ELSEVIER

Journal of Chromatography A, 662 (1994) 414–419

JOURNAL OF CHROMATOGRAPHY A

Short Communication

Competitive adsorption of α-lactalbumin and bovine serum albumin to a sulfopropyl ion-exchange membrane

Wendy F. Weinbrenner[a], Mark R. Etzel*[,b]

[a]Department of Chemical Engineering, 1415 Johnson Drive, University of Wisconsin, Madison, WI 53706-1619, USA
[b]Department of Food Science, 1605 Linden Drive, University of Wisconsin, Madison, WI 53706-1519, USA

(First received September 22nd, 1993; revised manuscript received November 23rd, 1993)

Abstract

Breakthrough curves were measured for pure solutions of α-lactalbumin (ALA) and bovine serum albumin (BSA) individually, and for a binary mixture of the proteins, using a sulfopropyl ion-exchange membrane. The breakthrough curves were qualitatively consistent with local-equilibrium theory predictions. Competitive adsorption caused displacement of bound BSA monomer by the more strongly binding BSA dimer, illustrating that even apparently single-protein systems may display multicomponent competitive behavior. In the two-protein experiment, ALA was competitively displaced by the more strongly binding BSA monomer and dimer, indicating that the binding strength was in the order: BSA dimer > BSA monomer > ALA.

1. Introduction

Conventional ion-exchange protein separation protocols utilize a packed column containing porous beads onto which the ligand is immobilized. Increasing the rate at which equilibrium between the protein solution and ion-exchange adsorbent is approached is of great practical importance because it maximizes the throughput of the process. In packed columns, the separation rate is typically limited by slow intra-bead diffusion for large beads, or low axial velocities and high column pressure drops for small beads. The rate at which the protein binds to the ion-exchange site is usually fast enough not to limit the overall rate. Only under ideal conditions do packed columns operate at local equilibrium and are separations based solely on differences in the equilibrium sorption isotherms [1–3].

Ion-exchange membranes are a promising new bioseparation technology designed to overcome the limitations of conventional packed columns. In ion-exchange membranes, pressure drop limitations are negligible because the membranes are thin, whereas intra-bead diffusional limitations are negligible because the feed solution flows by convection through the fine pores (pore size ≈ 1 μm) of the membrane. Consequently, properly designed and operated ion-exchange membranes may eliminate the mass-transfer and flow limitations associated with packed columns, and may approach local-equilibrium behavior.

Competitive ion-exchange sorption has been analyzed extensively, both theoretically and ex-

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1260-7

perimentally, for packed columns [4–8], but not for ion-exchange membranes. But, packed columns cannot be used to cleanly test the assumptions of local-equilibrium theory for protein adsorption because of complications arising from slow and restricted intra-bead diffusion.

In this work, predictions from local-equilibrium theory will be compared qualitatively to experimental data from single-protein and binary-protein ion-exchange membrane separations, and used to determine the extent to which ion-exchange membranes approach local-equilibrium behavior.

2. Experimental

2.1. Materials

Bovine serum albumin (BSA) (A 0281) and α-lactalbumin (ALA) (L 6010) were obtained from Sigma (St. Louis, MO, USA) and used without further purification. The sulfopropyl (SP) MemSep 1010 ion-exchange membrane chromatography cartridge (CISP 15H 01) was supplied by Millipore (Bedford, MA, USA). It contained a stack of 72 regenerated cellulose membranes, with a 1.2 μm pore size [9,10]. The stack was 1 cm high, 4.9 ml in volume, and the porosity was 85%. The ion-exchange capacity was 2.3 meq per cartridge [10]. Thin polypropylene gasket rings were inserted by Millipore between every three or four membranes in the stack to avoid lateral flow towards the walls of the housing. The membrane stack was then compressed by Millipore to seal the gaskets, and to eliminate gaps between the individual membranes. Millipore placed a non-woven polypropylene disc filter on top of the first membrane in the stack to evenly distribute the flow [10].

The loading/washing buffer was 0.1 *M* NaOAc, adjusted to pH 3.0 with HCl, the elution buffer was 0.375 *M* Tris, pH 8.8, and both buffers contained 0.05% NaN_3. All solutions were vacuum filtered and degassed before use.

2.2. Methods

Standard procedures

The cartridge was cleaned and regenerated prior to each use by pumping in sequence 25 ml each of 0.1 *M* NaOH, loading buffer, and 0.1 *M* HCl through the membrane. It was then washed and equilibrated to pH 3.0 by pumping 50 ml of loading buffer through the membrane. A UV detector (Model 111 with a 10 μl volume, 2 mm pathlength flow cell, Gilson, Middleton, WI, USA) measured the absorbance of the stream exiting the membrane. A fraction collector (Retriever II, Isco, Lincoln, NE, USA) collected 1–2 ml fractions. All experiments were performed at 4.0 ml/min.

