Yingjie Li*
Kevin W. Mattison
Paul L. Dubin†
Department of Chemistry
Indiana University–
Purdue University
Indianapolis, IN 46202

Henry A. Havel
Shun L. Edwards
Biopharmaceutical
Development
Eli Lilly and Co.
Indianapolis, IN 46285

Light Scattering Studies of the Binding of Bovine Serum Albumin to a Cationic Polyelectrolyte

Poly(dimethyldiallylammonium chloride) (PDMDAAC) exhibits a strong electrostatic interaction with bovine serum albumin (BSA) at pH 8.0 in 0.16M NaCl. Electrophoretic, dynamic, and static light scattering suggest that the mode of binding of BSA to PDMDAAC depends upon the weight concentration ratio (r) of BSA to PDMDAAC. When r is smaller than ca. 10, the system exhibits characteristics of cooperative binding, in that the BSA molecules are inhomogeneously distributed among the polymer chains, and free PDMDAAC molecules coexist with complex. When r reaches ca. 10, the amount of free PDMDAAC is too small to be observed. Further increase in r leads to a secondary binding process along with an increase in the amount of free protein. Hydrophobic interactions among the bound BSA are proposed as the driving force for the cooperative binding. © *1996 John Wiley & Sons, Inc.*

INTRODUCTION

Strong interactions between proteins and polyelectrolytes have been the subject of numerous research efforts.[1–43] These forces may result in the formation of soluble complexes,[19,26,43] complex coacervates,[42] or precipitates.[5,11,14,37] The study of these interactions is important for at least three reasons. (1) Polyelectrolyte–enzyme complex formation may be used to stabilize or immobilize enzymes[43,44]; (2) polyelectrolyte–protein complexes may serve as model systems for the study of nonspecific coulombic interactions between DNA and proteins[45,46]; and (3) selective precipitation or coacervation of proteins by polyelectrolytes has great potential in large-scale protein purification techniques.[42] Techniques to investigate the structure or properties of coacervated or precipitated polyelectrolyte–protein complexes are clearly very limited. However, soluble complexes can be studied by a wide range of methods well known in colloid and polymer chemistry, including turbidimetry, dynamic, static, and electrophoretic light scattering, fluorescence spectroscopy, ultracentrifugation, size exclusion chromatography, and CD.

We have studied the interaction between strong

Received June 12, 1995; accepted August 16, 1995.

* Present address: Century International, 802 Harmon Ave., Columbus, OH 43223.
† To whom correspondence should be addressed.

Biopolymers, Vol. 38, 527–533 (1996)

CCC 0006-3525/96/040527-07

polyelectrolytes such as poly(dimethyldiallylammonium chloride) (PDMDAAC) and globular proteins such as ribonuclease (RNAse), bovine serum albumin (BSA), and lysozyme.[22-24,31-36] The magnitude of the interaction has been found to depend on the nature of the polyelectrolyte and the protein, as well as on the solution conditions (pH and ionic strength).[43] However, the mechanism of protein–polyelectrolyte binding is still not completely understood. Fundamental questions concerning the magnitude of the binding constant and the cooperativity of binding still remain to be answered. Related questions are as follows: Can free polyelectrolyte, free protein, and protein–polyelectrolyte complex coexist? How does the bulk mass ratio of protein to polymer (r) affect the binding mechanism? This paper attempts to address these questions through a combination of electrophoretic, dynamic, and static light scattering techniques. To facilitate the interpretation of the results, narrow distribution PDMDAAC fractions were used.

EXPERIMENTAL

Materials and Solution Preparation

A commercial sample of PDMDAAC, "Merquat 100" from Calgon corporation (Pittsburgh PA) with a nominal molecular weight of 200 K and polydispersity of $M_w/M_n \approx 10$, was fractionated via preparative gel permeable chromatography (GPC). The fractions were characterized by static and dynamic light scattering, viscosity, GPC, and electrophoretic light scattering.[47] Three fractions with weight average molecular weights (M_w) of 1200, 1250, and 1300 K were used. All three fractions had a polydispersity index of less than 1.2, and all were dialyzed and freeze dried before use. BSA (>98.5% pure) with pI = 4.9 was purchased from Boehringer Mannheim and used without further purification. The pH was adjusted with 0.1 M NaOH and 0.1 M HCl from Fisher, and the ionic strength was adjusted to 0.16 M with NaCl from Fisher. Milli-Q water was used throughout the study, and all samples were filtered with Whatman 0.45 μm filters.

