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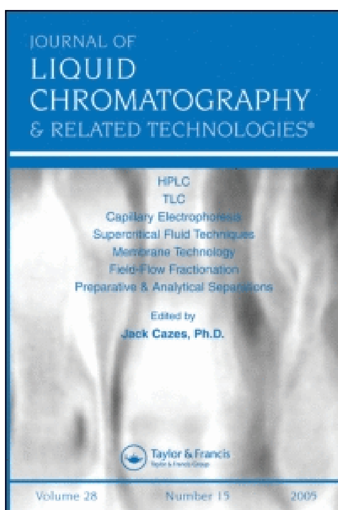

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STUDY OF ION-ION INTERACTION FOR PROTEIN-DRUG BINDING USING A NEWLY DEVELOPED GUANIDINO-BONDED PHASE IN LIQUID CHROMATOGRAPHY

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ABSTRACT

The chromatographic behavior of acidic drugs was studied using a guanidino phase in liquid chromatography. The quaternary anion-exchange group, i.e., the guanidino group, acted as a weak anion exchanger and the chromatographic retention capacity was similar to that of a DEAE ion exchanger. Acidic drugs were poorly retained on guanidino phase in a 50mM sodium phosphate buffer at pH7.4.

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This finding explains why drug-protein binding constants are generally related to the hydrophobicity of drugs and why the ion-ion interaction has seemed not to contribute to the molecular interaction. The computational chemical analysis of the retention mechanism indicated that mainly electrostatic force contributes to the retention of drugs on the guanidino phase.

INTRODUCTION

Albumin is thé most abundant plasma protein and often accounts for the entire drug binding in plasma. The binding process to albumin has physiological significance in the transport, modulation, and inactivation of metabolites and drug activities, and as a protective device in the binding and inactivation of potential toxic compounds to which the body is exposed. The majority of drugs bind to serum albumin quantitatively. Since drug binding to albumin is readily reversible, the albumin-drug complex serves as a circulating drug reservoir that releases more drug as the free drug is biotransformed or excreted. Albumin binding thus decreases the maximum intensity but increases the duration of action of many drugs.

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The forces available for the binding of drugs to albumin are electrostatic interaction, induced-electrostatic interaction, hydrogen bonding, and hydrophobic interaction. The primary force responsible for the tertiary structure of the protein is, undoubtedly, the hydrophobic interaction. Albumin has a net negative charge at the pH of serum (pH 7.4) but can interact with positive and negative charges on drugs. Theoretically drugs charge at the pH of serum, but many charges are seemed to be buried or protected in the albumin molecule. It is considered that the ionic bonds between drugs and albumin are generally not strong and there is little correlation between the net charge on albumin and the degree of binding of most drugs. Many highly albumin-binding drugs are rather insoluble in water, and for such drugs the hydrophobic interaction at hydrophobic sites on albumin is often important. While the earliest attraction and specificity of orientation of a drug molecule towards its binding site of albumin may be electrostatic, this interaction is reinforced by hydrogen bonding and hydrophobic interaction.

In vitro studies of drug-albumin interactions should establish not only the extent of drug binding but also the number of binding sites and their affinity for the drug. More detailed studies can determine the nature of the binding forces and the influence of factors such as temperature, pH, and ionic strength. The physiological importance and the relative ease with which a drug can be isolated and purified on a large scale have prompted a great number of binding studies.[1-5]

Drug-protein binding constants have also been measured using liquid chromatographic methods in which a variety of columns were used, such as aqueous size-exclusion, protein coated- and bonded-phases, reversed-phase, and internal surface reversed-phase columns. The study of drug-protein binding in liquid chromatography using protein-bonded and coated packing materials has not been satisfactory due to the different conformation of proteins. For example, the binding of proteins may eliminate the active site of proteins which is important for drug binding. The addition of proteins in a carrier solution in electrophoresis has not been a sufficient remedy, due to the denaturation of protein under the high current.

