行政院國家科學委員會補助專題研究計畫成果報告

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執行期間： 88 年 8 月 1 日至 89 年 7 月 31 日

計畫主持人：吳俊弘

計畫參與人員：李能佳、柳勝文、劉學穎、呂定蓉

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□赴國外出差或研習心得報告一份

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□國際合作研究計畫國外研究報告書一份

執行單位： 淡江大學化學系

中　華　民　國　90　年　2　月　21　日

行政院國家科學委員會專題研究計畫成果報告

毛細管電泳之有效電荷測量
Determination of Effective Charge by Capillary Electrophoresis

計畫編號： NSC 89-2113-M-032 -013
執行期限：88 年 8 月 1 日至 89 年 7 月 31 日
主持人：吳俊弘　　淡江大學化學系

一、中文摘要

在此計畫中，我們利用毛細管電泳技術來測量胺基酸和蛋白質在不同pH值緩衝溶液中以及蛋白質經界面活性劑變性後的有效電荷。我們探討了樣品濃度，蛋白質分子量和等電點，毛細管塗層，緩衝溶液pH值，以及電場強度等因素對於有效電荷測量之影響。

在本報告的第二部份，我們提出了一個嶄新的方法，利用毛細管電泳儀測量各種類型與蛋白質結合的界面活性劑的臨界微胞濃度。臨界微胞濃度可由在高於及低於此值的濃度區域之不同黏度線性外插測得。

關鍵詞：毛細管電泳，有效電荷，臨界微胞濃度。

Abstract:

Capillary electrophoresis (CE) technique provided a direct access to simultaneous measures of the protein electrophoretic mobility and the diffusion coefficient, and therefore the estimation of the effective charge. In this report, the subjects under investigations included amino acids with different charge polarities, proteins differing in pI and in molecular weight, and protein-SDS complexes in buffer solutions with different pH values and ionic strengths.

In part II of this report we presented a navel method for the critical micelle concentration (cmc) measurements of several protein-binding surfactants. The determination of cmc was based on the distinct viscosity properties of the surfactants before and after micellar formation.

Keywords: capillary electrophoresis, effective charge, critical micelle concentration

二、緣由與目的

The electrostatic properties of proteins play an important role in their biological functions. Therefore, the measurements of their effective charges are very useful in understanding their biological properties. The experimental methods used to estimate protein charges include isoionic point method [1], Donna potential measurement [2,3], and time dependent polyacrylamide gradient gel electrophoresis method [4-6]. These methods have disadvantages in several aspects, such as large amount of sample consumption, inaccuracy resulted from sample impurities, and requiring complicated model fitting. Recently the research group in Harvard University combined affinity capillary electrophoresis technique and protein charge ladder to measure the effective charges of various proteins [7-10]. In their method, a series of charged protein-binding ligands must be synthesized and then covalently bound to the protein in order to form a set of protein charge ladder. In this project we used capillary electrophoresis technique to simultaneously measure the electrophoretic

mobility and diffusion coefficient of charged species, and thus obtained a good estimate of the effective charge. The effective charge, Q, can be described by the following equation

$$Q = \frac{k_B T \mu}{D}$$

where, k_B is Boltzmann's Constant, T is temperature(K), μ is electrophoretic mobility and D is diffusion coefficient.

In this project we investigated some variables that would affect the determination of effective charge. These factors included sample concentration, protein molecular weight and isoelectric point, capillary coating, buffer pH value and electric field strength.

三、結果與討論

Part I. Effective charge measurements of amino acids, proteins, and protein-SDS complexes:

In this report, the subjects under investigations included amino acids with different charge polarities, proteins differing in pI and in molecular weight, and protein-SDS complexes in buffer solutions with different pH values and ionic strengths. To overcome the tailing and adsorption problems, especially for the basic proteins, cationic polymer coating on capillary inner wall was carried out. In the measurements of electrophoretic mobilities, electric field strength of less than 100v/cm was used to avoid systematic deviation resulted from conformational distortions of analytes and joule heat effect. This effect was shown in Figure 1. Table 1 showed that the diffusion coefficients of 3 amino acids and 6 proteins measured by hydrodynamic dispersion method were within 2% relative difference when compared with tabulated values. The effective charges of six amino acids at pH 2.05 were listed in Table 2. All the amino acids studied were positively charged under this pH value. As expected, the measured effective charges decreased with decreasing pI values. The experimental results of effective charge measurements for ten proteins were shown in Table 3. The measured diffusion coefficients decreased exponentially with increasing protein molecular weights. Unlike in the case of amino acids, the measured effective charges for proteins did not vary orderly with their pI values. This was probably due to the more complicated structures of proteins. We investigated the pH effect on protein effective charge by measuring the effective charge of Carbonic Anhydrase B prepared in buffer solutions with pH values ranged from 2.05 to 10.88. The resulting curve shown in Figure 2 was very similar to that reported in literature [11]. In the study of protein-surfactant binding behavior, we found that the effective charge of Myoglobin could increase more than twenty times when denatured by SDS.

