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Binding of anticancer drugs to human serum albumin studied by reversed-phase chromatography

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Abstract

The interaction of eight commercial anticancer drugs with human serum albumin (HSA) was studied by charge-transfer reversed-phase thin-layer chromatography in neutral, acidic, basic and ionic environments (NaCl and $CaCl_2$) and the relative strength of interaction was calculated. Each drug interacted with HSA in a neutral environment, and the pH and the presence of mono- and divalent cations markedly affected the strength of interaction. The capacity of anticancer drugs to interact with HSA depended considerably on their molecular structure. Various multivariate statistical methods such as principal component analysis and cluster analysis indicated that the steric parameters of anticancer drugs have a considerable impact on their capacity to bind to HSA. The influence of electronic parameters on the HSA–drug interaction was of secondary importance.

1. Introduction

Much effort has been devoted to the elucidation of the mode of action of various anticancer drugs. It has been established that they can bind to different biomolecules such as model and native membranes [1], DNAs [2,3] and various proteins [4]. The binding of anticancer drugs to proteins may modify the protein structure [5] and can increase or decrease the enzyme activity [6,7], resulting in modified biological efficiency of the drugs [8].

Until now, mainly reversed-phase high performance liquid chromatography was used for the study of the interaction of various drugs with proteins [9], because the mobility of proteins is very low on traditional reversed-phase thin-layer chromatographic (RP-TLC) plates. However, it has been found that serum albumins show adequate mobility on recently developed reversed-phase (RP-18W/UV_{254}) plates. This effect has been exploited for the enantiomeric separation of various compounds by adding bovine serum albumin at a concentration of 3–6% to aqueous eluents [10]. The same reversed-phase plates have been used for the successful enantiomeric separation of dansylated amino acids [11].

Charge-transfer chromatography carried out on RP-TLC layers has previously been applied to study the interaction between taxol and other anticancer drugs with acetyl-β-cyclodextrin [12] and hydroxypropyl-β-cyclodextrin [13]. To elucidate the role of individual amino acids in the binding of anticancer drugs to proteins, the interaction of amino acids with anticancer drugs has also been studied by this method and the involvement of hydrophilic forces in the drug–amino acid interaction has been stressed [14].

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Principal component analysis (PCA) and cluster analysis (CA) have frequently been used to extract maximum information from retention data matrices of considerable dimensions [15,16]. The advantages of the application of PCA is that it allows a reduction in the number of variables whilst maintaining most of information content. PCA is suitable not only for calculations of two–two variables relationships, but also for the simultaneous study of all-variables relationships. For the easier visualization of the multi-dimensional results of PCA, the two-dimensional nonlinear map [17] and cluster analysis (CA) [18] can be applied.

The objectives of this work were to study the interaction of some anticancer drugs with human serum albumin (HSA) by means of charge-transfer chromatography, to study the effect of various environmental conditions (pH, NaCl and $CaCl_2$), to determine the relative strength of interaction and to elucidate the role of various molecular parameters of drugs that influence their binding to HSA. The use of various additives was motivated by the fact that the pH of the environment and the presence of various salts considerably influence the strength of interaction between bioactive molecules [19].

2. Experimental

RP-18W/UV_{254} plates were purchased from Macherey–Nagel (Düren, Germany) and used as received. HSA (electrophoretic purity over 95%) was purchased from Reanal (Budapest, Hungary) and used as received. The IUPAC and common names and the structures of anticancer drugs are shown in Table 1 and Fig. 1, respectively.

The drugs were dissolved in methanol at a concentration of 3 mg/ml and 2-μl volumes of the solutions were spotted separately on the plates. The eluent systems were aqueous solutions containing HSA in the concentration range 0–50 mg/ml. Owing to the relatively high molecular mass, higher concentrations of HSA could be used because the eluent became extremely viscous, resulting in a very low mobility of the eluent front. As the object was to study the binding of drugs to HSA and not to study the effect of HSA on the separation of anticancer drugs, the drugs were separately spotted on the plates. In this way the HSA: drug ratio was the same for each drug. Developments were carried out in sandwich chambers ($22 \times 22 \times 3$ cm) at room temperature, the distance of development being about 16 cm. After development, the plates were dried at 105°C and the spots of anticancer drugs were revealed by their UV adsorption and with iodine vapour. Each determination was run in quadruplicate. To study the effect of pH and the presence of salts, each experiment was run also in eluents containing 0.16 *M* sodium acetate, acetic acid, NaCl and $CaCl_2$ (the pH of the $CaCl_2$ solution was set at 7).