Measurement of system dispersion

Dispersion was determined from the breakthrough curve for 1.0 mg/ml BSA in elution buffer. The cartridge was washed with 50 ml of elution buffer, then 35 ml of the protein solution was loaded, followed by washing with elution buffer.

Breakthrough curve measurement

An aliquot of 150 ml of the two-protein solution, containing 1.0 mg/ml each of BSA and ALA in loading buffer, and 360 ml of each single-protein solution, containing 1.0 mg/ml of either BSA or ALA in loading buffer, was loaded. Loading buffer was then pumped through the system to wash unadsorbed protein from the membrane system. The adsorbed protein was eluted from the membrane until the absorbance returned to baseline.

Determination of protein concentration

Excluding the ALA single-protein experiment, fractions were analyzed by HPLC (Model 2350 pump and Model V^4 detector, Isco, Lincoln, NE, USA) at a wavelength of 280 nm. A 50-μl injection loop was used with a mobile phase buffer consisting of 0.2 *M* Na_2HPO_4 pH 7.0 at 2.0 ml/min through a gel filtration column (ZORBAX GF-250, E.I. du Pont de Nemours, Wilmington, DE, USA). Peaks were integrated

by a computer-based system, calibrated using pure samples of each protein in solutions of known concentration.

In experiments involving only one protein, a ratio of the UV detector voltage due to the feed solution, without the membrane in the system, to the concentration of the feed solution was used to convert all voltages to concentration. The concentration of the feed solution was calculated from the absorbance at 280 nm. The extinction coefficient of ALA was 1.78 ml mg^{-1} cm^{-1}, and of BSA was 0.6 ml mg^{-1} cm^{-1} at pH 3.0 and 20°C.

3. Results

3.1. System dispersion

In the dispersion experiment, BSA emerged in the effluent after 5.2 ml were loaded. This is the system dead volume, which includes the membrane void volume of 4.2 ml. Next, an additional 2.8 ml was loaded during which the effluent concentration rose to the feed concentration. If there were no dispersion in the system, the effluent concentration would have instantly risen to the feed concentration. An aliquot of 4.4 ml was required during washing for the effluent concentration to return to zero.

3.2. BSA breakthrough curve

Fig. 1 contains the breakthrough curve for 1.0 mg/ml BSA in loading buffer. The effluent concentration rose quickly after an effluent volume of 70 ml. Later, the breakthrough curve was very broad and asymmetric. After an effluent volume of 350 ml, the effluent concentration of BSA rose to only 96% of the feed solution concentration. Based on the area above the breakthrough curve, 102 mg of BSA were removed from the feed solution.

The compositions of the effluent fractions were determined by gel filtration HPLC and are plotted in Fig. 2. The feed solutions were essentially binary solute solutions because approximately 20% of the BSA existed as a dimer, and 80% existed as a monomer. After an effluent volume of 70 ml, BSA monomer only emerged from the membrane. Then, the BSA monomer concentration in the effluent rose to 9% above the feed solution concentration of BSA monomer. After an effluent volume of 140 ml, BSA dimer emerged in the effluent, and the BSA monomer concentration in the effluent decreased to the concentration of BSA monomer in the feed solution, and remained there. As loading continued, the BSA dimer concentration in the effluent continued to rise slowly, but did not

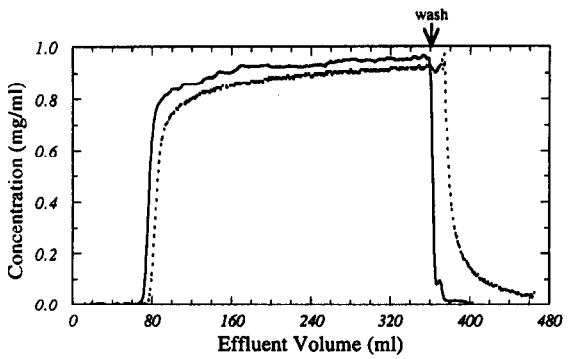

Fig. 1. Single-protein breakthrough curves from the UV detector for ALA (dashed line) and BSA (solid line). The feed solution contained 1 mg/ml of either ALA or BSA in 0.1 *M* NaOAc pH 3 buffer. The flow-rate was 4 ml/min, and 360 ml of each protein solution were loaded onto the membrane.

