Separation of denatured proteins in free solution on a microchip based on differential binding of alkyl sulfates with different carbon chain lengths†

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Denatured proteins were separated in free solution on a microfluidic chip without any sieving matrix after carrying out the denaturation with a mixture of alkyl sulfates with different carbon chain lengths.

Electrophoresis of polyelectrolytes such as DNA and denatured proteins is usually performed in chemical or physical gels instead of in free solution, except in some special cases.[1] It has been generally accepted that proteins with molecular weights more than 10 kDa have a constant free solution mobility that is independent of their molecular weights, after they are fully denatured by sodium dodecyl sulfate (SDS) and a reducing agent.[2] This phenomenon is generally attributed to the constant charge density along the polypeptide chain. The coating of the negatively charged surfactant makes the intrinsic charge of the proteins insignificant. For similar reasons, DNA fragments longer than 10–20 bp have the same free solution mobilities regardless of fragment size or base composition.[3] In this report, we demonstrate that denatured proteins have different electrophoretic mobilities in free solution after the denaturation is carried out using a mixture of alkyl sulfates with different carbon chain lengths. Furthermore, the free solution mobilities are not correlated with the molecular weights of the proteins. In this work, the free solution electrophoresis was carried out on a glass microchip.

In our experiments, surfactants with different compositions were used for the denaturation of proteins. The surfactants used in different experiments included a commercial alkyl sulfate mixture from Pierce (Rockford, IL) (referred as Pierce SDS in the text, No. 28364, lot analysis: C_{12} 73%, C_{14} 24%, C_{16} 3%, and C_{10} + C_{18} <1%, w/w.), SDS (99%) from Sigma (St. Louis, MO), sodium tetradecyl sulfate (STS) (99%) from Lancaster Synthesis (Pelham, NH), and a binary mixture of SDS (99%) and STS (99%) (w/w = 3:1). In the experiments involving Pierce SDS, pure SDS, and SDS/STS, the sample buffer for the denaturation was prepared in 2.5 mM sodium borate (pH ~ 9.2) and contained 1% 2-mercaptoethanol and 1.45% (w/v) surfactant. STS has a very low solubility. In the case of denaturation using STS, the sample buffer contained 0.59% (w/v) STS. The protein mixture was denatured by heating in the sample buffer at 95 °C for 5 min. After the denaturation, the proteins were labeled using a fluorogenic dye FQ (3-(2-furoyl)quinoline-2-carbaldehyde) from Molecular Probes (Eugene, OR). The labeling procedure was similar to the protocol in the literature.[4] The protein separation was performed using microchip electrophoresis in free solution with laser induced fluorescence detection. The details of the design and performance of the microchip are available in the ESI.† The running buffer consisted of 2.5 mM sodium borate and 5 mM of the surfactant or surfactant mixture used in the denaturation. The small concentration of surfactants was used to maintain the coating on the protein surface during electrophoresis, and the concentration is below the critical micelle concentration (CMC).[5]

After the denaturation, the negatively charged detergent coating overwhelmed the intrinsic charge of proteins and the charge of the dye molecules, the heterogeneity of multiple labeling was suppressed. A single peak was observed for each denatured protein. In the first experiment, we denatured four model proteins: α-lactalbumin (14 kDa), ovalbumin (45 kDa), conalbumin (78 kDa), and β-galactosidase (116 kDa), using Pierce SDS which was a commercial sulfate mixture with multiple chain lengths. Fig. 1 shows that with an electric field of 436 V cm^{-1} in the separation channel and 12 cm from the injection point, the four denatured proteins were completely separated in free solution. The peaks were assigned by the comigration of a single protein with the mixture. The elution sequence of the proteins was not correlated with the molecular weights, indicating that the separation mechanism is not based on differences in the molecular size.

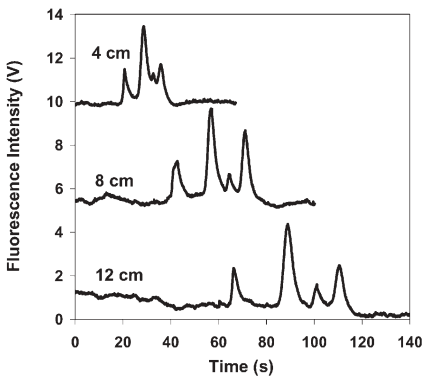

Fig. 1 Free solution electrophoresis of four proteins denatured with Pierce SDS. The protein mixture had concentrations around 1 × 10^{-6} M for each protein. The separation was driven by 436 V cm^{-1} and the running buffer was 2.5 mM sodium borate + 0.14% (w/v) Pierce SDS. The fluorescence was detected at 4, 8 and 12 cm from the injection. The peaks were assigned as follows (12 cm): β-galactosidase (67 s); conalbumin (89 s); α-lactalbumin (101 s); ovalbumin (111 s).

