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Study of the binding of antibiotics to human serum albumin by charge-transfer chromatography

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Abstract

The interaction of 13 antibiotics with human serum albumin was studied by charge-transfer reversed-phase thin-layer chromatography in neutral, acidic, basic and ionic environments (NaCl and $MgCl_2$) and the relative strength of interaction was calculated. The pH and the presence of mono- and divalent cations markedly influenced the strength of interaction. The capacity of antibiotics to interact with HSA also considerably depended on their chemical structure. © 1997 Elsevier Science B.V.

Keywords: Charge-transfer chromatography; Principal component analysis; Mobile phase composition; Antibiotics; Albumin

1. Introduction

It has been proven many times that various xenobiotics [1], drugs [2] and other bioactive compounds [3] readily bind to serum albumin. The interaction of drugs with human serum albumin (HSA) modifies the biological efficacy [4,5] and stability of the drugs [6]. The drug–HSA interaction influences not only the application parameters of drugs but also the structure of HSA [7]. The binding of a wide variety of drugs such as cefalosporin [8], tolmetin and zomepirac [9], carprofen [10,11], N-methylated barbiturates [12], ceftriaxone and cefatoxime [13] and fantofarone, a novel Ca^{2+} antagonist [14] to HSA was investigated.

Many physical and physicochemical methods have been used for the elucidation of the various aspects of drug–HSA interaction. Thus, dialysis [15], potentiometric ion probe technique [16], circular dichroism [17], and various spectroscopic methods [18,19] have been successfully used for the study of such types of interactions. Due to their high separation power and versatility chromatographic methods have also found application in the assessment of binding of drugs to HSA [20]. Due to the low mobility of HSA on traditional reversed-phase thin-layer chromatographic (RP-TLC) plates until now RP-TLC has not been used for the study for the elucidation of HSA–drug interactions. It has been recently established that serum albumins show adequate mobility on newly developed reversed-phase (RP-18 W/UV_{254}, Macherey–Nagel, Dürren, Germany) plates. This finding has been exploited for the enantiomeric separation of various compounds by adding bovine serum albumin to the eluent [21,22], for the elucidation of the binding of anticancer drugs to HSA [23] and for the study of the interaction of commercial pesticides with human, bovine and egg albumins [24].

Principal component analysis (PCA) has frequently been used to extract maximum information from retention data matrices of considerable dimensions [25]. The advantages of the application of PCA is that it allows a reduction in the number of variables

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whilst maintaining most of the 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 matrices of PC loadings and PC variables, the two-dimensional non-linear mapping technique can be applied [26].

The objectives of this work were to study the interaction of some antibiotics with HSA by means of charge-transfer chromatography, to study the effect of various experimental conditions (pH, NaCl and $MgCl_2$), to determine the relative strength of interaction and to elucidate the role of various molecular parameters of antibiotics which influence their binding to HSA. The use of various additives were motivated by the fact that pH values of the environment and the presence of various salts considerably influences the strength of interaction between bioactive molecules [27].

2. Experimental

RP-18 W/UV_{254} plates were purchased from Macherey–Nagel and used as received. HSA (electrophoretic purity over 95%) was purchased from Reanal Fine Chemicals (Budapest, Hungary) and used without further purification. The IUPAC and common names and chemical structures of antibiotics are shown in Table 1. Antibiotics were selected to form a non-homologous series of compounds and to have a practical importance in up-to-date health care. Antibiotics were dissolved in methanol at a concentration of 3 mg ml^{-1}, and 2 μl of solutions were spotted separately on the plates. The eluent systems were aqueous solutions containing HSA in the concentration range of 0–30 mg ml^{-1}. Due to the relatively high molecular mass higher concentrations of HSA cannot be used because the eluent became extremely viscous resulting in very low mobility of the eluent front. We are well aware that the concentration of HSA in the eluent can be increased by adding 2–3% 2-propanol to the eluent [28]. As the dependence of the strength of interaction on the concentration of 2-propanol in the eluent is not entirely elucidated we prefer to use pure water with the assumption that in this instance the weaker interactions between HSA and antibiotics cannot be determined. As the object was to study the binding of drugs to HSA and not the study of the effect of HSA on the separation of antibiotics, the drugs were separately spotted on the plates. Developments were carried out in sandwich chambers (22×22×3 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 antibiotics were revealed by their UV adsorption and by iodine vapour. 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 $MgCl_2$. As the molar concentration of HSA in the eluents was very low, we assume that the presence of this low concentration of HSA did not influence the pH of the eluent. Each determination was run in quadruplicate. When the coefficient of variation between the parallel determinations was higher than 8% the data were omitted from the following calculations. The R_M value characterizing molecular lipophilicity in RP-TLC by $R_M = \log(1/R_F - 1)$ separately for each antibiotics and eluent system. The dependence of R_M value of each drug on the concentration of HSA was calculated by the following equation:

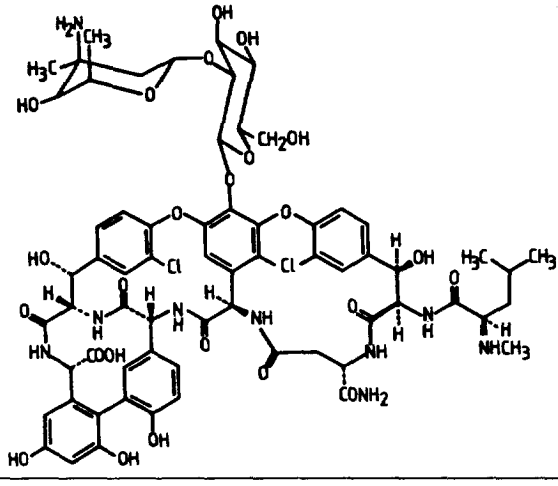
$$R_M = R_{M0} + bC \quad (1)$$

where $R_M = R_M$ value for a drug determined at a given HSA concentration, $R_{M0} = R_M$ value extrapolated to zero HSA concentration, b is the decrease in the R_M value caused by a unit change of HSA concentration in the eluent (related to the strength of HSA–drug interaction) and C is the concentration of HSA in the eluent (mg ml^{-1}).

To find the similarities and dissimilarities between the relative strength of HSA–antibiotic interactions determined in neutral, basic, acidic and ionic environments and the hydrophobicity parameters of drugs principal component analysis was applied. The slope (b) values of Eq. (1) and the hydrophobicity (R_{M0}) and specific hydrophobic surface area (SHSA) of drugs were the variables (7 altogether) and the drugs were the observations. The total variance explained was set to 99%. The R_{M0} and SHSA values of drugs determined by means of reversed-phase thin-layer chromatography were taken from [29]. As the hydrophobicity parameters of antibiotics

Table 1
Common and IUPAC names of antibiotics

No.	Common name	IUPAC name
1	Ampicillin	6-[(Aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
2	Cefotaxime	3-[(Acetyloxy)methyl]-7-[[(2-amino-4-thiazo-lyl)(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
3	Cephalexin	7-[(Aminophenylacetyl)amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
4	Cephalotin	3-[(Acetyloxy)methyl]-8-oxo-7-[(2-thienylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2- ene-carboxylic acid
5	Chloramphenicol	2,2-Dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]acetamide
6	Kanamycin	O-3-Amino-3-deoxy-α-D-glucopyranosyl-(1-6)-O-[6-amino-6-deoxy-α-D-glucopyranosyl-(1-4)]-2-deoxy-D-streptamine
7	Methicillin	6-(2,6-Dimethoxybenzamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]-heptane-2-carboxylic acid
8	Novobiocin	N-[7-[[3-O-(Aminocarbonyl)-6-deoxy-5-C-methyl-4-O-methyl-ß-L-lyxopyranosyl]oxy]-4-hydroxy-8-methyl-2-oxo-2H-1-benzopyran-3-yl]- 4-hydroxy-3-(3-methyl-2-butenyl)benzamide
9	Oxytetracyclin	4-(Dimethylamino)-1,4,4α,5,5α,6,11,12α-octahydro-3,5,6,10,12,12α-hexahydroxy-6-methyl- 1,11-dioxo-2-naphthacenecarboxamide
10	Oxacillin	[[(5-Methyl-3-phenyl-4-isoxazolyl)carbonyl]amino]-3,3-dimethyl-6-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid
11	Penicillin G	3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
12	Trichotecin	12,13-Epoxy-4-[(1-oxo-2-butenyl)-oxy]trichotech-9-en-8-one
13	Vancomycin B	



2, 6 and 10 were not determined they were omitted from the calculations. For the easier visualization of the results the two-dimensional non-linear maps of PC loadings and variables were also calculated. The iteration was carried out to the point when the difference between the two last iterations was lower than 10^{-8}.