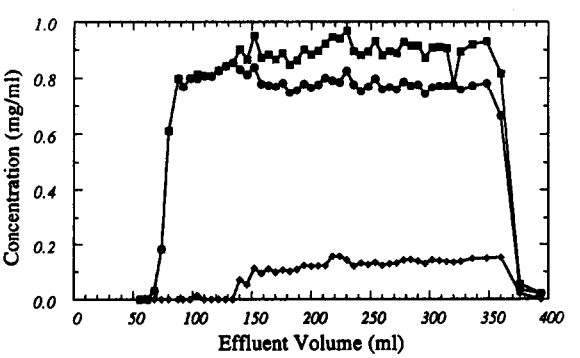

Fig. 2. BSA breakthrough curves for monomer (●), dimer (◆) and total (■). The total curve is the sum of the monomer and dimer curves. Fractions were collected from the BSA experiment of Fig. 1 and analyzed by HPLC.

reach the concentration of BSA dimer in the feed solution.

3.3. ALA breakthrough curve

Fig. 1 contains the breakthrough curve for a pure solution of ALA in loading buffer. The effluent concentration rose quickly after an effluent volume of 75 ml. Later, the breakthrough curve was very broad and asymmetric. After an effluent volume of 350 ml, the effluent concentration rose to only 92% of the feed concentration. Based on the area above the breakthrough curve, 94 mg of ALA were removed from the feed solution.

3.4. Two-protein breakthrough curve

Fig. 3 contains the breakthrough curve for a feed solution containing 1.0 mg/ml each of BSA and ALA in loading buffer. ALA emerged in the effluent after an effluent volume of 42 ml. For an effluent volume from 42 to 52 ml, the effluent contained pure ALA. The ALA concentration in the effluent peaked at 145% of the feed concentration of ALA at an effluent volume of 52 ml. After this point, BSA monomer emerged in the effluent, and the ALA concentration decreased to that in the feed solution. At an effluent volume of 120 ml, the BSA monomer concentration in the effluent peaked at 107% of the feed concentration of BSA monomer. Then it quickly decreased to an asymptote equal to the feed concentration of BSA monomer. At this point, BSA dimer emerged in the effluent. However, the feed solution concentration of BSA dimer was not reached. By subtracting the amount of protein in the effluent from that which was loaded, it was estimated that the membrane bound 38 mg of ALA, 43 mg of BSA monomer, and 24 mg of BSA dimer.

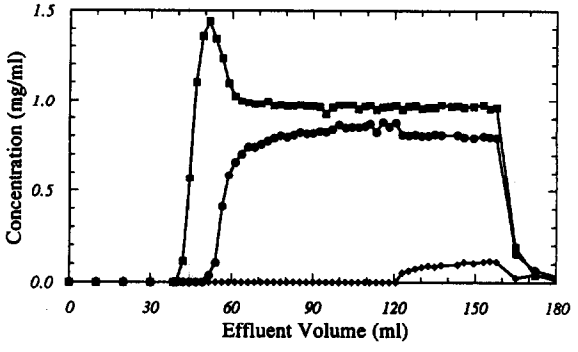

Fig. 3. Breakthrough curves for a mixture of BSA monomer (●), BSA dimer (◆) and ALA (■). The feed solution contained 1 mg/ml each of ALA and BSA in 0.1 *M* NaOAc pH 3 buffer. The flow-rate was 4 ml/min, and 150 ml of each protein solution were loaded onto the membrane. Fractions were analyzed by HPLC.

4. Discussion

The experimental results from this work will be qualitatively compared to the predictions of local-equilibrium theory, in which separation dynamics are described by purely thermodynamic factors [8,11–14]. In local-equilibrium theory, the mobile phase and the stationary phase are assumed to be in equilibrium at any point. For a single step increase in influent composition for a multiprotein feed solution containing *i* solutes, the predicted breakthrough curves contain *i* composition plateaus [11–13,15]. Initially, all the proteins are completely removed from the feed solution, and the effluent is free of protein. Then, the first plateau emerges in the effluent and contains only the lowest-affinity protein at a concentration usually greater than the feed concentration. Subsequent plateaus emerge in the effluent and contain the previous proteins plus the next lowest-affinity protein. Each transition between plateaus is sharp and vertical. The last plateau emerges in the effluent after saturation of the membrane, and has the same composition as the feed solution.

4.1. Multicomponent adsorption behavior in single-protein systems

If local-equilibrium theory for single solutes is applicable, then a single sharp transition to the feed concentration should occur in the breakthrough curve. However, during loading of a pure solution of BSA, as plotted in Fig. 2, BSA monomer and dimer appeared to compete for

adsorption sites, with BSA dimer displacing the more weakly bound BSA monomer. This displacement probably caused the peak in the BSA monomer concentration. Then, after an effluent volume of 140 ml, BSA dimer emerged in the effluent and BSA monomer displacement gradually ceased. Without displacement, the BSA monomer concentration in the effluent returned to the feed concentration.

