163f Protein Adsorption Kinetics Measurements with Radioactive Tracers

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The determination of the rates and mechanisms of protein mass transfer in adsorbent particles is important as mass transfer is typically the determining factor in column efficiency, especially at the process scale. Considerable advances in our understanding of protein adsorption kinetics have been made in recent years by means of microscopy techniques, which allow a visualization of protein concentration profiles in the adsorbent using either colored or fuorescently labeled proteins. However, a complementary technique is based on the use of radioactive tracers. With such tracers measurements can be conducted with optically opaque particles and mass transfer rates can be determined directly. Moreover mass transfer kinetics can be determined readily under both gradient and tracer diffusion conditions. In this work we have used I125 labeled lysozyme and ?-chymotrypsinogen to determine the adsorption kinetics in three different cation exchangers: SP-Sepharose-FF, SP-Sepharose-XL, and S-HyperD. The radiolabeled proteins were found to be stable and indistinguishable from the unlabeled proteins in high-resolution gradient elution cation exchange chromatography. Measurements of adsorption kinetics were conducted with a shallow-bed placed directly in the lead-shielded chamber of a radioactivity detector. With this arrangement protein uptake and desorption curves are obtained directly at a constant protein concentration by following the bed radioactivity as a function of time. Very different mass transfer rates were obtained for the three different adsorbents considered in this work. Moreover there was a profound difference in the dependence of the rate on salt concentration. A comparison of gradient and tracer diffusion kinetics is especially interesting. In gradient diffusion experiments, initially clean particles are exposed to a protein solution containing the radioactive tracer. Conversely, in tracer diffusion experiments, particles initially saturated with the unlabeled protein are exposed to a protein solution containing the radioactive tracer. In the first case mass transfer is potentially affected by electrokinetic contributions, while in the second case, mass transfer should be determined by diffusion alone. Further, comparison of these measurements can provide clues regarding the diffusional mobility of these molecules in the adsorbed phase. At low ionic strengths we observed large differences between the gradient diffusion and the tracer diffusion kinetics for all three media. However, at higher ionic strengths this difference became less pronounced and disappeared altogether for SP-Sepharose-FF and SP-Sepharose-XL. The difference remained however quite pronounced for S-HyperD suggesting significant differences in the transport mechanism. Even more interesting results were obtained by comparing tracer diffusion kinetics in the opposite direction; i.e. by exposing particles presaturated with protein and radiolabeled tracer to a solution containing the unlabeled protein alone. An interpretation of these results in terms of pore size and charge of the media, and of diffusion models will be presented providing insight in the mechanism of protein transport in these high-capacity cation exchangers.