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Charge transfer chromatographic study of the binding of commercial pesticides to various albumins ☆

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Abstract

The interaction of 28 commercial pesticides with human and bovine serum albumin as well as with egg albumin was studied by charge-transfer reversed-phase thin-layer chromatography and the relative strength of the interaction was calculated. Only one pesticide interacted with egg albumin whereas the majority of pesticides bound both to bovine and human serum albumins. Stepwise regression analysis proved that the hydrophobicity parameters of pesticides exert a significant impact on their capacity to bind to serum albumins. These findings support the hypothesis that the binding of pesticides to albumins may involve hydrophilic forces occurring between the corresponding apolar substructures of pesticides and amino acid side chains. No linear correlation was found between the capacities of human and bovine serum albumins to bind pesticides.

1. Introduction

Pesticides can bind to a wide variety of proteins, e.g. to GTP-binding proteins [1], to cytochrome P-450 dependent mono-oxygenases [2], to aminotransferase in *Pseudosuccinea columella* [3], and to gut enzymes in tropical grassland earthworm species [4]. The strength of the interaction between the pesticides and the various proteins may have a marked impact on their activity [5]. The binding generally is reversible [6], however, covalent binding of the carbodiimide product of diafenthiuron to two mitochondrial proteins has also been reported [7]. Pesticides can also bind to various enzymes in humans. These interactions may modify the activity of plasma cholinesterase [8,9], hepatic cytochrome P-450 steroidal hydroxylase [10], and that of serum cholinesterase [11].

The hydrophobic or hydrophilic (electrostatic) character of the forces involved in the binding of pesticides to proteins has been vigorously discussed. The lipophilicity of pesticides exerts a considerable influence on their capacity to activate mouse liver microsomal glutathione S-transferase [12]. Not only the lipophilicity but also the electronic properties and dielectric moment of diphenyl ether herbicides influence their activity towards protoporphyrinogen oxidase [13]. Hydrogen bonds appear to be involved in the binding of organophosphorus insecticides to insect juvenile hormone esterase [14]. The binding of pesticide to proteins has been exploited in the construction of a pesticide biosensor [15].

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Numerous methods such as fluorescence spectroscopy [16], circular dichroism [17], various filtration methods [18,19] etc. have been used to study the binding of bioactive molecules to proteins. Other pysichochemical methods such as Raman spectroscopy [20] and NMR [21] have found only limited application in the study of such interactions because the evaluation of the complicated spectra of proteins with high molecular mass is difficult, and the exact determination of the hydrophilic or hydrophobic amino acid side chains involved in the interaction is often impossible.

Chromatographic methods have been frequently used for the determination of various molecular interactions [22]. Charge-transfer chromatography carried out on reversed-phase thin layers has been successfully applied for the study of complex formation between bioactive compounds of low molecular mass [23]. As the mobility of polymers is generally negligible on traditional reversed-phase plates their interactions cannot be studied with reversed-phase thin-layer chromatographic (RP-TLC) methods. Only the determination of the complex forming capacity of water-soluble β-cyclodextrin polymer (molecular mass under 5 kDa) with chlorophenols [24], barbituric acid derivatives [25] and nonionic surfactants [26] has been reported. It has been recently established that albumins readily move in aqueous eluents on RP-18W/UV_{254} plates (Macherey-Nagel, Dürren, Germany) [27]. This finding has been exploited for the enantiomeric separation of dansylated amino acids with added bovine albumin in the eluent [28].

The objectives of the present work were the determination of the binding of various commercial pesticides to human and bovine serum albumin as well as to egg albumin, to calculate the relative strength of the albumin–pesticide interaction and to determine which physicochemical parameters of the pesticides exert a significant impact on the strength of the interaction. The inclusion of egg albumin in the experiments was motivated by the finding that pesticides can also accumulate in eggs [29].