The coincidence of BSA dimer emergence in the effluent, and simultaneously the BSA monomer concentration in the effluent returning to the feed concentration is characteristic of multicomponent local-equilibrium behavior. Evidently, even apparently single-protein systems may display multicomponent behavior.

The slow approach to the inlet concentration in the BSA breakthrough curve has been reported for sulfopropyl gel-bead packed columns at pH 5.0 [5]. It was attributed to slow dimer formation between adsorbed and free BSA. The slow formation of dimers on the surface should result in removal of monomer from solution, leading to a slow approach of the monomer concentration to that in the inlet. This did not occur in Fig. 2. Instead, there was a slow approach of the dimer concentration to that in the inlet. This probably was caused by the slow continued removal of BSA dimer from solution. BSA dimers do not form in solution in the absence of Cu(II) at pH 3.0 [16], which may explain the difference between these results and those for the packed column at pH 5.0.

4.2. Multicomponent adsorption and competition in two-protein systems

In the two-protein breakthrough curve of Fig. 3, all proteins were removed from the feed solution for the first 42 ml of effluent volume. Then bound ALA, the weakest binding protein, was displaced from the membrane by BSA monomer and dimer. This caused the ALA concentration in the effluent to peak at 145% of the feed concentration of ALA. Simultaneous to the peak in the ALA concentration, BSA monomer emerged in the effluent. The BSA monomer concentration peaked when BSA dimer emerged in the effluent. The peak in the BSA monomer concentration in the effluent probably resulted from displacement of bound BSA monomer from the membrane by the more strongly binding BSA dimer. These results are in good agreement with multicomponent local-equilibrium theory. Based on the order of emergence in the effluent, the binding strength to the SP ion-exchange membrane was in the order: BSA dimer > BSA monomer > ALA.

In summary, based on the results of this work, ion-exchange membranes qualitatively display local-equilibrium behavior. Thus, ion-exchange membranes overcome many of the limitations of conventional packed columns. Because of the relative absence of non-equilibrium effects, ion-exchange membranes are a useful tool for examining competitive protein sorption behavior. Based on the data, even apparently single-proteins systems may display multicomponent competitive behavior.

5. Acknowledgements

Funding for this work was provided by the Wisconsin Center for Dairy Research, and the Wisconsin Milk Marketing Board. Shing-Yi Suen supplied important technical advice while reviewing the manuscript.

6. References

[1] C.K. Lee, Q. Yu, S.U. Kim and N.-H.L. Wang, *J. Chromatogr.*, 484 (1989) 29–59.
[2] F.D. Antia and C. Horváth, in C.A. Costa and J.S. Cabral (Editors), *Chromatographic and Membrane Processes in Biotechnology*, Kluwer, Boston, 1991, pp. 115–136.
[3] C.A. Costa, in C.A. Costa and J.S. Cabral (Editors), *Chromatographic and Membrane Processes in Biotechnology*, Kluwer, Boston, 1991, pp. 3–24.
[4] G.L. Skidmore and H.A. Chase, *J. Chromatogr.*, 505 (1990) 329–347.
[5] G.L. Skidmore, B.J. Horstmann and H.A. Chase, *J. Chromatogr.*, 498 (1990) 113–128.
[6] A. Velayudhan and C. Horváth, *J. Chromatogr.*, 443 (1988) 13–29.

[7] R.D. Whitley, R. Wachter, F. Liu and N.-H.L. Wang, *J. Chromatogr.*, 465 (1989) 137–156.
[8] Q. Yu and N.-H.L. Wang, *Sep. Purif. Methods*, 15 (1986) 127–158.
[9] M.S. Le and J.A. Sanderson, *US Pat.*, 4 895 806 (1990).
[10] *SP MemSep ion exchange membrane chromatography cartridge: operating instructions, Cat. No. MSGF 147*, Millipore, Bedford, MA, 1991.
[11] E. Glückauf, *Proc. R. Soc. London A*, 186 (1946) 35–57.
[12] F. Helfferich and G. Klein, *Multicomponent Chromatography. Theory of Interference*, Marcel Dekker, New York, 1970.
[13] H.-K. Rhee, R. Aris and N.R. Amundson, *Philos. Trans. R. Soc. London A*, 267 (1970) 419–455.
[14] D.D. Frey, *Chem. Eng. Sci.*, 45 (1990) 131–142.
[15] S.-Y. Suen, M. Caracotsios and M.R. Etzel, *Chem. Eng. Sci.*, 48 (1993) 1801–1812.
[16] J.F. Foster, in V.M. Rosenoer, M. Oratz and M.A. Rothschild (Editors), *Albumin Structure, Function and Uses*, Pergamon Press, Oxford, 1977, p. 59.