However, the reference values of binding parameters have varied, even if they were measured by the same method, due to the existence of endogenous substances such as fatty acids and bilirubin. One reason for the variation in reference values is the existence of glycosylated albumin in human serum albumin. After the purification of a commercial human serum albumin (fraction V) by affinity chromatography using borate resin, the purified albumin containing only 3.2% of glycosylated human serum albumin was used to measure the drug binding parameters. The correlations of the binding parameters of several drugs such as sulfamethoxazole, salicylic acid, tolbutamide, furosemide, warfarin, naproxen, and phenylbutazone with their respective octanol/water partition coefficients (logP values) were examined; the correlation coefficient was 0.954 (n=7).[6] Thus, the logP values were related to the logk values measured in reversed-phase liquid chromatography.

The question arises as to why such constants were related to the retention factors obtained in reversed-phase liquid chromatography using an octadecyl-bonded silica gel as the packing material. The surface of a packing material is not like a protein, even if the three-dimensional structure of the protein is neglected in the analysis. It therefore seems that the ion-ion interaction was negligible.

Therefore, the basic study of drug-protein binding cannot be performed without studying the work of guanidyl groups, because the guanidyl group of arginine is an important anion-exchange group of proteins. A guanidino-bonded silica gel was, therefore, developed in the present study to clarify the ion-ion interaction between guanidyl groups and drugs in liquid chromatography.

The stereophonic effect of proteins cannot be examined, but the ion-ion interaction between acidic drugs and guanidyl groups can be examined on this bonded-silica gel. The interaction between a drug-guanidino-phase was investigated with a computational chemical analysis using a molecular mechanics calculation program (MM2).

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EXPERIMENTAL

Reagents and Materials

The furosemide, naproxen, phenylbutazone, sulfamethoxazole, tolbutamide, warfarin, tolazamide, and indomethacin were obtained from Sigma (St. Louis, MO, USA), and the procaine hydrochloride, diazepam, salicylic acid, benzoic acid, and other chemicals were purchased from Wako Chemical Co. (Osaka, Japan). The HPLC-grade acetonitrile was obtained from Kanto-Kagaku (Tokyo, Japan). The water used was MiliQ grade water.

Liquid Chromatograph

The liquid chromatograph was constructed with two model LC-1OAD pumps and a model SIL-l0AXL auto-injector, a model SPD-l0AV UV detector from Shimadzu (Kyoto, Japan), and a model ERC-3522 degasser from ERC (Tokyo). The aluminum block column heater for micro-columns was made to specification and controlled with a model 965 temperature & process controller from Sakaguchi E.H Voc Co. (Tokyo). The operation and chromatographic data analysis were performed by a model CLASS-LC10 work-station from Shimadzu.

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Preparation of the Guanidino-Bonded Column

A guanidino-bonded phase was synthesized from propylamine-bonded phase. O-Methylisourea hydrogen sulfate and amino-propyl silica gel were suspended in 100mL of water. The mixture was heated to 80 - 90°C for 41 hours, and then filtered and washed sequentially with water and lmM aq. NaOH and acetone. The dried guanidino-bonded silica gel was packed in 150 mm long, 2 mm I.D. stainless steel columns.

Computational Chemical Calculation

The computer used for the calculations was a Macintosh 8100/100, and the software program was CAChe™ from Sony-Tektronix (Tokyo). The chemical calculation was performed without modification of the program. The geometry of a molecule and the ionization of a molecule, created using CAChe™ molecular editor, was first optimized using the molecular mechanics calculation (MM2).

The properties used for the calculation were bond stretch, bond angle, dihedral angle, improper torsion, van der Waals, electrostatic and hydrogen bond forces. The cut-off distance for the van der Waals interaction was 9Å.[7] The Cricket-Graph™ program from Computer Associates (San Diego, CA, USA) was used for data handling.

