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Journal of Chromatography B, 768 (2002) 211–214

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

Study on the multiple sites binding of human serum albumin and porphyrin by affinity capillary electrophoresis

Weibing Zhang[a], Lihua Zhang[a], Guichen Ping[a], Yukui Zhang[a,*], A. Kettrup[b]

[a]*National Chromatographic R&A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 161 Zhongshan Road, Dalian 116011, PR China*

[b]*GSF-National Research Center for Environment and Health, Institute of Ecological Chemistry, Ingolstädter Landstrasse 1, D-85764 Neuherberg, Germany*

Abstract

Equations to describe the two sites binding between proteins and ligands were deduced. According to these equations, not only the binding constants, but also the mole fraction of proteins in different forms could be obtained. Using the published data on the interaction between human serum albumin (HSA) and three kinds of porphyrin (coproporphyrin (CP), uroporphyrin I (UP) and protoporphyrin (PP)), a further study on their binding was carried out. It was concluded that there may exist two binding sites with the binding constants at the first site, proved to be the preferential one, being 6.50×10^5, 1.94×10^6 and 8.94×10^5, respectively. In addition, it was also demonstrated that the two binding sites of HSA with CP and UP might be of different kinds, though those of HSA and PP were of the same kind but at different positions.

Keywords: Affinity capillary electrophoresis; Protein binding; Human serum albumin; Porphyrin

1. Introduction

Research on the interaction between biological macromolecules and drugs is one of the most important topics in the field of life science because the binding sites and constants can provide important information about the transportation and metabolism of small molecules in human bodies. Since affinity capillary electrophoresis (ACE) combines the high efficiency of capillary electrophoresis (CE) and the special selectivity of biological identification, it has been used more and more in the study of molecule interaction [1–3].

As far as the interaction and binding sites of proteins and drugs are concerned, they are commonly studied directly or indirectly by binding curve through quantitative analysis or by variation on migration velocities of analyte. Liu et al. have worked on the interaction between protein, Hsc 70, and deoxidized spermatin. The binding constants under different pH and the possible forms of metabolites were obtained [4]. Busch et al. have compared the interaction between antigen and antibody by frontal analysis and traditional methods [5]. Shibukawa et al. have studied the characteristics of globulin [6]. Ding et al. have obtained the binding constants of HSA and porphyrin by studying the change in electrophoretic mobility of HSA in buffer

*Corresponding author. Tel.: +86-41-1369-3427; fax: +86-41-1369-3427.

E-mail address: ykzhang@ms.dicp.ac.cn (Y. Zhang).

1570-0232/02/$ – see front matter
PII: S0378-4347(01)00501-1

with porphyrin [7,8]. Chu et al. have measured the binding constant of carbonic anhydrase B and benzanesul-bonamides using a similar method [9].

If there is only one binding site between proteins and drugs, the binding constant could be obtained by Scatchard equation, which is a very useful method for obtaining a lot of important information. However, when there is more than one binding site, other equations should be taken into consideration.

In this paper, considering the possible multiple sites interaction between proteins and ligands, we proposed equations to describe two sites interaction and applied them to studying the binding of HSA and porphyrin by ACE.

2. Theory

Based on the structure characteristic of proteins, there may exist different kinds or several of the same kind of binding sites between proteins and drugs. Here the situation with two binding sites is discussed.

Compared to proteins, drugs are rather small. Accordingly, it could be considered that the binding constant between protein and the first ligand is the same as that between their complex and the second one. Therefore, the equilibrium between proteins, drugs and their complex could be expressed by the following equations.

$$P + L \overset{k_1}{\Leftrightarrow} PL_1(1) \tag{1}$$

$$P + L \overset{k_2}{\Leftrightarrow} PL_1(2) \tag{2}$$

$$PL_1(1) + L \overset{k_2}{\Leftrightarrow} PL_2(12) \tag{3}$$

$$PL_1(2) + L \overset{k_1}{\Leftrightarrow} PL_2(21) \tag{4}$$

If the velocities of mass transference in Eqs. (1)–(4) were fast enough, it could be considered that the equilibriums of the above mentioned procedures could be realized in a short time. Therefore, the following equations could be obtained.