Quasielastic Light Scattering

Quasielastic light scattering (QELS) measurements were carried out at 24 ± 1°C and at a 90° scattering angle using a Brookhaven Instruments system equipped with a 72-channel digital correlator (BI-2030AT) and an Omnichrome 200 mW argon laser operating at a vacuum wavelength (λ_o) of 488 nm. In the self-beating mode of dynamic light scattering, the measured photoelectron count autocorrelation function $G^{(2)}(\tau, q)$ for a detector with a finite effective photocathode area has the form[48]

$$G^{(2)}(\tau, q) = N_s\langle n\rangle^2(1 + b|g^{(1)}(\tau, q)|^2) \quad (1)$$

where $g^{(1)}(\tau, q)$ is the first-order scattered electric field (E_s) time correlation function, τ is the delay time, $\langle n\rangle$ is the mean counts per sample, N_s is the total number of samples, and b is a spatial coherence factor that depends upon the experimental setup and is taken as an unknown parameter in the data fitting procedure; q, the scattering vector, $= (4\pi n/\lambda_o)\sin(\theta/2)$, where n and θ are the refractive index of the scattering medium and the scattering angle, respectively. For a solution of polydisperse particles, $g^{(1)}(\tau, q)$ has the form

$$g^{(1)}(\tau, q)| = \int_0^\infty G(\Gamma, q)e^{-\Gamma(q)\tau}d\Gamma \quad (2)$$

where $G(\Gamma, q)$ is the normalized distribution of line width Γ measured at a fixed value of q. In the present study, a CONTIN algorithm was used to obtain the average Γ, denoted as Γ_{av} and its distribution of both the complex and the free micelle modes.[49,50] The apparent translational diffusion coefficient (D) is related to Γ_{av} by $D = \Gamma_{av}/q^2$. The apparent hydrodynamic radius R_h can be estimated via the Stokes–Einstein equation

$$D_o = k_BT/(6\pi\eta R_h) \quad (3)$$

where k_B is the Boltzman constant, T is the absolute temperature, and η is the solvent viscosity.

Static Light Scattering

Static light scattering measurements were performed at 24 ± 1°C and at a 90° scattering angle on the same solutions described above. Utilizing the Brookhaven Instrument with automatic rejection of dust events, 30 total intensity readings were collected at 2 − s intervals and averaged. Three of these averaged values were collected for each sample to yield a total population of 90 readings.

Electrophoretic Light Scattering

Electrophoretic light scattering (ELS) was carried out at 25°C and at four angles (8.6°, 17.1°, 25.6°, 34.2°) using a DELSA 440 instrument from Coulter Instrument Co. The electric field was applied in a constant current mode. The electrophoretic cell had a rectangular cross section connecting the hemispherical cavities in each electrode, and the total sample volume was about 1 mL. The measured electrophoretic mobility (U) was the average value at the stationary layer measured at the above angles. More details concerning electrophoretic light scattering can be found in Ref. 51.

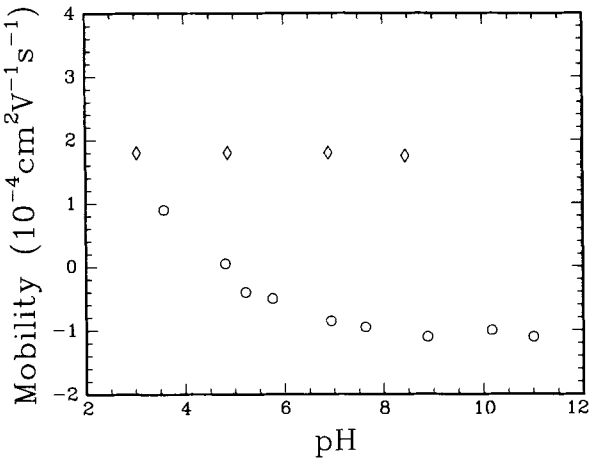

FIGURE 1 Electrophoretic mobility of BSA (○) and PDMDAAC (◇) in 0.16 *M* NaCl as a function of pH.