2. Experimental

RP-18W/UV_{254} plates were purchased from Macherey-Nagel, (Dürren, Germany) and used as received. 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 without further purification. The commercial and IUPAC name as well as the biological activity of pesticides are compiled in Table 1. Pesticides were dissolved in dioxane at a concentration of 5 mg/ml, and 2 μl of the solutions were spotted separately on the plates. The eluent systems used were aqueous solutions of the albumin listed above with a concentration range of 0–1 mM. Due to their relatively high molecular mass higher concentrations of albumins cannot be used because the eluent became extremely visçous resulting in very low mobility of the eluent front. As the object was to study the complex formation between the pesticides and albumins and not the study of the effect of albumins on the separation of pesticides, the pesticides were separately spotted on the plates. In this way the ratio albumin:pesticide was the same for each pesticide. Development was performed 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 105°C and the pesticide spots were detected by their UV adsorption spectra. Each determination was run in quadruplicate.

The R_M value calculated as usual by $\log(1/R_F - 1)$, which characterizes the molecular lipophilicity in RP-TLC was determined for each pesticide and eluent.

The dependence of the R_M value of each pesticide on the concentration of albumins was calculated by the following equation:

$$R_M = R_{M0} + b \cdot C \quad (1)$$

where R_M is the R_M value for a pesticide determined at a given albumin concentration, R_{M0} is the R_M value extrapolated to zero albumin concentration, b is the decrease in the R_M value

Table 1
IUPAC name and biological activities of pesticides

No.	Activity	Common name	IUPAC name
1	F	Cymoxanyl	1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea
2	I	Chlofentezine	3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine
3	I	Methiocarb	4-Methylthio-3,5-xylyl methylcarbamate
4	H	Diphenamid	N,N-Dimethyldiphenylacetamide
5	H	Isoproturon	3-(4-Isopropylphenyl)-1,1-dimethyl urea
6	H	Chlorotoluron	3-(3-Chloro-*p*-tolyl)-1,1-dimethyl urea
7	H	Linuron	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea
8	H	Chlorbromuron	3-(4-Bromo-3-chlorophenyl)-1-methoxy-1-methylurea
9	F	Thiophanate-methyl	4,4′-*o*-Phenylenebis(3-thioallophanic acid)dimethylester
10	M	Chlorfenson	4-Chlorobenzene sulfonic acid-4-chlorophenylester
11	F	Prochloraz	N-Propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]imidazole-1-carboxamide
12	I	Endosulfan	6,7,8,9,10,10-Hexachloro-1,5,5α,6,9,9α-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
13	F	Benomyl	1-[(Butylamino)carbonyl]-1H-benzimidazole-2-yl-carbamic acid methylester
14	H	Oxabetrinil	(Z)-1,3-Dioxolan-2-ylmethoxyimino-(phenyl)acetonitrile
15	F	Oxadixyl	2-Methoxy-N-(2-oxo-1,3-oxazolidin-3-yl)acet-2′6′-xylidide
16	F	Flutriafol	(*RS*)-2,4′-Difluoro-α-(1H-1,2,4-triazol-1-ylmethyl)benzhydryl alcohol
17	I	Buprofezin	2-*tert.*-Butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one
18	F	Carboxin	5,6-Dihydro-2-methyl-1,4-oxa-thiine-3-carboxanilide
19	H	Terbacil	3-*tert.*-Butyl-5-chloro-6-methyluracyl
20	H	Lenacil	3-Cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(3H)-dione
21	H	Atrazin	6-Chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine
22	H	Terbutylazine	N^2-*tert.*-Butyl-6-chloro-N^4-ethyl-1,3,5-triazine-2,4-diamine
23	H	Terbutryn	N^2-*tert.*-Butyl-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine
24	H	Aziprotryne	4-Azido-N-isopropyl-6-methylthio-1,3,5-triazine-2-ylamine
25	H	Triasulfuron	1-[2-(2-Chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea

(*continued on p. 288*)

Table 1 (*continued*)

No.	Activity	Common name	IUPAC name
26	H	Fuberidazole	2-(2-Furyl)benzimidazole
27	H	Ethofumasate	(±)-2-Ethoxy-2,3-dihydro-3,3-dimethyl benzofuran-5-yl methanesulfonate
28	F	Captafol	3a,4,7,7a-Tetrahydro-2-[(1,1,2,2-tetrachloroethyl)thio]-1H-isoindole-1,3(2H)dione

A = acaricide; F = fungicide; H = herbicide; I = insecticide; M = miticide.

caused by a unit change in the albumin concentration in the eluent (related to the strength of interaction between albumin and pesticide), and C is the concentration of albumin in the eluent (mM).