The R_M value characterizing molecular lipophilicity in RP-TLC was calculated from the equation by $R_M = \log(1/R_F - 1)$ separately for each drug and eluent system. The dependence of the R_M value of each drug on the concentration of HSA was calculated by the following equation:

$$R_M = R_{M0} + bc \quad (1)$$

where R_M is the R_M value for a drug determined at a given HSA concentration, R_{M0} is the R_M value extrapolated to zero HSA concentration b is the decrease in the R_M value caused by a unit change in HSA concentration in the eluent (related to the strength of HSA–drug interaction) and C is the concentration of HSA in the eluent (m*M*).

To find the similarities and dissimilarities between the relative strength of HSA–drug interactions determined in neutral, basic, acidic and ionic environments and the physico-chemical parameters of the drugs, PCA was applied. The slope (b) value in Eq. 1 and the various physico-chemical parameters of drugs were the variables (fourteen altogether) and the drugs were the observations. The total variance explained was set to 99%. The physico-chemical parameters included in the calculation were π = Hansch–Fujita substituent constant characterizing hydrophobicity, *H-Ac* and *H-Do* = indicator variables

Table 1
IUPAC names of anticancer drugs

No.	Common name	Systematic name	Provenance
1	Ftorafur	N-(2-Furanidyl)-5-*fluorouracil*	Medexport (Russia)
2	Bicnu	N,N-Bis(2-chloroethyl)-N-nitrosourea	Laboratoire Bristol (France)
3	Vumon	4′-O-Demethyl-1-O-(4,6-O-2-thenylidene-β-D-glucopyranosyl)epipodophyllotoxin	Bristol-Arzneimittel (Germany)
4	Natulan	N-(1-Methylethyl)4-[(2-methylhydrazino)methyl]benzamide	Roche (Switzerland)
5	Alexan	4-Amino-1-β-D-arabifuranosyl-2(14)-pyrimidine	Mack (Germany)
6	Mitomycin C	[1-a*R*]-6-Amino-8-[(aminocarbonyl)oxymethyl]-1, 1a, 2, 8, 8a, 8b-hexahydro-8a-methoxy-5-*methylazirino*[2′,3′:3,4]pyrrolo[1,1*a*]indole-4,7-dione	Kyowa (Japan)
7	Deticene	5-(3,3-Dimethyl-1-triazenyl)-1-*H*-imidazole-4-carboxamide	Rhône-Poulenc (France)
8	Metotrexate	2,4-Diamino-10-methylpteroylglutamic acid	Lachema (Czech Republic)