$$[PL_1(1)] = k_1[P][L] \tag{5}$$

$$[PL_1(2)] = k_2[P][L] \tag{6}$$

$$[PL_2(12)] = k_1k_2[P][L]^2 = [PL_2(21)] \tag{7}$$

Although $PL(1)$ and $PL(2)$ might be a little different in structure, they have the same charge numbers and molecular masses. Therefore, their migration in electric field should also be the same. Also $PL_2(12)$ and $PL_2(21)$ are the same compounds. Accordingly, the mole fractions of $P(\chi_P)$, $PL(\chi_{PL})$ and $PL_2(\chi_{PL_2})$ should be expressed, respectively, as follows.

$$\chi_P = \frac{1}{1 + (k_1 + k_2)[L] + 2k_1k_2[L]^2} \tag{8}$$

$$\chi_{PL} = \frac{(k_1 + k_2)[L]}{1 + (k_1 + k_2)[L] + 2k_1k_2[L]^2} \tag{9}$$

$$\chi_{PL_2} = \frac{2k_1k_2[L]^2}{1 + (k_1 + k_2)[L] + 2k_1k_2[L]^2} \tag{10}$$

With the electrophoretic mobilities of P, PL and PL_2 expressed by μ_P, μ_{PL} and μ_{PL_2}, the total electrophoretic mobility could be obtained according to Eq. (11):

$$\mu = \sum \mu_{PL_i}\chi_{PL_i} = \frac{\mu_P + (k_1 + k_2)[L]\mu_{PL} + 2k_1k_2[L]^2\mu_{PL_2}}{(k_1 + k_2)[L] + 2k_1k_2[L]^2 + 1} \tag{11}$$

Based on this equation, by multivariate regression between μ and $[L]$, not only the equilibrium constants between proteins and drugs but also the migration of different forms of complex could be obtained.

In addition, the number of binding sites could be calculated by the following equation,

$$n = \sum \chi_{PL_i} n_{PL_1} \tag{12}$$

where $n_{PL_1} = 1$ and $n_{PL_2} = 2$.

3. Results and discussion

Ding et al. have studied the interaction between HSA and CP, UP and PP by ACE [7,8]. A self-made polyacrylamide coated capillary of 50 μm I.D. and 57 cm length and a commercial sulfonic coated capillary (Eatontown, NJ, USA) of 75 μm I.D. and

44.5 cm length were used. All experiments were carried out on Beckman P/ACE 5500 instrument with UV detection wavelength at 214 nm. With different concentrations of porphyrin in the buffer (C), different migration velocities of HSA, and through further calculation, different electrophoretic mobilities (μ_{exp}) were obtained, as shown in the first three columns in Table 1. In their work, through Scatchard regression, it was concluded that there was only one binding site between HSA and porphyrin.

According to the equations deduced above, further study on the binding of HSA and porphyrin was carried out.

Based on Eq. (11), through the regression between μ_{exp} and C under different situations, χ_{PL_i} and n could be obtained, as shown in Table 1, from which it can be seen that with a little porphyrin in buffer, χ_P decreased and χ_{PL} increased very quickly, which meant the binding between HSA and porphyrin at the ratio of 1:1 was quite easy. However, with more porphyrin in buffer, the number of binding sites of HSA and porphyrin increased gradually and approached 2 when the concentration of porphyrin was high enough, which showed that after the first binding site was nearly saturated, binding at the second site began. Under ultimate conditions, most HSA existed in the form of PL_2.

From Table 1, it can also be seen that the concentrations of CP, UP and PP when the numbers of binding sites was ~1.5 were 4, 10 and 0.15 mM, respectively. Under such low concentrations, it was impossible to form the dimer of porphyrin so that n listed in Table 1 only reflects the binding of HSA and the monomer of porphyrin.