RESULTS AND DISCUSSION

Analysis of Chromatography

The amount of carbon of the original propylamine-bonded silica gel was 7.7 w/ w%, and that of the final products, guanidyl- bonded silica gel, was 10 w/w%. The amount of propylamino groups should be the maximum compared to that of octyl and octadecyl-bonded silica gels whose surface was inert for pyridine test. This result indicates that about 33% of the amino groups were converted to guanidyl groups. The conversion ratio should be the maximum due to the computational chemical analysis of the molecular mass of guanidino and amino groups. The ion-exchange capacity was 0.07 meq./g by titration. This guanidyl-bonded silica gel was used for the chromatography of saccharides in aqueous acetonitrile. The saccharides were quantitatively recovered from the column as the guanidyl-bonded polyvinylalcohol gel.[8] This result indicated that the guanidyl groups acted predominantly for the chromatography of saccharides. This guanidino-bonded silica gel column was applied to the ion-exchange liquid chromatography of acidic drugs. The retention factors listed in Table 1 were measured in a 50mM sodium phosphate buffer at pH 2.0 - 8.0 at 37°C. The buffer solutions were mixtures of 50mM phosphoric acid, monosodium dihydrogenphosphate, and disodium hydrogenphosphate. The chromatographic capability of the guanidino-bonded phase was analyzed from the chromatographic behavior of benzoic acid, which was measured on a strong anion-exchange (quaternary amine) resin made from polystyrene gel and a weak anion-exchanger (diethylaminoethyl; DEAE) made from silica gel.[9]

The retention factor of benzoic acid was constant in higher pH range than dissociation constant (pKa) value of benzoic acid on the strong anion-exchange resin; however, that decreased on the DEAE anion-exchanger in higher pH range than pKa value of benzoic acid due to the ion-exclusion effect of the strong anion and phosphoric ion of the buffer components. The chromatographic behavior of benzoic acid on the guanidino phase was similar to that on the DEAE anion exchanger. The chromatographic behavior of acidic drugs was similar to that of benzoic acid. In the case of acidic drugs, the retention factors were maximum in eluents whose pH was about the pKa values. The retention factors were very small on this guanidino phase in a 50mM sodium

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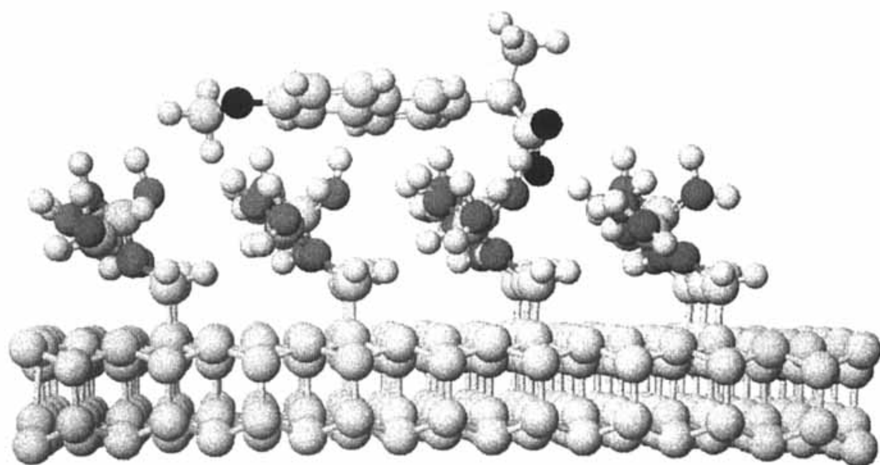

Figure 1. Complex of naproxen with guanidino-bonded phase optimized by molecular mechanics calculation. Small and large gray balls: hydrogen and carbon atoms; dark gray balls: nitrogen atoms; black balls: oxygen atoms.