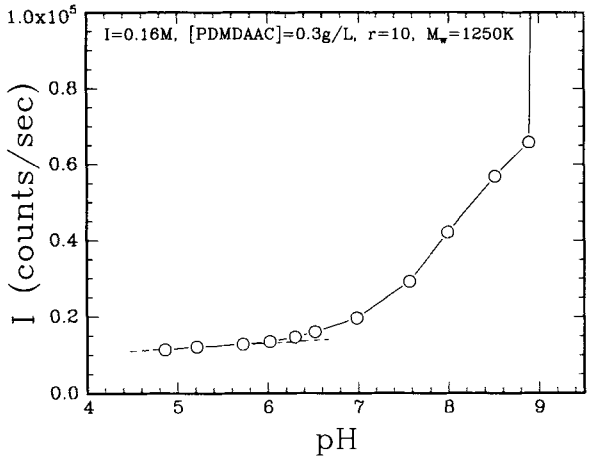

FIGURE 3 CONTIN distribution of BSA/PDMDAAC as a function of r. For clarity, only selected distributions are shown.

RESULTS AND DISCUSSION

Figure 1 shows the electrophoretic mobility of BSA and PDMDAAC as a function of pH. As expected, PDMDAAC shows no pH dependence, while BSA displays an increase in negative charge with increasing pH, the electrophoretic mobility changing from 0.9×10^{-4} to -1.1×10^{-4} cm^2 V^{-1} s^{-1} as the pH is increased from 3.5 to 11.0, with an isoelectric point at ca. pH 4.9. PDMDAAC and BSA have electrophoretic mobilities of 1.9×10^{-4} and -1.0×10^{-4} cm^2 V^{-1} s^{-1}, respectively, at pH 8.0 and 0.16 *M* NaCl, where most of the current studies were conducted. These conditions were chosen so as to ensure soluble complex formation. Figure 2 shows the total scattering intensity of BSA/PDMDAAC in 0.16 *M* NaCl as a function of pH. Above pH 6.0 (defined as pH_c), a significant increase in the scattering intensity was observed. This increase corresponds to the onset of complex formation between BSA and PDMDAAC. Above pH 8.9 (defined as pH_ϕ), macroscopic phase separation takes place and the system becomes turbid. pH_c and pH_ϕ represent the boundaries of the soluble complex region. Mattison et al.[52] showed that both pH_c and pH_ϕ were independent of PDMDAAC molecular weight, and that while pH_ϕ exhibited an inverse dependence on r, pH_c was independent of r. The dependence of scattering intensity on pH clearly increases as the pH increases, and one may discern discontinuities at pH 7.0 and 7.5. It is still not known whether these discontinuities represent regions of abrupt increase in the intrinsic binding constant or an increase in the extent of aggregation. In this study pH 8.0 was chosen to ensure that complex formation would be reasonably complete while still avoiding the more intense aggregation indicated in Figure 2 for pH values greater than 9.0.

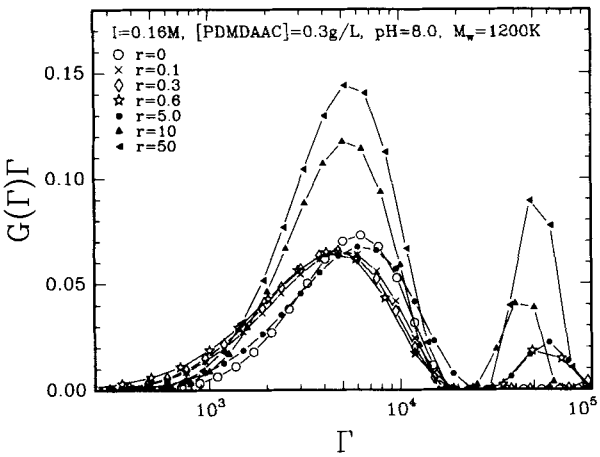

FIGURE 2 Total scattered intensity of BSA/PDMDAAC as a function of pH. Soluble BSA/PDMDAAC complexes can be observed at pH 6.0–8.9. Broken line indicates scattered intensity due to BSA alone.

