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Note

The adsorption of proteins to pharmaceutical container surfaces

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Summary

The adsorption of a variety of proteins to different pharmaceutical container surfaces was investigated. No correlation was found between the amount adsorbed and molecular mass or isoelectric point, although glass surfaces appeared to bind more protein under the experimental conditions examined.

The interaction of proteins with the surfaces of their storage containers is a potentially significant problem in biotechnology. The amphipathic nature of protein molecules results in their adsorption to a wide variety of surfaces and can result in both their loss and destabilization (Felgner and Wilson, 1976; Andrade, 1985; Stella, 1986; Van der Oetelaar et al., 1989; Wu and Chen, 1989). This problem can be acute at low protein concentration where a substantial portion of what is usually assumed to be solution state protein may actually be adsorbed to container walls. We therefore examined the amount of surface adsorption of a number of proteins ranging in molecular mass from 6.5 to 670 kDa and isoelectric point (p*I*) from 4.3 to 10.5 to several commonly used container surfaces.

Protein solutions containing 6.2 mM sodium phosphate, 0.15 M NaCl, pH 7.2, were stored at concentrations of 1, 5, 10, and 20 μg/ml for 24 h at 4°C in 15-ml 2-cm diameter cylindrical vials. All proteins were obtained from Sigma Chemical Co., except for acidic fibroblast growth factor and transforming growth factor α-*Pseudomonas* exotoxin conjugate which were obtained from Merck and Co. (West Point, PA). The two latter proteins are highly homogeneous as determined from previous isolation procedures (Heimbrook et al., 1990; Volkin et al., 1992). The glass vials are either untreated, siliconed, sulfur-treated or Purcoat®-treated. The plastic vials used are polyester + 0.3%, polyester 5 × 0, polypropylene, and nylon. All vials were supplied by the West Company (Phoenixville, PA) and washed and sterilized prior to use. A 5 ml volume of protein