† Electronic supplementary information (ESI) available: Microchip electrophoresis details. See http://www.rsc.org/suppdata/cc/b4/b411697f/

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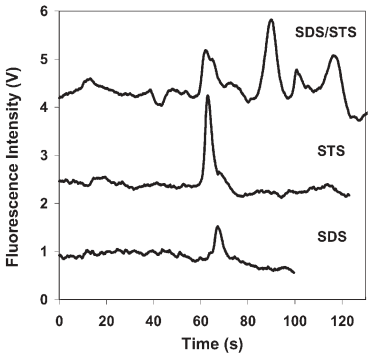

Fig. 2 Free solution electrophoresis of four proteins denatured with SDS, STS, and a binary mixture SDS/STS (3:1, w/w). The running buffer contained 2.5 mM sodium borate and 0.14% (w/v) surfactant when SDS or SDS/STS was used. In the STS experiment, the running buffer had 0.04% (w/v) STS. The separation was driven by 436 V cm^{-1}. The fluorescence was detected at 12 cm from the injection.

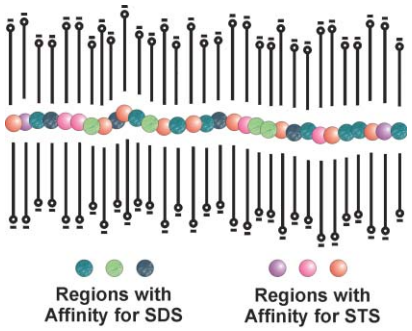

Fig. 3 A schematic illustration of the differential binding of SDS and STS on the protein surface and its dependence on the amino acid sequence. The longer surfactant is STS. The different color spheres represent amino acid residue clusters with different hydrophobicities. The drawing does not reflect the actual packing density of the surfactants.

Fig. 2 shows that we were also able to separate the proteins by free solution electrophoresis when they were denatured by a binary mixture made of pure SDS and pure STS. The ratio between the two in the mixture was similar to that in Pierce SDS. This result indicates that the separation was not caused by any minor impurity in Pierce SDS and a binary mixture is enough to yield separation. Experiments were also conducted with the sulfate mixture replaced by either pure SDS or pure STS. Proteins denatured by single component sulfate could not be separated by free solution electrophoresis. This agrees with the previous reports that SDS–protein complexes had identical electrophoretic mobilities in free solution when the molecular weights are larger than 10 kDa.[2] Our results indicate that STS can also form STS–protein complexes with a constant charge-to-size ratio.

Henry has derived the electrophoretic mobility of an infinitely long, charged, insulating cylinder in an aqueous salt solution.[6] He showed that the mobility could be described by eqn. (1)

$$\mu = \frac{\varepsilon_b \varepsilon_0 \zeta}{4\pi\eta} \quad (1)$$

where ε_b is the dielectric constant, ε_0 is the permittivity of vacuum, ζ is the surface potential of the charged cylinder, and η is the viscosity of the solution. Stigter showed that in the case of a charged, insulting cylinder of finite length L and radius a, $L/a \gg 1$, while the radius a is of the same order as Debye length $1/\kappa$, eqn. (1) was still a good approximation.[7] The Debye length $1/\kappa$ is given by eqn. (2)

$$\kappa^{-1} = \left(\frac{\varepsilon_b \varepsilon_0 kT}{e^2 \sum_k z_i^2 C_i} \right)^{1/2} \quad (2)$$

where k is the Boltzmann constant, T is the absolute temperature, e is the electron charge, and C_i and z_i are the concentration and charge valence of species i, respectively. Using the Debye–Hückel approximation, the surface potential ζ can be related to the surface charge density σ [eqn. (3)]

$$\zeta = \frac{\sigma}{\varepsilon_b \varepsilon_0 \kappa} \quad (3)$$

Combining eqns (1) and (3), we have

$$\mu = \frac{\sigma}{4\pi\eta\kappa} \quad (4)$$

Eqn. (4) is by no means a rigorous theoretical treatment. However, it gives important suggestions about how to interpret our results. According to this result, the difference in the surface charge density is the main reason for different free solution mobilities of denatured proteins. In our case, the packing density of the surfactants determines the surface charge density.

It has been reported that the presence of some impurities in SDS, mostly longer-chained alkyl sulfates such as STS and sodium hexadecyl sulfate (SHS), could alter the results of protein gel electrophoresis in terms of the resolution and the electrophoretic mobilities in gels.[8] Although the mechanism was not completely understood, the results suggest that a coating of mixed alkyl sulfates was formed on the polypeptide surface when the proteins were denatured using a mixture of alkyl sulfates.

Based on the literature and our results, we propose that the separation of denatured proteins in free solution is due to the differential binding of alkyl sulfates with different carbon chain lengths on the polypeptide surface (Fig. 3). Longer-chained alkyl sulfates such as STS and SHS have higher hydrophobicity than SDS. Some amino acid residues are more hydrophobic than the others. Conceivably, the incorporation of alkyl sulfates with mixed chain lengths into the protein-sulfate complex generates heterogeneity in the surface coating, which is determined by the amino acid sequence. The specific distribution of different alkyl sulfates is unique to each protein and determines the packing density of surfactants, and therefore, the free solution mobility. From our data, the free solution mobilities of the proteins denatured by the binary mixture SDS/STS were different from those of the proteins denatured by either pure SDS or pure STS. This suggests that the molecular packing density in the mixed coating is significantly different from that of a single surfactant coating. This novel mechanism provides a fast and simple method for protein analysis. Further work is in progress to compare the efficiency of this method with those of other electrophoresis tools and establish the relationship between the amino acid sequence and the free solution mobility.

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