Figure 3 shows the distribution of Γ as a function of r. At $r = 0$, the single peak corresponds to PDMDAAC with $R_h = 25$ nm. At $r = 0.6$, two peaks were observed: the high Γ peak ($R_h = 3.5$ nm) corresponds to free BSA, and the low Γ peak may correspond to both complex and free PDMDAAC (the coexistence of free and bound PDMDAAC will be discussed later). The scatter-

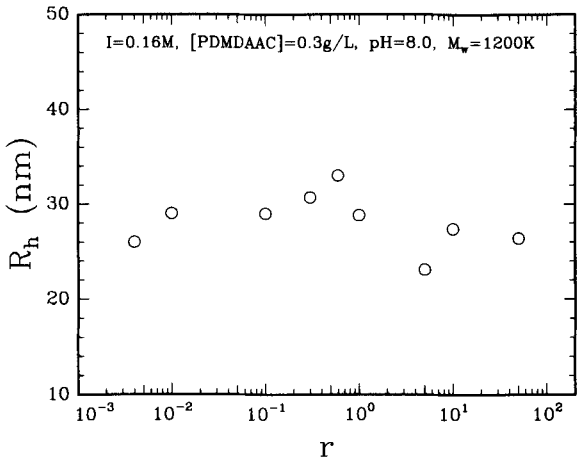

FIGURE 4 Average apparent hydrodynamic radius of BSA/PDMDAAC and free PDMDAAC as a function of r. The measured R_h values for PDMDAAC and BSA alone are 25 and 3.5 nm, respectively.

ing intensity of free PDMDAAC depends on its molecular weight and relative concentration, so that in mixtures of BSA and lower molecular weight PDMDAAC previously studied, only the fast mode for BSA diffusion was detected below pH 4.5, where complexation does not occur.[23] Under the current conditions with high molecular weight PDMDAAC, both BSA and PDMDAAC can be detected at pH < 4.5. Although the meaning of R_h for the low Γ peak is not entirely clear, it can at least be concluded that the size of the complex does not change significantly with increasing r. This observation is consistent with an intrapolymer complex in which the randomly coiled confirmation is retained. Since PDMDAAC and BSA are oppositely charged at this pH, complex formation represents partial charge neutralization. This charge neutralization makes the complex less hydrophilic than either free BSA or free polymer, resulting in a rather compact conformation for the complex. The ca. 25% decrease in R_h for the complex in the region r = 1–50 (Figure 4) is consistent with a partial collapse of the polyion chain with progressive protein binding. This behavior might be the most fundamental difference between protein polyelectrolyte complexes and proteins complexed with nonionic polymers. In the latter, complex formation represents charge accumulation, and the repulsion between the charged groups leads to conformational expansion.[33]

Figure 5 shows the electrophoretic mobility of the BSA/PDMDAAC system as a function of r. In contrast to QELS, the component that provides the weakest signal in ELS and hence is not detected is free protein, whereas both complex and free polymer are observed.[31,32] In this sense, QELS and ELS are complementary techniques. At $r \leq 5$, the system shows two electrophoretic mobilities. The value at ca. 1.9×10^{-4} cm^2 V^{-1} s^{-1} corresponds precisely to free PDMDAAC, and the lower value at ca. 1.4×10^{-4} cm^2 V^{-1} s^{-1} represents the soluble complex. Neither value shows an significant r dependence up to r = 5. These results suggest that for r values less than 10, the PDMDAAC molecules are either complexed with BSA or free, an indication of a cooperative binding mechanism. In this model, an increase in r gives rise to an increase in the number of complexes and a simultaneous decrease in the number of free PDMDAAC molecules. The degree of binding β, defined as the molecular mass ratio of BSA to PDMDAAC in the complex, would therefore be constant. Using sedimentation, a similar nonuniform distribution of BSA among polyelectrolyte "hosts" was observed earlier by Kabanov et al.[9] Cooperative binding has also been observed in complexes of native calf thymus DNA with cationic polypeptides such as poly-L-ornithine, poly-L-lysine, poly-L-arginine, and poly-L-homoarginine.[53] It is also interesting to note that, on the basis of the results in Figures 3 and 5, free polymer and free protein coexist in the range $0.5 < r < 5$. This observation indicates that the binding constant is not very large at the pH and ionic strength conditions employed here.