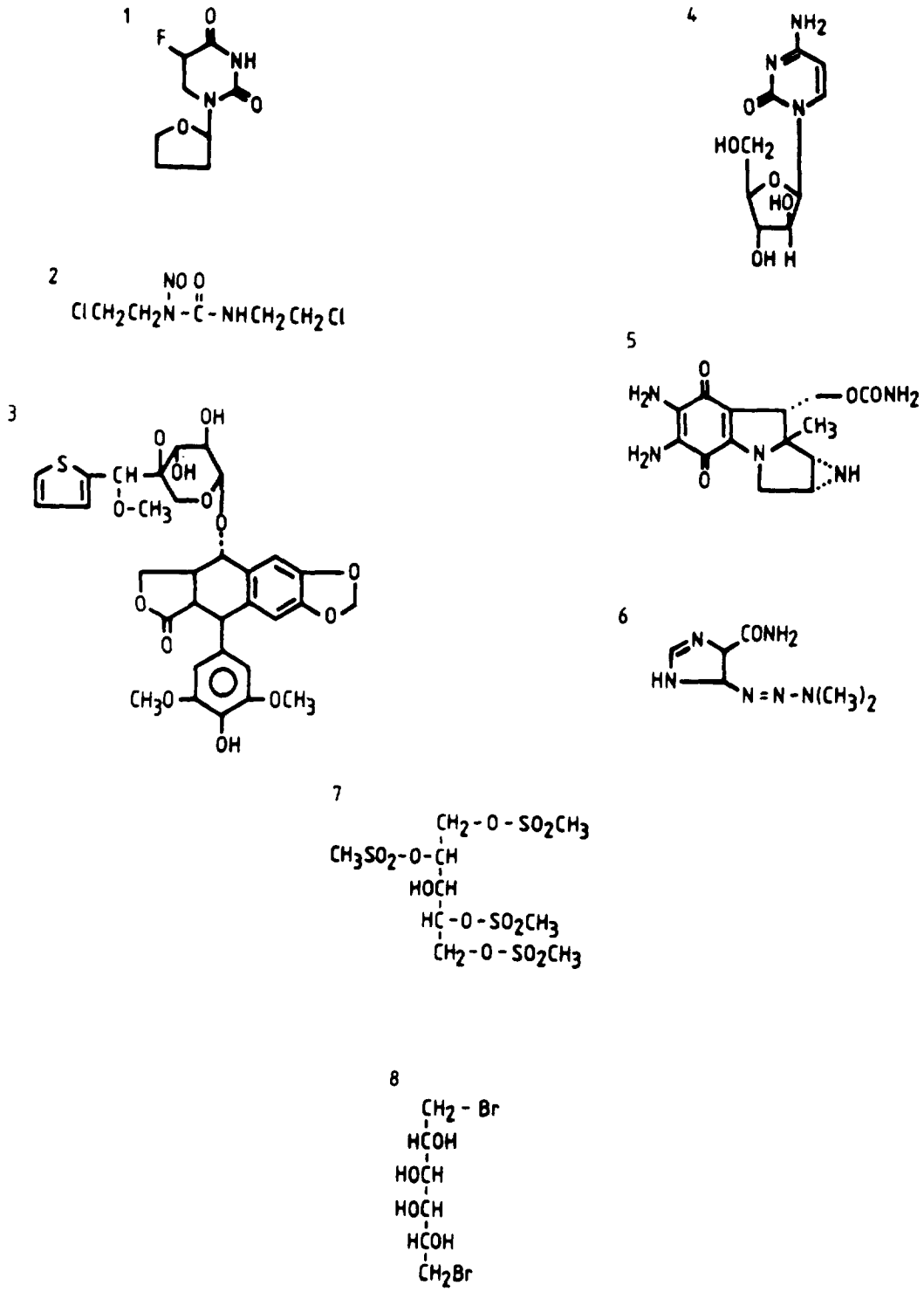

Fig. 1. Structures of anticancer drugs.

for proton acceptor and proton donor properties, respectively, *M-RE* = molar refractivity, *F* and *R* = Swain–Lupton electronic parameters characterizing the inductive and resonance effect, respectively, σ = Hammett's constant, characterizing the electron-withdrawing power of the substituent, *Es* = Taft's constant, characterizing steric effects of the substituent, and B_1 and B_4 = Sterimol width parameters determined by the distance of substituents at their maximum point perpendicular to attachment.

Both two-dimensional non-linear mapping and CA were applied to the PC loadings and PC variables to visualize the multi-dimensional results of PCA and to compare their information content.

3. Results and discussion

In most instances the R_M value of the anticancer drugs decreased with increasing concentration of HSA in the eluent. This result indicates the binding of drugs to HSA; the more hydrophilic HSA makes the drugs less lipophilic by interacting with them. The direct binding of the drugs to HSA may modify the concentration of free drugs in the blood, their mobility, accessibility to the target organ or organs, their distribution between the hydrophobic and hydrophilic compartments of the cell, etc.

The parameters of Eq. 1 are compiled in Table 2. Each anticancer drug showed an interaction with HSA. Eq. 1 fits the experimental data well, the significance level being over 95%. Eq. 1 accounted for 60.12–98.23% of the total variance, suggesting that HSA has a significant impact on the hydrophobicity parameters of the drugs. The relative strength of interaction (*b*) shows a high divergence depending both on the type of drug and on the pH and salts. This finding supports the supposition that not only the molecular structure but also the physiological conditions of the environment may have a significant influence on the binding of anticancer drugs to HSA. The interaction strength of drugs containing basic –NH– or $-NH_2$ groups was higher in the presence of $CaCl_2$ than in an ion-free neutral environment. It is probable that with these drugs the formation of salt bridges between the corresponding substructures of HSA and the drugs contribute to the binding.

The results of PCA are given in Table 3. Five principal components were sufficient to explain most of the variance (96.29%), which means that the original fourteen variables can be substituted by five background (imaginary) variables without a considerable loss of information. Unfortunately, PCA does not define these five background variables as concrete physico-chemical entities, it only indicates their mathematical possibility. Except for the interactive forces determined in the presence of $CaCl_2$, the other interactive forces, the steric (*Es*, B_1, *M-RE* and B_4) characteristics and electronic (*R*) parameter of the drugs have high loadings in the first principal component, indicating that these physico-chemical parameters may have a marked impact on the HSA–drug interaction. This result can be explained by the supposition that steric parameters of the drugs influence the accessibility of the active centers on the surface of the HSA molecule. These active centers are probably hydrophilic, and the direct binding of drugs to HSA occurs by hydrophilic forces (probably hydrogen bond formation). The conclusions drawn from the two-dimensional non-linear map (Fig. 2) and cluster dendogram of PC loadings (Fig. 3) entirely support our previous conclusions: the relative strengths of interaction determined in neutral, acidic, basic and NaCl-containing environments form a cluster with steric parameters of drugs. We must stress that other physico-chemical parameters of anticancer drugs not included in the calculations may also have some influence on the HSA–drug interactions and our conclusions are valid only for the parameters included in these calculations.