To find the physicochemical parameters of pesticides significantly influencing their capacity to bind to albumins stepwise regression analysis was applied [30]. The relative strength of interaction (b) was the dependent variable, whereas the hydrophobicity (R_{M0}) and the specific hydrophobic surface area (b_1) taken from Ref. [31], as well as the complex hydrophobicity parameter R_{M0}/b_1 [32] were independent variables, respectively. The number of accepted independent variables was not limited and the acceptance limit was set to the 95% significance level.

To compare the binding capacity of human and bovine serum albumins towards pesticides, the linear correlation was calculated between the relative strength of interaction of pesticides with human and bovine serum albumins.

3. Results and discussion

Compound 17 showed a very low mobility in the eluent systems used and therefore its interaction cannot be determined by this method.

Only compound 26 showed significant interaction with egg albumin:

$$R_{M0} = 1.85 - (0.303 \pm 0.08) \cdot C_{\text{egg albumin (m}M)}$$

$$r_{\text{calc.}} = 0.7905 \quad r_{99\%} = 0.7646$$

This finding indicates that pesticides have a lower tendency to bind to egg albumin than to bind to human and bovine serum albumins.

The parameters of Eq. (1) for bovine and human serum albumins are compiled in Tables 2 and 3, respectively. Only the pesticides showing significant interaction are included in the Tables.

Eq. (1) fits well to the experimental data (see $r_{\text{calc.}}$ values), the significance level being over 95%. Eq. (1) accounted for 35.84–89.23% of the total variance. In some instances albumins in the eluent decrease the retention of pesticides. This phenomenon suggests that the pesticide–albumin complex can be less hydrophobic than the uncomplexed pesticide molecule. Modification of

Table 2
Parameters of the linear correlation between the retention (R_{M0}) of commercial pesticides and the concentration of bovine serum albumin (C, mM) in the mobile phase

$R_{M0} = a + bC$

Compound	a	$-b \cdot 10$	$s_b \cdot 10$	$r_{\text{calc.}}$
2	2.20	6.13	2.04	0.8326
6	1.89	3.50	0.97	0.7869
7	1.99	6.17	2.15	0.7884
8	1.92	5.49	1.36	0.8545
9	1.76	2.66	0.90	0.7245
10	2.14	8.40	2.18	0.8440
12	1.38	2.31	0.86	0.6869
14	2.07	7.20	2.69	0.7612
23	1.79	4.07	0.85	0.8614
24	1.66	3.01	0.69	0.8387
25	1.61	3.70	0.73	0.8724
26	1.85	6.00	0.74	0.9447
27	2.27	8.37	1.74	0.9237
28	2.58	11.00	4.16	0.7632

Compound numbers refer to pesticides in Table 1.

Table 3
Parameters of the linear correlation between the retention (R_{M0}) of commercial pesticides and the concentration of human serum albumin (C, mM) in the mobile phase

$R_{M0} = a + bC$

Compound	a	$-b \cdot 10$	$s_b \cdot 10$	r_{calc}
1	1.18	2.35	1.05	0.5987
2	2.02	3.59	0.68	0.9355
3	1.62	2.20	0.82	0.6647
4	1.89	4.00	0.77	0.9333
5	2.13	6.42	2.30	0.7516
6	1.87	4.15	0.82	0.8595
7	2.32	9.05	1.94	0.8858
8	2.06	6.24	1.69	0.8128
9	1.81	3.20	0.90	0.7644
10	2.08	5.16	1.02	0.8999
11	2.47	10.58	3.93	0.7694
12	1.39	2.55	0.76	0.7477
13	1.73	3.07	0.83	0.7755
14	2.28	8.02	2.27	0.8450
15	1.53	2.12	0.84	0.6660
16	1.83	2.84	1.06	0.7108
18	1.56	3.38	0.98	0.7554
19	1.50	3.23	1.38	0.6381
20	2.52	11.63	3.67	0.8458
21	2.19	7.19	2.47	0.8243
23	1.82	3.21	0.74	0.8210
25	1.65	5.81	0.98	0.8915
26	1.93	6.55	1.11	0.8908
27	1.95	4.47	0.84	0.9080
28	1.94	3.74	1.38	0.7707