When r reaches 10 in Figure 5, the free PDMDAAC signal disappears, and the mobility of the complex begins to decrease with increasing r.

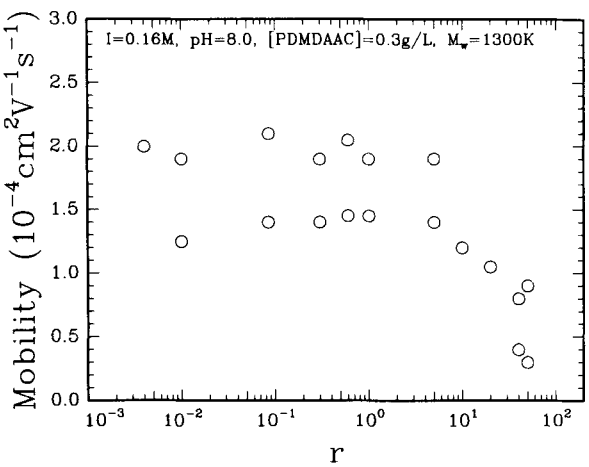

FIGURE 5 Mobility of BSA/PDMDAAC as a function of r. The mobility of free BSA in these solutions in not detectable. Two mobilities are observed at $r <$ s.

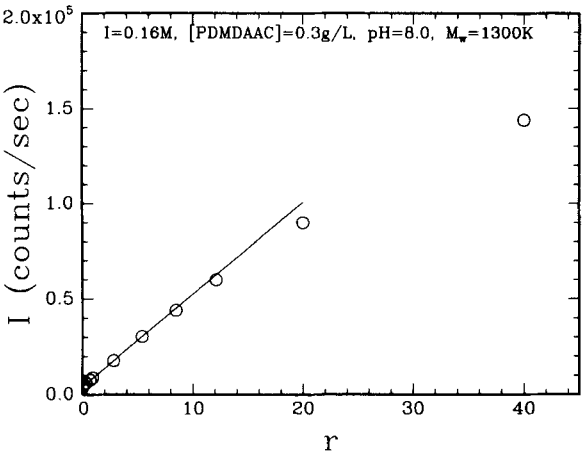

FIGURE 6 Total scattered intensity of BSA/PDMDAAC as a function of r.

This decrease implies a secondary binding process, driven by mass-action effects, wherein the degree of binding (β) is a function of r. The incomplete binding at low r may arise from the excluded volume of the bound BSA molecules. However, a subsequent increase in β takes place only after the free PDMDAAC concentration becomes very low and a large amount of free BSA exists in the solution (see Figure 3, $r > 5$).

Figure 6 shows the total scattering intensity as a function of r. Similar to the results of the previous figures, the total scattering intensity indicates a transition between $r = 5$ and 10. Below $r = 10$, a linear relationship between the total intensity and r is observed. Above $r = 10$, however, the total intensity begins to level off. The linearity of $I(r)$ is consistent with the results of Figure 5, if we assume that the structure of the complex is invariant, and only number of complexes (n_x) changes with r. If n_x is linear with r, then $I(r)$ may be linear, also (see below).