Part II. Cmc measurements of protein binding surfactants

Critical micelle concentration can be obtained by measuring the distinct physical properties of surfactant molecules in the monomer state and in the associate(or aggregate) state. In this report, two linear increments of viscosities were found when increasing surfactant concentrations from dilute to above cmc, which was then decided by linear extrapolation of the two concentration regions. With capillary electrophoresis instrument and according to Poiseuille's law, viscosity can be obtained by measuring the retention time of a dilute marker in the hydrodynamic flow of the fluid. This method was verified by the measurements of a set of standards with known viscosities. The results were shown in Figure 3. The determination of cmc value was demonstrated in Figure 4. The cmc values of various types of surfactants including anionic, cationic, zwitterionic, and non-ionic ones were determined and the results were listed in Table 4. For all types of surfactants, the cmc values measured were all in good agreement with those reported in literatures. As shown in Table 4, this method was also demonstrated to be applicable to cmc measurement of aqueous surfactant with

organic or electrolyte additive.

四、計劃成果自評

The results of this proposal provided us a new method for effective charge measurements of amino acids and proteins under different aqueous environments. This would make us better understand the electrostatic properties of proteins and thus their binding behaviors with other species. Moreover, the results were useful in optimizing CZE conditions for the separation and identification of amino acids and proteins. Furthermore, in this project we also developed a retention time method for convenient and accurate cmc measurements.

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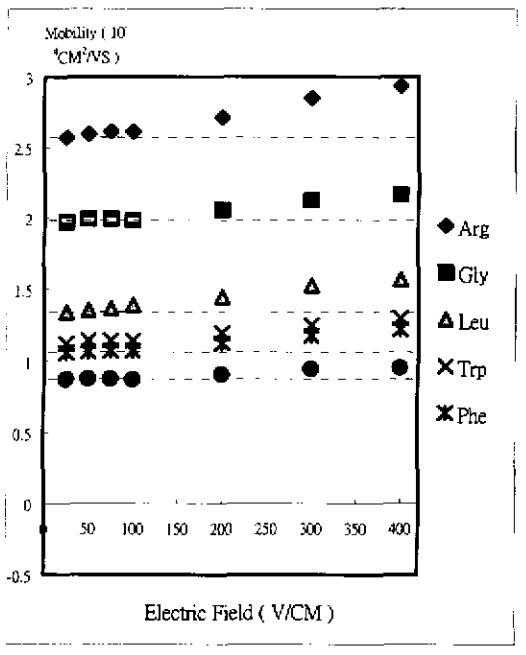

Figure 1. Effect of electric field strength on mobility measurements of six amino acids.

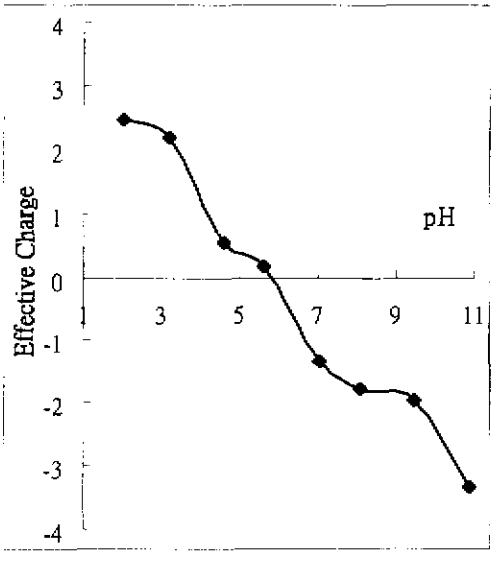

Figure 2. pH dependence of the measured effective charge of Carbonic Anhydrase B.