Compound numbers refer to pesticides in Table 1.

the hydrophobicity of pesticides may result in different mobility, uptake, adsorption capacity and decomposition rate of the pesticides, thus enhancing or lessening its biological efficiency. The relative strength of interaction shows a wide range from 0 to 1.16. Unfortunately, charge-transfer chromatography can not be used to give any information about the stoichiometry of the complex and therefore the differences in the relative strengths of interaction may be due to different stoichiometry of the pesticide–albumin complexes. The data further suggest that the structures of the pesticides strongly influence their capacity to interact with albumins.

Stepwise regression analysis selected only one independent variable accounting for the binding capacity of pesticides both for human and bovine serum albumin:

$$b_{\text{human albumin}} = 2.64 \cdot 10^{-2} + 0.265\ R_{M0}$$

$$r_{calc} = 0.4607\ r_{95\%} = 0.4555$$

$$b_{\text{bovine albumin}} = -0.352 + 0.255 \cdot \text{Specific hydrophobic surface area}$$

$$r_{calc} = 0.8179\ r_{99\%} = 0.7977$$

The hydrophobicity parameters of pesticides exert a significant influence on their capacity to bind to serum albumins (Figs. 1 and 2). This indicates that hydrophobic forces are involved in the interaction, the less polar substructures of pesticides binding to the hydrophobic amino acid side chains. However, we have to emphasize that the ratio of variance explained by the equations is fairly low. This finding suggests that other than hydrophobic forces may have a considerable impact on the strength of the pesticide–albumin interactions.

No significant linear correlation was found between the binding capacity of human and bovine serum albumins indicating that the two proteins bind the pesticides in a different way and that the binding constants determined for

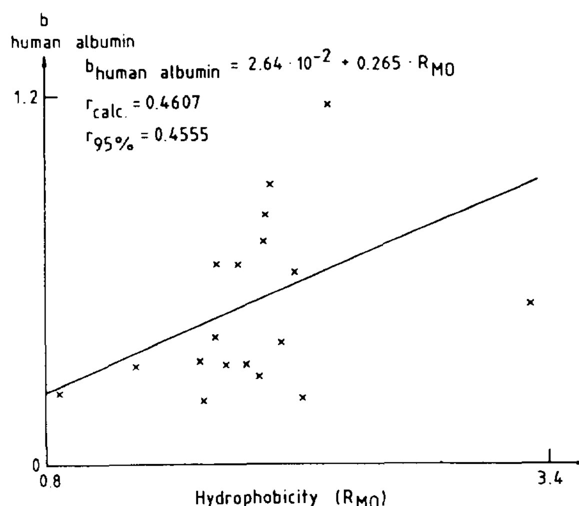

Fig. 1. Relationship between the hydrophobicity of pesticides and their capacity to bind to human serum albumin.

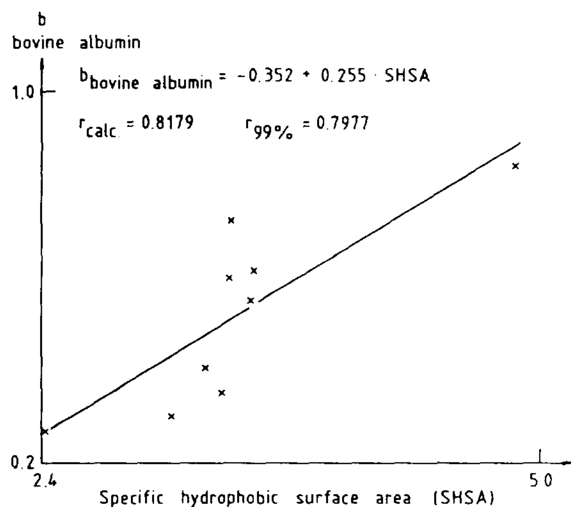

Fig. 2. Relationship between the specific hydrophobic surface area of pesticides and their capacity to bind to bovine serum albumin.

one albumin cannot be extrapolated to the other albumin.

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