3. Results and discussion

In many cases the R_M value of antibiotics 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 antibiotics 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. Eq. (1) fits well to the experimental data the significance level being over 95%. Eq. (1) accounted for

Table 2
Parameters of the linear correlation between the retention (R_M) of antibiotics and the concentration of HSA (C mg ml^{-1}) in the mobile phase ($R_M = R_{M0} + bC$)

Eluent compound	R_{M0}	$-b \cdot 10^2$	$s_b \cdot 10^3$	$r_{calc.}$
Neutral				
2	0.59	1.17	1.88	0.9518
4	0.63	1.20	1.29	0.9778
5	1.02	0.45	1.12	0.9186
6	0.65	1.60	1.96	0.9781
8	2.22	1.46	2.09	0.9801
10	1.45	1.26	1.08	0.9855
11	0.70	0.75	1.12	0.9581
0.16 *M* acetic acid end concentration				
2	1.38	2.00	3.31	0.9378
4	1.18	1.93	4.13	0.9021
6	1.38	1.88	3.16	0.9360
7	1.39	2.59	4.35	0.9362
10	1.87	2.53	4.11	0.9510
11	1.44	2.76	2.54	0.9838
12	0.98	0.51	1.35	0.8831
0.16 *M* sodium acetate end concentration				
2	1.27	0.37	0.66	0.9298
4	1.40	0.39	1.16	0.8293
6	1.33	0.32	1.04	0.8108
10	1.69	0.87	3.13	0.7795
0.16 *M* NaCl end concentration				
2	0.27	1.26	3.81	0.8552
4	0.47	0.82	2.39	0.8931
6	0.29	1.02	2.99	0.8619
10	1.18	0.97	2.19	0.9315
11	0.51	1.54	3.74	0.9216
0.16 *M* $MgCl_2$ end concentration				
2	1.50	0.91	1.94	0.9024
3	0.65	0.39	1.24	0.8119
4	1.52	1.26	1.56	0.9635
5	0.95	0.71	1.26	0.9285
6	1.55	0.70	2.04	0.8376
7	0.72	0.74	0.63	0.9827
9	1.01	−0.64	0.76	0.9665
10	1.80	1.19	3.78	0.8157
11	1.30	0.91	2.53	0.8502
12	1.09	0.60	0.62	0.9744

Numbers refer to antibiotics in Table 1. (Only antibiotics showing significant interaction with HSA are listed).

60.75–97.12% of total variance suggesting that HSA has a significant impact on the hydrophobicity parameters of the drugs. The relative strength of interaction (b) shows high diversity depending both on the type of the antibiotics and that of the pH and salts. This finding supports the supposition that not only the chemical structure but the physiological conditions of the environment may have a significant influence on the binding of antibiotics to HSA.

The results of PCA are compiled in Table 3. Three principal components were sufficient to explain most of the variance (81.33%) that means that the original 7 variables can be substituted by 3 background (imaginary) variables without considerable loss of information. Unfortunately, PCA does not define these three background variables as concrete physicochemical entities, it only indicates their mathematical possibility. The relative strengths of interaction have a high loading in the first PC indicating their similarity. The hydrophobicity parameters have the highest loadings in the second PC. This finding suggests that the influence of hydrophobic forces is relatively low in the determination of the strength of interaction between antibiotics and HSA. The two-dimensional non-linear map of PC loadings supports our previous qualitative conclusions (Fig. 1). The hydrophobicity parameters are near to each other and far from the interaction parameters indicating again the negligible influence of hydrophobic forces on the interaction. Moreover, the relative strengths of interaction do not form separate clusters suggesting that both the pH and the presence of monovalent or divalent cations exert a different influence on the strength of interaction.