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Table 1

pH Effect of Eluents on Retention Factors of Drugs

Drug	pKa pH	k/pH 2.0	3.0	35	4.0	4.5	5.0	5.6	6.5	7.0	7.5	8.0
1 Benzoic acid	4.2	0.83	0.85	1.51	2.19	2.40	1.59	0.93	0.54	-0.02	0.06	0.01
2 Diazepam	3.4	-0.23	0.04	0.43	0.60	1.15	1.03	0.96	0.71	0.70	0.82	0.41
3 Furosemide	3.9	1.48	2.22	3.93	4.08	4.15	2.72	1.47	0.98	0.65	0.27	0.09
4 Indomethacin	4.5	2.33	2.97	4.03	5.65	6.03	3.87	2.14	1.29	0.33	0.37	0.10
5 Naproxen	5.0	1.69	1.86	3.57	4.59	6.12	4.31	2.56	1.66	1.00	0.49	0.18
6 Phenylbutazone	4.5	0.80	1.27	2.25	3.18	3.94	3.14	2.04	1.25	0.70	0.30	0.10
7 Procaine	9.0	-0.34	-0.53	-0.17	-0.15	-0.07	-0.01	0.60	0.89	1.82	3.19	2.25
8 Salicyclic acid	3.0	3.19	4.07	4.45	4.16	2.55	1.55	0.89	0.50	0.17	0.05	0.02
9 Sulfamethoxazole	5.6	0.02	0.33	0.35	0.45	0.58	0.72	0.72	0.76	0.42	0.15	0.06
10 Tolazamide	3.1/5.7	0.51	0.71	0.82	0.95	1.82	1.83	1.94	2.00	1.21	0.70	0.21
11 Tolbutamide	5.3	1.06	0.79	1.13	1.33	2.46	2.51	2.02	1.45	0.84	0.48	0.11
12 Warfarin	5.0	0.62	0.85	1.10	1.93	2.92	2.90	2.05	1.61	0.33	0.34	0.12

Column: *150 x 2.0* mm I.D. packed with 5mm guanidino phase silica gel; Eluent: 50 mM sodium phosphate buffer; Flow rate: 0.2 mL/min at 37°C.

Table 2

Energy Values of Ionized Drugs and Their Complexes with Ionized Guanidino Phase

Drug	VW*	ES*	HB*	FS*	VW#	ES#	HB#	FS#	ΔES
1 Benzoic acid	4.75	0.00	0.00	-2.55	250.14	-178.06	-23.72	1812.12	-24.22
2 Diazepam	17.87	1.65	0.00	18.37	258.18	-156.43	-31.68	1840.86	-4.24
3 Furosemide	10.15	0.95	-2.23	21.45	239.91	-168.34	-36.68	1819.46	-15.45
4 Indomethacin	5.99	-4.51	-1.50	-10.09	242.48	-181.67	-37.63	1784.82	-23.32
5 Naproxen	6.68	3.16	0.00	-13.54	241.72	-175.29	-32.71	1782.01	-24.61
6 Phenylbutazone	13.94	-9.03	0.00	5.78	249.53	-168.15	-32.75	1814.84	-5.28
7 Procaine	13.31	-2.29	0.00	16.16	246.58	-149.52	-29.33	1844.95	6.61
8 Salicylic acid	5.23	-0.15	--1.49	-4.15	246.39	-172.73	-32.28	1805.62	-18.74
9 Sulfamethoxazole	3.54	-0.24	-2.43	4.89	245.16	-161.45	-31.00	1827.46	-7.37
10 Tolazamide	8.25	-14.06	-3.81	-5.35	239.65	-175.48	-34.58	1804.50	-7.58
11 Tolbutamide	4.68	-23.61	-3.75	-27.92	244.26	-189.20	-30.31	1790.10	-11.75
12 Warfarin	8.84	-7.66	0.00	-10.54	244.57	-181.78	-29.90	1790.72	-20.28
Guanidino phase	247.16	-153.84	-23.33	1840.88					

Unit: kcal/mol, * energy values of ionized drugs, # energy values of complex, VW: van der Waals energy, ES: electrostatic energy, HB: hydrogen bonding energy, FS: energy of final structure.

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phosphate buffer at pH 7.4 which was used for the measurement of drug-protein binding. The retention factors were related to the pH of the eluents that are listed in Table 1. This finding explains why the binding parameters of several drugs measured by the modified Hummel-Dreyer method were directly related to their logP values and their ion-ion interactions were neglected.[6] The ion-ion interaction may not be an important driving force of drug-protein binding for acidic drugs in pH7.4 solution. If the pH of the blood is acidic, the ion-ion interaction will contribute the drug-protein binding.