From Eq. (11), μ_{PL_i}, k_1, and k_2 could also be obtained (as shown in Table 2). Taking the binding at the first site into consideration, our calculated values were similar to those reported, 1.3×10^5,

Table 1
Electrophoretic mobilities, mole fraction and average number of binding points of HSA and its combination with porphyrin

Ligands	C (μM)	μ_{exp} ($cm^2\ min^{-1}\ kV^{-1}$)	χ_P	χ_{PL}	χ_{PL_2}	n
CP	0	14.90	1	0	0	0
	0.5	15.67	9.15×10^{-4}	0.89	0.12	1.11
	1	15.98	4.12×10^{-4}	0.80	0.20	1.20
	2	16.16	1.71×10^{-4}	0.66	0.34	1.34
	4	16.35	6.42×10^{-5}	0.50	0.50	1.50
	8	16.52	2.14×10^{-5}	0.33	0.67	1.67
	20	16.90	4.27×10^{-6}	0.17	0.83	1.83
	30	16.97	2.01×10^{-6}	0.12	0.88	1.88
	50	17.07	7.58×10^{-7}	0.073	0.93	1.93
	100	17.06	1.97×10^{-7}	0.038	0.96	1.96
UP	0	15.20	1	0	0	0
	10	15.62	8.15×10^{-5}	0.53	0.47	1.47
	20	15.79	2.77×10^{-5}	0.36	0.64	1.64
	30	15.86	1.41×10^{-5}	0.27	0.73	1.73
	50	15.94	5.66×10^{-6}	0.18	0.82	1.82
	75	15.96	2.68×10^{-6}	0.13	0.87	1.87
	100	16.03	1.56×10^{-6}	0.10	0.90	1.90
	150	16.06	7.17×10^{-7}	0.070	0.93	1.93
PP	0	14.688	1	0	0	0
	0.02	15.375	4.50×10^{-2}	0.81	0.14	1.10
	0.15	17.328	3.20×10^{-3}	0.44	0.56	1.56
	0.60	18.678	3.00×10^{-4}	0.164	0.84	1.84
	0.75	18.766	1.99×10^{-4}	0.13	0.87	1.87
	1.50	18.956	5.32×10^{-5}	0.072	0.93	1.93
	3.75	19.288	8.90×10^{-6}	0.030	0.97	1.97

Table 2
Electrophoretic mobilities of HSA and its complex as well as the binding constants

Ligands	μ_P ($cm^2\ min^{-1}\ kV^{-1}$)	μ_{PL} ($cm^2\ min^{-1}\ kV^{-1}$)	μ_{PL_2} ($cm^2\ min^{-1}\ kV^{-1}$)	k_1 ($\times 10^5$)	k_2	k_1/k_2 ($\times 10^3$)
CP	14.90	15.20	16.10	6.50	4.44	146
UP	15.20	15.58	17.14	19.38	12.6	149
PP	14.67	14.72	19.38	8.94	429	2.08

2.0×10^4 and 0.5×10^6 or 1.3×10^6 [10–12] for HSA with CP, UP and PP, respectively. In addition, from the value of k_1/k_2, it could be seen there might exist two different kinds of binding sites between HSA and CP and UP. However, those between HSA and PP were of the same kind but at different positions.

In addition, since porphyrin was charged under the separation conditions, μ_{PL_i} increased with more ligands bound to it. It could be seen that the increase was rather large when HSA was bound to the second PP compared to CP and UP, which might be caused by the special binding sites and the effects of ligands on the structure of HSA.

Assuming that there was only one binding site between HSA and porphyrin, with Scatchard equation, an unusual result against linear regression was obtained, which called that assumption into question [7]. However, with the two binding sites theory, no exception existed, which demonstrated that if there was an unusual phenomenon when Scatchard equation was applied, there might exist more than one binding site between proteins and ligands.

4. Conclusions

According to the characteristics of multiple sites binding between proteins and drugs, equations to obtain binding constants as well as the electrophoretic mobilities of protein and its complex were deduced. Based on published data on the interaction between HSA and porphyrin and the theory proposed in this paper, it was demonstrated that there might exist two binding sites between them. Also the mole fraction of protein in different forms and the average number of binding sites could be obtained.

Acknowledgements

The authors are grateful for financial support from the National Natural Science Foundation of China and a Scholarship for the Joined-Education Program from DAAD (Germany).

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