The two-dimensional non-linear map of PC variables is shown in Fig. 2. Except compounds 9 and 12, antibiotics do not form distinct clusters on the map. This result can be tentatively explained by the supposition that more than one substructure of antibiotics is involved in the interaction and the distribution of antibiotics on the map is the result of the interplay of various interactive forces between the corresponding substructures of antibiotics and HSA.

Unfortunately, charge transfer chromatography carried out on RP-TLC plates does not make possible the determination of the stoichiometry of the interaction [30] or the exact determination of the binding sites of antibiotics on the HSA molecule [31].

It can be concluded from the data that the majority of antibiotics can bind to HSA. The strength of the interaction considerably depends on the chemical structure of antibiotics and on the environmental

Table 3
Relationship between the relative strength of interaction of antibiotics with HSA and their hydrophobicity parameters, results of principal component analysis.

Number of PC	Eigen value	Variance explained (%)	Total variance explained (%)
1	3.33	47.51	47.51
2	1.49	21.35	68.86
3	0.87	12.47	81.33
Parameters	Principal component loadings		
	1	2	3
b_{H2O}	0.59	0.22	−0.71
b_{NaCl}	0.82	−0.27	−0.01
$b_{CH3COOH}$	0.82	−0.40	0.26
$b_{CH3COONa}$	0.74	0.33	−0.19
R_{M0}	−0.42	0.75	0.20
SHSA	0.49	0.72	0.13

For symbols see Section 2.

conditions (acidic or alkaline pH, presence of monovalent or divalent cations). The marked influence of pH and salt concentrations indicates the involvement of hydrophilic forces in the interaction.

Acknowledgments

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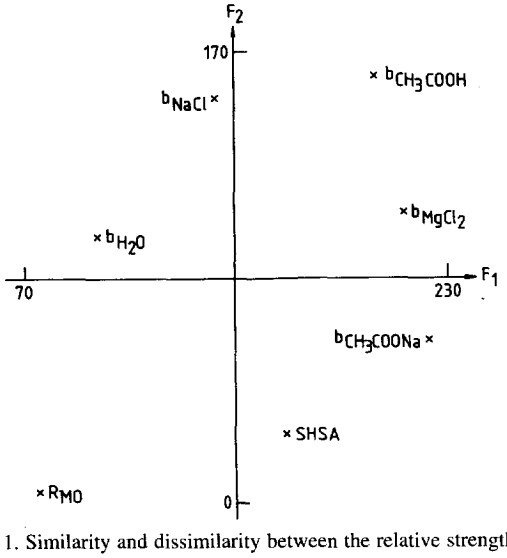

Fig. 1. Similarity and dissimilarity between the relative strength of antibiotic–HSA interaction and the hydrophobicity parameters of antibiotics. Two-dimensional non-linear map of principal component loadings. Number of iterations: 84, Max. error: $3.85 \cdot 10^{-2}$. For symbols see Section 2.

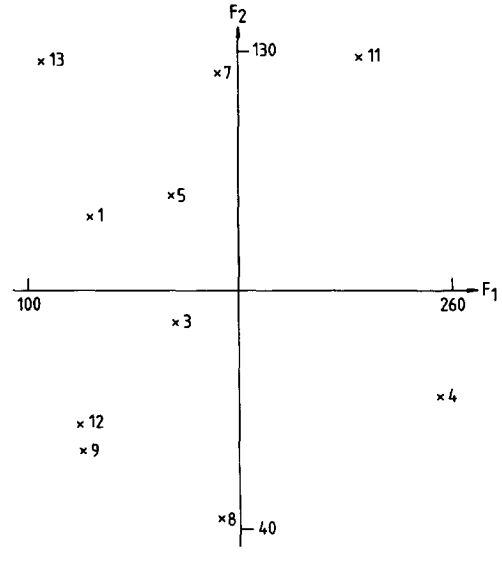

Fig. 2. Similarity and dissimilarity between antibiotics. Two-dimensional non-linear map of principal component variables. Number of iterations: 82, Max. error: $3.08 \cdot 10^{-2}$. Numbers refer to antibiotics in Table 1.

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