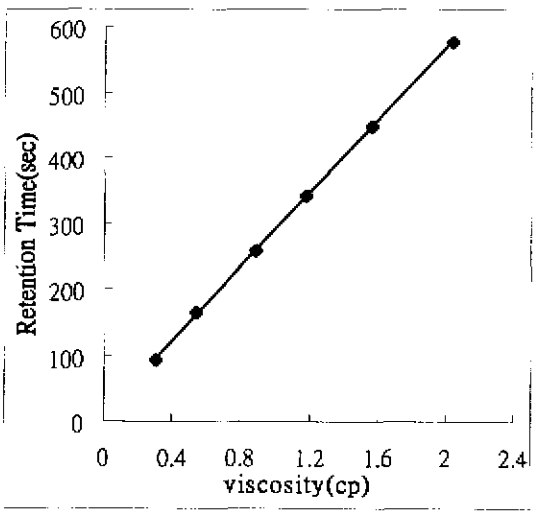

Figure 3. The linear relationship between viscosity and retention time for six standards.

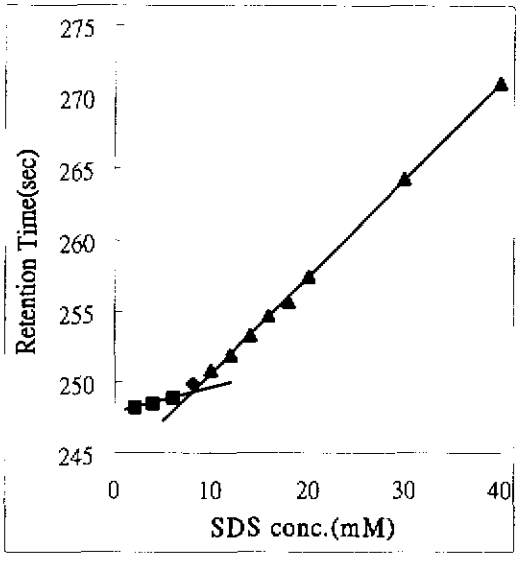

Figure 4. Cmc determination of SDS by linear extrapolation of the two concentration dependent viscosity regions.

Table 1. Comparisons of measured diffusion coefficients with literature values

Amino acid or Protein	$D_{exp}(10^{-6})$	$D_{table}(10^{-6})$	R.D.(%)
Glycine	10.268	10.50	2.21
Phenylalanine	6.990	7.05	0.85
Tryptophan	6.583	6.59	0.11
Insulin	1.474	1.5	1.73
Lysozyme	1.147	1.13	1.51
Myoglobin	1.129	1.13	0.18
α -Chymotrypsinogen	0.987	0.95	3.89
Ovalbumin	0.774	0.776	0.26
Hemoglobin	0.702	0.69	1.74

Table 2. Results of effective charge measurements of six amino acids at pH 2.05

Amino acid	pI	$\mu(10^{-4})$	$D(10^{-6})$	E.C.
Arg	10.76	2.613	6.651	+0.996
Gly	5.97	1.982	10.77	+0.477
Leu	5.98	1.389	7.390	+0.465
Trp	5.89	1.140	6.422	+0.456
Phe	5.48	1.073	6.704	+0.414
Asp	2.96	0.873	7.847	+0.291

μ = Mobility ($10^{-4}cm^2/Vs$)
D = Diffusion coefficient ($10^{-6}cm^2/s$)
E.C. = Effective charge (Valence)

Table 3. Experimental results of effective charge measurements for ten proteins

Protein	PI	$\mu(10^{-4})$	$D(10^{-6})$	E.C.
Insulin	5.3	1.291	1.474	-2.252
Lysozyme	10.9	1.811	1.147	+4.118
Myoglobin	6.8	0.288	1.129	-0.654
α-Ch A	9.8	0.508	0.987	+1.376
CAA	5.9	0.699	0.935	-1.921
CAB	5.9	0.541	0.935	-1.487
CA II	5.9	0.534	0.981	-1.401
Ovalbumin	5.1	1.737	0.774	-5.767
Hemoglobin	6.8	0.564	0.702	-2.066
G6P- DH	6.4	1.008	0.605	-4.282

Table 4. Comparisons of experimental and tabulated cmc values of anionic, cationic, zwitterionic, and non-ionic surfactants

Surfacant/Solvent	**Experimental values(mM)**	**Literature values(mM)**	**References**
SDS/water	7.95	8.0	12
SDS/3M Urea	9.74	9.85	13
SDS/10mM NaCl	5.07	5.29	14
Sodium Decanesulfonate	32.25	32.6	15
Sodium Octanoate	341.8	351	15
DoTAB	15.41	15.5	16
CTAB	1.16	1	15
CHAPS	10.03	6~10	15
N-Tertradecyl-N,N-Dimethyl-3 -ammonio-1-propanesolfonate	0.35	0.1~0.4	15
Decanoyl-N-methyl-glucamide	7.03	6~7	15
F127	6.7(g/L)	6.2(g/L)	17
Triton X-100	0.26	0.24	15