If it is assumed that the angular dependence and interparticle interactions are insignificant, the total scattering intensity (I) from a solution of complex, free PDMDAAC, and free BSA, after a blank correction for the 0.16 M NaCl, can be expressed as[48]

$$I = K_x C_x M_{w,x} + K_p C_{p,f} M_{w,p} + K_{pr} C_{pr,f} M_{w,pr} \quad (4)$$

where K_x, K_p, and K_{pr} are the contrast factors for complex, PDMDAAC, and BSA; C_x, $C_{p,f}$, and $C_{pr,f}$ are the mass concentrations of complex, PDMDAAC, and BSA; and $M_{w,x}$, $M_{w,p}$, and $M_{w,pr}$ are the weight average molar masses of complex, PDMDAAC, and BSA. In a solution where intrapolymer complex predominates, if we define $\beta = C_{pr,B}/C_{p,B}$, and $r = C_{pr,T}/C_{p,T}$, where $C_{pr,B}$, $C_{p,B}$, $C_{pr,T}$, and $C_{p,T}$ are the mass concentrations of bound BSA, bound PDMDAAC, total BSA, and total PDMDAAC, Eq. (4) can be rewritten as follows:

$$\begin{aligned} I = [&K_x(1+\beta)^2 M_{w,p} \\ &- K_p M_{w,p} - K_{pr}\beta M_{w,pr}] C_{p,B} \\ &+ [K_p C_{p,T} M_{w,p} + (K_{pr} C_{p,T} M_{w,pr}) r] \end{aligned} \quad (5)$$

In Eq. (5), the second term gives rise to a straight line for I vs r with a slope of $K_{pr}C_{p,T}M_{w,pr}$. The variables in the first term are β and $C_{p,B}$. Because of the constant mobility, it can be assumed that β remains essentially constant for r values less than 10. The requirement for linearity of I vs r is then that $C_{p,B} \propto r$, i.e., $\propto C_{pr,T}$ (since $C_{p,T}$ is nearly constant). This means that each incremental addition of protein produces a proportional incremental increase in the mass of complex; and as long as the structure of the complex is constant (constant β), then $C_{p,B}$ will increase proportionally as well. This progressive increase in complex concentration, without change in complex structure, with increasing added protein is consistent with cooperative binding.

Some speculations can be made regarding the inferred cooperative binding mechanism. Proteins may have localized hydrophobic regions on their surface, and BSA is known to be particularly hydrophobic.[54-58] When BSA binds to an oppositely charged polyelectrolyte, charge neutralization makes the bound protein more hydrophobic. Consequently, hydrophobic interactions between adjacent bound proteins can be a driving force for the cooperative behavior observed in this study. Although the present results do not provide any direct information on the local structure of protein–polyelectrolyte complexes or the orientation of BSA therein, given the highly asymmetric geometry of this protein (axial ratio ca. 2.5), and the highly hydrophobic fatty acid binding site, some speculations concerning complex structure can be advanced. In the low r binding region ($r < 5$), the orientation of proteins with respect to polymer is largely random, and the complex retains a large measure of orientational entropy. Nevertheless, the domain within the complex is hydrophobic relative to the bulk solvent, and preferential partitioning of free BSA into this domain produces the appearance of cooperative binding. At higher r values (no free polymer), an increase in the number of bound proteins per polymer chain may be sur-

mised from the decrease in mobility, but the size of the complex, according to the CONTIN plots of Figure 3, remains relatively unchanged. This constant size implies that denser structures are formed at $r > 10$. These may encompass bound proteins in sufficient proximity such that their orientation with respect to each other is no longer random, corresponding to the "secondary binding" process alluded to above.

CONCLUSION

Electrophoretic, dynamic, and static light scattering suggest that the mechanism of binding BSA to PDMDAAC depends upon the concentration ratio of BSA to PDMDAAC (r). At low $r(<10)$, the binding of BSA to PDMDAAC is cooperative, in that the BSA molecules are inhomogeneously distributed among the PDMDAAC molecules, and free PDMDAAC molecules exist. At high $r(>10)$, the amount of free PDMDAAC is too small to detect, but large amounts of free protein are observed. In the presence of free protein, a secondary binding process is inferred. Hydrophobic interactions between the bound and free BSA are proposed to be the driving force for the cooperative binding.

The support of grant DMR9311433 from the National Science Foundation, jointly funded by the Divisions of Materials Research and Chemical Transport Systems, is gratefully acknowledged.

