyacetic acid binding significantly depended on the lipophilicity of the compounds in the case of human and bovine albumins (Figs. 1 and 2). The significant contribution of molecular lipophilicity to the strength of interaction suggests the involvement of hydrophobic interactive forces in the binding of phenoxyacetic acid congeners to serum albumins. It can be assumed that the hydrophobic ring structures of the compounds interact with the apolar side chains of amino acids. The ratios of the variance explained by the effect of lipophilicity are 70.71 and 76.30% for human and bovine serum albumins, respectively. This result indicates that other molecular parameters not included in the calculation may influence the binding forces between serum albumins and phenoxyacetic acid congeners.

Significant linear relationship was found between the strength of the binding of phenoxyacetic acid derivatives to human serum albumin (b_{HSA}) and the sum of substituents (SS) by stepwise regression analysis:

$$b_{\text{HSA}} = 18.36 - (3.41 \pm 1.32) \cdot \text{SS} \quad (3)$$

$$r_{\text{calc.}} = 0.6514 \quad r_{95\%} = 0.5760$$

The significant negative influence of the number of substituents on the binding indicates that steric parameters are probably involved in the interaction. This result can be tentatively explained by the supposition that the bulky phenoxyacetic acid congeners have a lower possibility to reach the adsorption center on the surface of albumins than the smaller ones. It has to be emphasized that the ratio of variance explained by Eq. (3) is relatively low. Unfortunately, the physicochemical parameters of phenoxyacetic acid congeners have not been determined, therefore, it was not possible to use a much more rigorous sterical binding model.

Significant linear correlation was found between the binding capacity of human and bovine serum

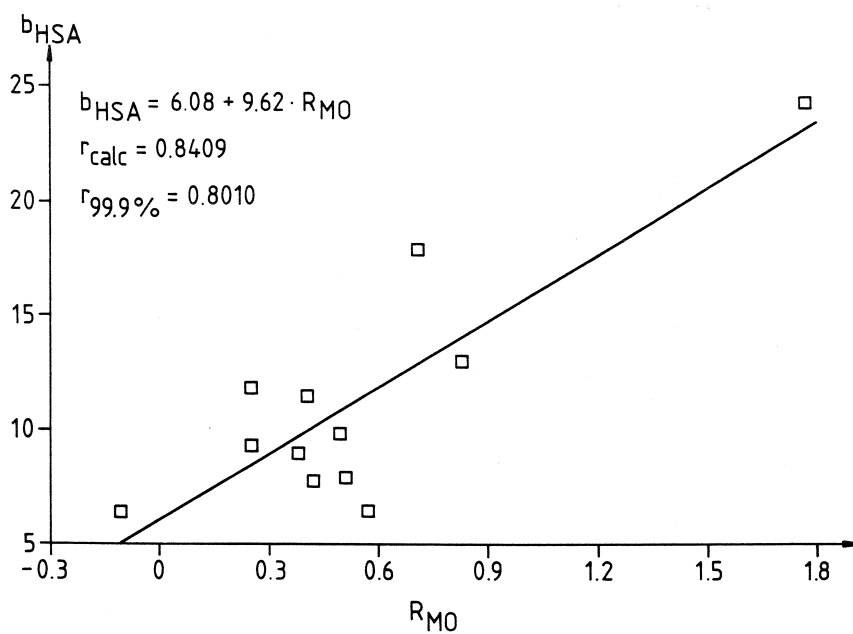


Fig. 1. Relationship between the relative strength of the binding of phenoxyacetic acid congeners to human serum albumin (b_{HSA}) and their lipophilicity (R_{M0}).

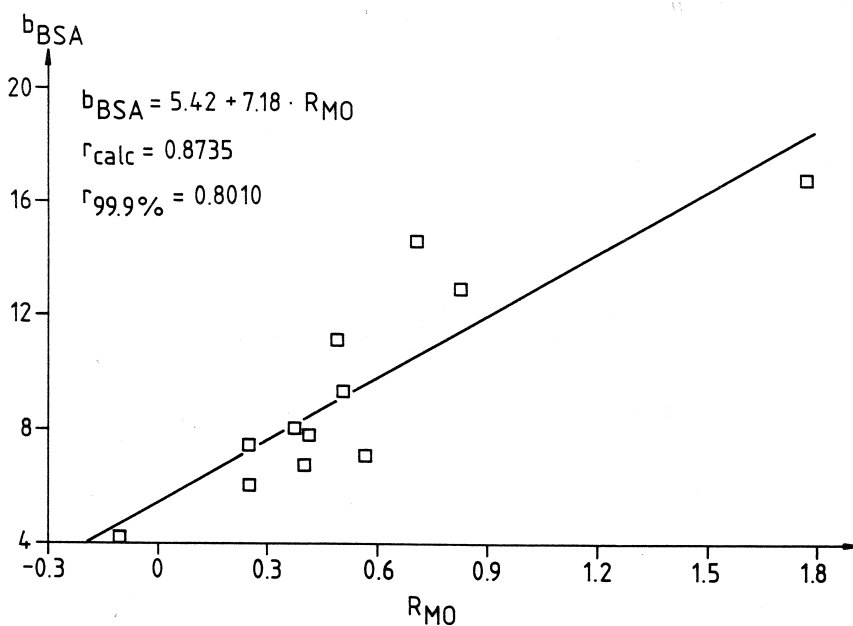


Fig. 2. Relationship between the relative strength of the binding of phenoxyacetic acid congeners to bovine serum albumin (b_{BSA}) and their lipophilicity (R_{M0}).

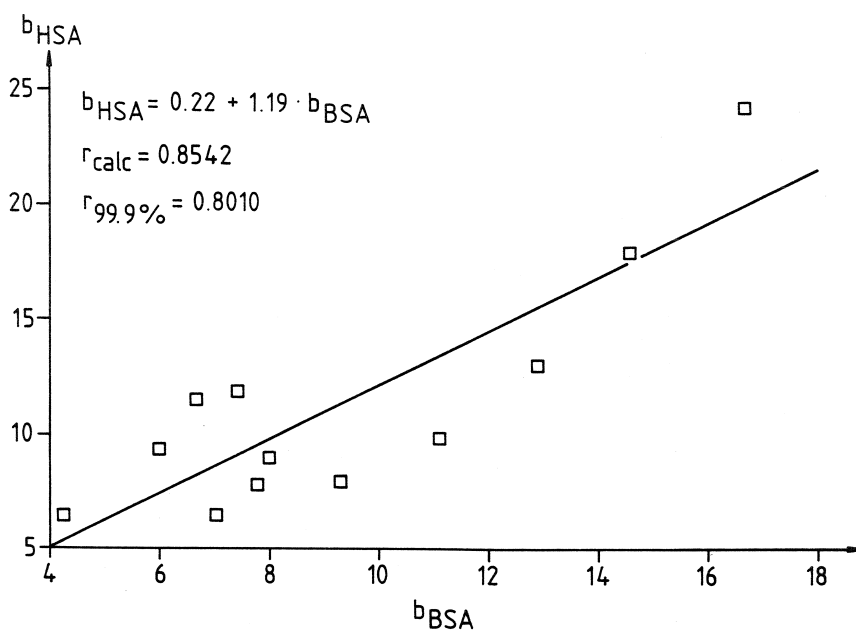


Fig. 3. Relationship between the relative strength of the binding of phenoxyacetic acid congeners to human (b_{HSA}) and bovine serum albumins (b_{BSA}).

albumins (Fig. 3) indicating that the two proteins bind the congeners in a similar way and the binding constants determined for one albumin can be extrapolated to the other albumin.

Acknowledgements

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