Computational Chemical Analysis

The retention factors of saccharides on the amino phase were generally related to the hydrogen bonding energy values..[10] Those on the guanidino phase were related to the electrostatic energy values.[8] Therefore, a *model guanidino* phase was constructed for studying drug-guanidino phase interactions.

The original amino phase consisted of 368 carbon, 30 nitrogen and 318 hydrogen atoms. The molecular weight was 5,154. The amino groups were converted to guanidyl groups. The final guanidino phase consisted of 316 carbon, 36 nitrogen and 71 hydrogen atoms due to the capacity of the computer and the size of the guanidino phase. The molecular weight was 4,367. The adsorption form of naproxen on the guanidino phase is shown in Fig. 1 as an

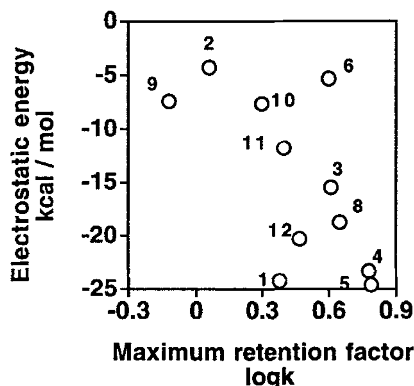

Figure 2. Retention factors related to energy values of optimized complex. symbol (1) Benzoic acid, (2) Diazepam, (3) Frosemide, (4) Indomethacin, (5) Naproxen, (6) Phenylbutazone, (8) Salicylic acid, (9) Sulfamethoxazole, (10) Tolazamide, (11) Tolbutamide, (12) Warfarin. (Procaine (7) is not included because of a basic drug).

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example of the adsorption of a drug on the guanidino phase. The retention factors obtained in liquid chromatography were related to their final (FS), hydrogen bonding (HB), electrostatic (ES), and van der Waals (VW) energy values calculated by MM2 and listed in Table 2.

After subtraction of the individual energies of the drugs and the guanidino phase from the molecular interaction energy values, the maximum retention factors measured between pH 3.5~6.5 were correlated with the electrostatic energy values (AES), as shown in Fig. 2. The molecular interaction for acidic drugs was mainly due to electrostatic energy and not hydrogen bonding or van der Waals energies on the guanidino phase.

CONCLUSIONS

The chromatographic results demonstrated that the hydrophobicity of drugs is the predominant force for protein-drug binding, and ion-ion interaction is negligible.

The computational chemical calculation suggested that electrostatic force contributes to the retention of drugs on the guanidino phase. These results encourage the further study of ion-ion interaction in protein-drug binding using a newly designed experimental system.

REFERENCES

1. R. H. McMenamy, "Albumin Binding Sites," in **Albumin Structure, Function and Uses**, V. M. Rosenoer, M. Oraty, M. A. Rothschild eds., Pergamon Press, Oxford, 1977, p. 143.

2. E. M. Sellers, J. Koch-Wester, "Clinical Implications of Drug-Albumin Interaction," in **Albumin Structure, Function and Uses**, 1977, p. 159.

3. U. Kragh-Hansen, "Molecular Aspects of Ligand Binding to Serum Albumin," Pharmacological Reviews, **33**, 17, 1981.

4. T. Peter Jr., "Serum Albumin," Advances in Protein Chemistry, **37**,161 (1985).

5. D. C. Carter, J. X. Ho, "Structure of Serum Albumin," Advances in Protein Chemistry, **45**, 153 (1994).

6. K. Koizumi, C. Ikeda, M. Ito, J. Suzaki, T. Kinoshita, K. Yasukawa, T. Hanai, Biomedical Chromatography, in press.

7. **Manual of CAChe™**, Sony-Tektronix (Tokyo) 1994.

8. T. Hanai, R. Miyazaki, J. Suzuki, T. Kinoshita, J. Liq. Chromatogr., **20**, 2941 (1997).

9. T. Hanai, J. Hubert, J. Chromatogr., **316**, 261 (1984).

10. T. Hanai, H. Hatano, N. Nimura, T. Kinoshita, J. Liq. Chromatogr., **17**, 241 (1994).

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