REFERENCES

1. Morawetz, H. & Hughes, W. L., Jr. (1952) *J. Phys. Chem.* **55,** 64.
2. Berdick, M. & Morawetz, H. (1954) *J. Biol. Chem.* **206,** 959.
3. Morawetz, H. & Sage, H. (1955) *Arch. Biochem. Biophys.* **56,** 103.
4. Samsonov, G. V., Ponomareva, R. B. & Luchko, R. G. (1969) *Biofizika* **14,** 634.
5. Sternberg, M. & Hershberger, D. (1974) *Biochim. Biophys. Acta* **342,** 195.
6. Hill, R. D. & Zadow, J. G. (1974) *J. Dairy Res.* **41,** 373.
7. Jendrisak, J. J. & Burgess, R. R. (1975) *Biochemistry* **14,** 4634.
8. Jendrisak, J. J. & Burgess, R. R. (1975) *Biochemistry* **14,** 4639.
9. Kabanov, V. A., Evdakov, V. P., Mustafaev, M. I. & Antipina, A. D. (1977) *Molekul. Biol.* **11,** 582–597.
10. Kabanov, V. A., Mustafaev, M. I., Belova, V. & Evdakov, V. P. (1978) *Molekul. Biol.* **12,** 1264–1277.
11. Kokufuta, E., Shimizu, H. & Nakamura, I. (1981) *Macromolecules* **14,** 1178.
12. Kuramoto, N., Sakamoto, M., Komiyama, J. & Iijima, T. (1982) *Int. J. Biol. Macromol.* **4,** 207.
13. Kuramoto, N., Sakamoto, M., Komiyama, J. & Iijima, T. (1984) *Int. J. Biol. Macromol.* **6,** 69.
14. Nguyen, T. Q. (1986) *Makromol. Chem.* **187,** 2567.
15. Shatayeva, L. K., Radzyavichyus, K. I. & Samsonov, G. V. (1985) *Polym. Sci. USSR* **27,** 782.
16. Barberousse, V., Sacco, D. & Dellacherie, E. (1986) *J. Chromatogr.* **369,** 244.
17. Anufrieva, Y. V., Pautov, V. D., Kuznetsova, N. P., Lushchik, V. B. & Krakovyak, M. K. (1987) *Polym. Sci. USSR* **29,** 663.
18. Clark, K. M. & Glatz, C. E. (1987) *Biotechnol. Prog.* **3,** 241.
19. Sacco, D., Bonneaux, F. & Dellacherie, E. (1988) *Int. J. Biol. Macromol.* **10,** 305.
20. Fisher, R. R. & Glatz, C. E. (1988) *Biotechnol. Bioeng.* **32,** 777.
21. Fisher, R. R. & Glatz, C. E. (1988) *Biotechnol. Bioeng.* **32,** 786.
22. Dubin, P. L., Ross, T. D., Sharma, I. & Yegerlehner, B. E. (1977) in *Ordered Media in Chemical Separations,* Hinz, Armstrong, D. W., Ed., American Chemical Society, Washington, DC.
23. Dubin, P. L. & Murrel, J. M. (1988) *Macromolecules* **21,** 2291.
24. Dubin, P. L., Strege, M. A. & West, J. (1990) in *Large Scale Protein Purification,* Ladish, M., Ed., American Chemical Society, Washington, DC, chap. 5.
25. Kokufuta, E. & Takahashi, K. (1990) *Polymer* **31,** 1177.
26. Dellacherie, E. (1991) *Polym. Prepr.* **32,** 602.
27. Shieh, J. & Glatz, C. E. (1991) *Polym. Prepr.* **32,** 606.
28. Bozzano, A. G., Andrea, G. & Glatz, C. E. (1991) *J. Membr. Sci.* **55,** 181.
29. Zaitsev, V. S., Izumrudov, V. A. & Zezin, A. B. (1992) *Polym. Sci. USSR* **34,** 54.
30. Chen, W., Walker, S. & Berg, J. C. (1992) *Chem. Eng. Sci.* **47,** 1039.
31. Park, J. M., Muhoberac, B. B., Dubin, P. L. & Xia, J. (1992) *Macromolecules* **25,** 290.
32. Xia, J., Dubin, P., Kim, Y., Muhoberac, B. & Klimkowski, V. (1993) *J. Phys. Chem.* **97,** 4528.
33. Xia, J., Dubin, P. L. & Kokufuta, E. (1993) *Macromolecules* **26,** 6688.
34. Xia, J., Dubin, P. L. & Dautzenberg, H. (1993) *Langmuir* **9,** 2015.
35. Ahmed, L., Xia, J., Dubin, P. & Kokufuta, E. (1994) *J. Macromol. Sci. Pure Appl. Chem.* **31.**
36. Xia, J., Morishima, Y., Sato, T., Dubin, P. L. & Muhoberac, B. B. (1995) *Biopolymers* **35,** 411.
37. Niederauer, M. Q. & Glatz, C. E. (1994) *J. Macromol. Sci. Pure Appl. Chem.* **31,** 127.