The two-dimensional non-linear map and cluster dendogram of PC variables are shown in Figs. 4 and 5, respectively. Anticancer drugs do not form distinct clusters on these maps. However, drugs with a basic substructure (compounds **3**, **6** and **8**) are slightly separated from the other. This again indicates the possible role of hydrophilic forces in the HSA–drug interactions. It

Table 2
Parameters of the linear correlation between the retention (R_M) of anticancer drugs and the concentration of human serum albumin (C mM) in the mobile phase ($R_M = R_{M0} + bc$)

Eluent	Compound[a]	R_{M0}	$-b \cdot 10$	$S_b \cdot 10$	r_{calc}
Neutral					
	1	0.85	1.34	0.93	0.9356
	2	2.55	10.10	0.96	0.9911
	3	1.74	4.11	1.49	0.7772
	4	2.07	6.78	2.26	0.8021
	5	0.44	6.22	1.41	0.9746
	6	2.15	6.32	1.98	0.8803
	7	1.19	3.30	1.11	0.8278
	8	1.66	13.71	2.43	0.9293
0.16 *M* acetic acid end concentration					
	1	0.73	3.17	0.83	0.8844
	2	2.26	6.57	1.75	0.8825
	3	1.64	3.82	1.39	0.7754
	4		Not significant		
	5		Not significant		
	6	2.02	3.83	1.43	0.7674
	7	1.06	4.84	1.16	0.9007
	8	1.85	8.62	2.02	0.9049
0.16 *M* sodium acetate end concentration					
	1		Not significant		
	2	2.15	6.35	2.16	0.8268
	3	1.71	3.41	1.18	0.7890
	4		Not significant		
	5		Not significant		
	6	1.96	5.36	0.55	0.9897
	7	1.07	4.29	0.91	0.9199
	8	1.73	10.97	1.50	0.9562
0.16 *M* NaCl end concentration					
	1	0.21	0.71	0.02	0.7878
	2	2.01	2.55	0.37	0.9501
	3	1.91	5.05	1.12	0.7932
	4		Not significant		
	5	0.12	2.77	0.58	0.9218
	6	1.65	2.86	0.34	0.9865
	7	0.99	7.76	1.04	0.9657
	8	1.86	14.49	4.32	0.9320
0.16 *M* $CaCl_2$ end concentration					
	1	1.15	6.37	1.20	0.9588
	2	2.18	4.94	1.04	0.8998
	3	1.75	3.87	0.89	0.8974
	4		Not significant		
	5	0.10	4.45	1.23	0.9583
	6	2.16	10.92	1.11	0.9080
	7	0.79	6.65	1.78	0.8096
	8		Not significant		

[a] See Table 1.

Table 3
Relationship between the retention parameters and physico-chemical parameters of anticancer drugs: results of principal component analysis

No. of PC	Eigenvalue	Variance explained (%)	Total variance explained (%)
1	6.18	44.18	44.18
2	2.63	18.82	63.00
3	1.94	13.93	76.92
4	1.45	10.38	87.30
5	1.26	8.99	96.29

Parameter		Principal component loadings			
		1	2	3	4,5
π	−0.21	0.68	−0.26	0.38	0.47
H-Do	0.36	−0.33	−0.11	−0.13	0.78
M-Re	0.54	0.45	−0.68	0.01	−0.01
F	−0.34	0.90	0.12	−0.11	0.12
R	−0.86	−0.05	0.26	0.28	0.25
σ	0.10	0.83	0.46	−0.19	0.03
Es	−0.93	−0.08	0.33	−0.05	0.01
B_1	0.98	0.03	0.01	−0.09	−0.15
B_4	0.88	0.14	−0.42	−0.07	0.04
b_{H_2O}	0.72	−0.38	0.39	−0.18	0.28
$b_{H\text{-}Ac}$	0.64	0.12	0.48	0.56	0.07
$b_{Na\text{-}Ac}$	0.82	−0.12	0.32	0.41	0.19
b_{NaCl}	0.70	0.25	0.38	0.20	−0.39
b_{CaCl_2}	−0.33	−0.23	−0.40	0.75	−0.17