38. Izumi, T., Hirata, M., Kokufuta, E., Cha, H. J. & Frank, C. W. (1994) *J. Macromol. Sci. Pure Appl. Chem.* **31,** 31.
39. Izumi, T., Hirata, M., Takahashi, K. & Kokufuta, E. (1994) *J. Macromol. Sci. Pure Appl. Chem.* **31,** 39.
40. Teramoto, A., Watanabe, M. & Iizuka, K. (1994) *J. Macromol. Sci. Pure Appl. Chem.* **31,** 53.
41. Shimabayashi, S., Hirao, K., Bando, J. & Shinohara, C. (1994) *J. Macromol. Sci. Pure Appl. Chem.* **31,** 65.
42. Dubin, P. L., Gao, J. & Mattison, K. (1994) *Sep. Purif. Methods* **23,** 1.
43. Xia, J. & Dubin, P. L. (1994) in *Macromolecular Complexes in Chemistry and Biology,* Dubin, P., Davis, R. M., Schultz, D. & Thies, C., Ed., Springer-Verlag, Berlin, chap. 15.
44. Margolin, A., Sherstyuk, S. F., Izumrudov, V. A., Zezin, A. B. & Kabanov, V. A. (1985) *Eur. J. Biochem.* **146,** 625.
45. Record, M., Jr., Anderson, C. F. & Lohman, T. M. (1978) *Quart. Rev. Biophys. II* **2,** 103.
46. von Hippel, P. H., Bear, D. G., Morgan, W. D. & McSwiggen, J. A. (1984) *Ann. Rev. Biochem.* **53,** 389.
47. Xia, J., Dubin, P. L., Edwards, S. L. & Havel, H. A. (1995) *J. Polym. Sci. Polym. Phys.* **33,** 1117.
48. Chu, B. (1991) *Laser Light Scattering,* Academic Press: New York.
49. Provencher, S. W. (1976) *J. Chem. Phys.* **64,** 2772.
50. Provencher, S. W. (1979) *Makromol. Chem.* **180,** 201.
51. Ware, B. & Haas, D. D. (1983) in *Fast Methods in Physical Biochemistry and Cell Biology,* Sha'afi, R. I., Fernandez, S. M., Eds., Elsevier: Amsterdam, chap. 8.
52. Mattison, K. W., Brittain, I. B. & Dubin, P. L. (1995) *Biotechnol. Progr.,* **11,** 632.
53. Olins, D. E., Olins, A. L. & von Hippel, P. H. (1967) *J. Mol. Biol.* **24,** 157.
54. Cantor, C. R. & Schimmel, P. R. (1980) *Biophysical Chemistry,* Part I; W. H. Freeman, New York, chap. 2.
55. Feng, L., Hu, C. Z. & Andrade, J. D. (1988) *J. Colloid Interface Sci.* **126,** 650.
56. Nozaki, Y. & Tanford, C. (1971) *J. Biol. Chem.* **7,** 2211.
57. Bigelow, C. (1967) *J. Theoret. Biol.* **16,** 187.
58. Cardamone, M. & Puri, N. K. (1992) *Biochem. J.* **282,** 589.