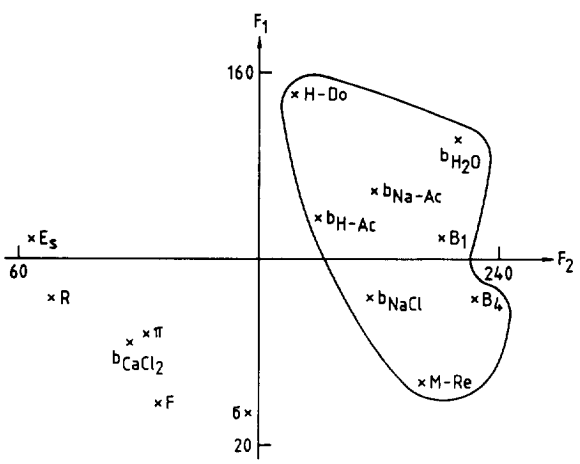

Fig. 2. Two-dimensional non-linear map of principal component loadings. Number of iterations, 77; maximum error, $4.54 \cdot 10^{-2}$. For symbols, see Experimental.

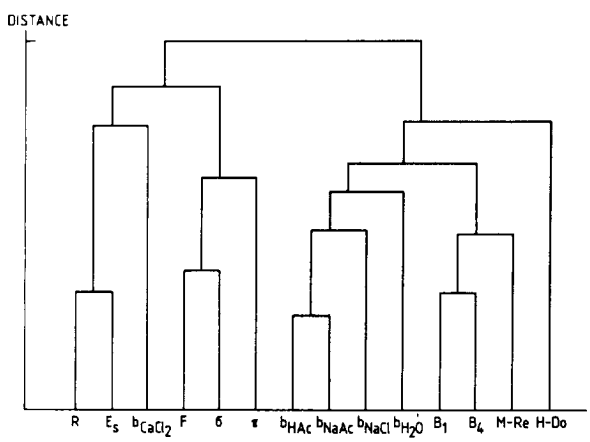

Fig. 3. Cluster dendogram of principal component loadings. For symbols, see Experimental.

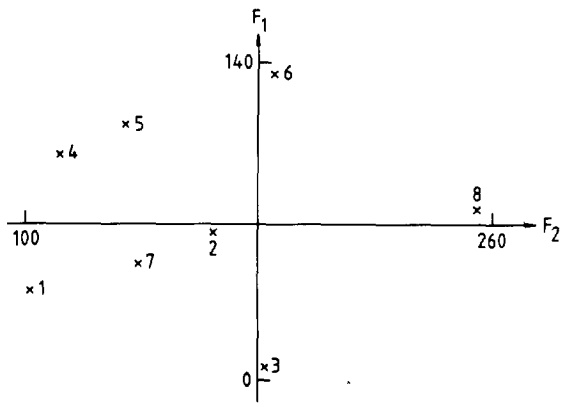

Fig. 4. Two-dimensional non-linear map of principal component variables. Number of iterations, 87; maximum error, $3.67 \cdot 10^{-2}$. Numbers refer to anticancer drugs in Fig. 1 and Table 1.

can be assumed that these basic structures can bind to secondary carboxyl groups of dicarboxyamino acids in HSA, contributing to the stability of the HSA–drug binding.

It can be concluded from the data that the anticancer drugs can bind to HSA, the strength of interaction depending on the pH and on the presence of dissociable salts. Multivariate mathematical statistical calculations indicated that the steric and electronic parameters of anticancer drugs have a marked impact on their capacity to interact with HSA. It is probable that the steric conditions govern the accessibility of polar binding centers on the HSA surface whereas hydrophilic forces (possibly hydrogen bonding) are the decisive factors in the formation of the various HSA–drug associates.

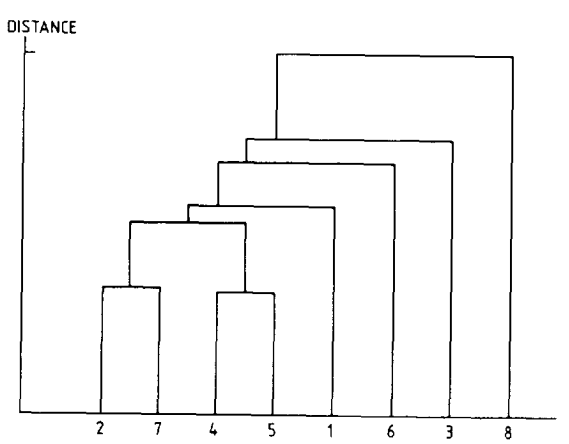

Fig. 5. Cluster dendogram of principal component variables. Numbers refer to anticancer drugs in Fig. 1 and Table 1.

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