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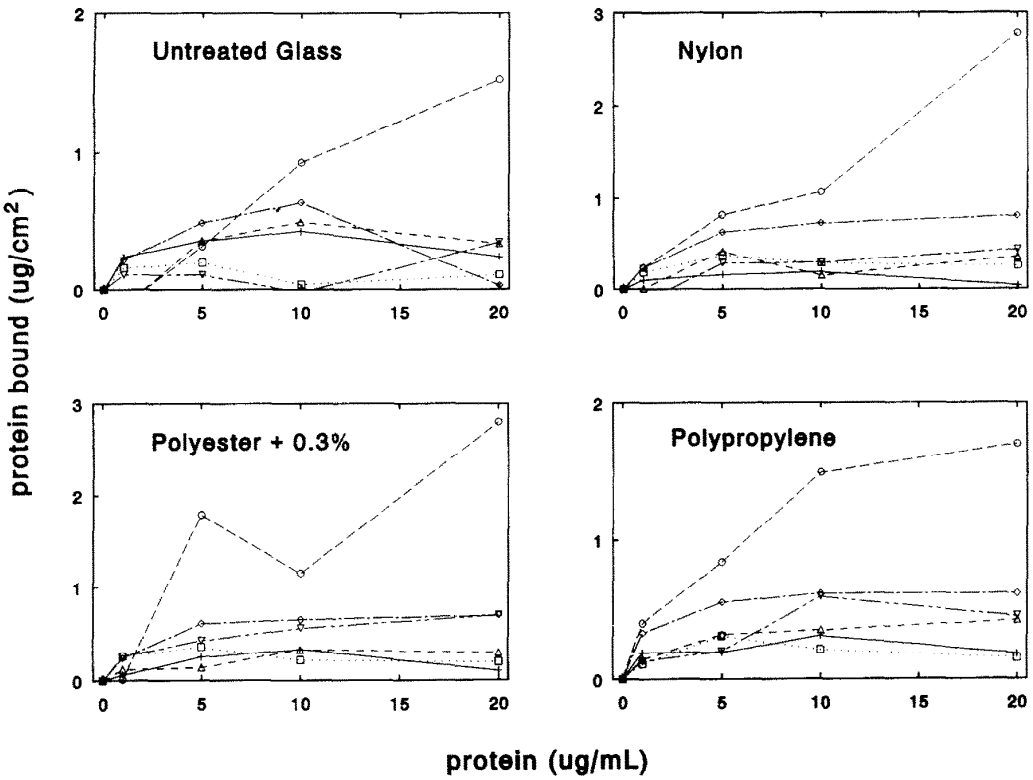
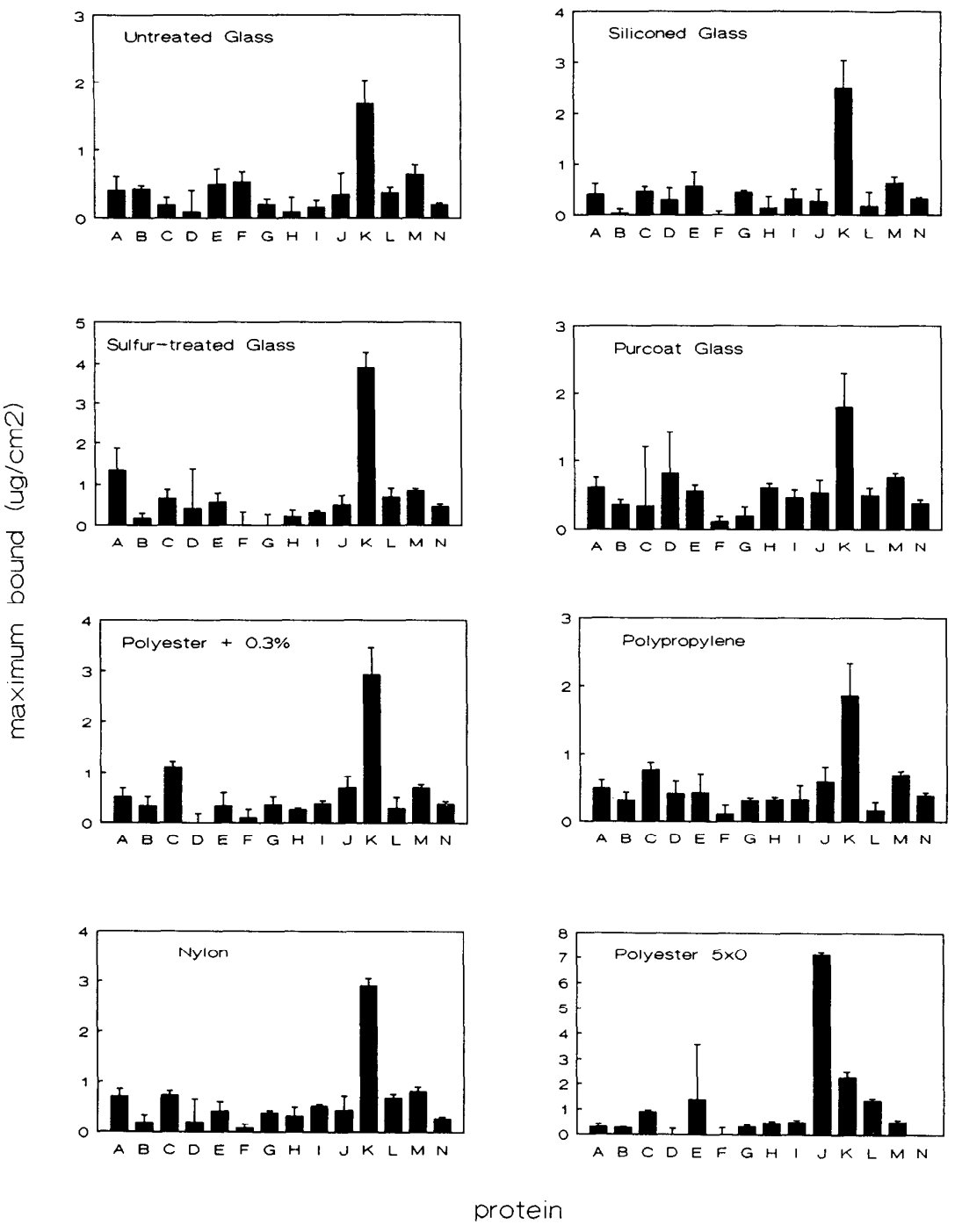


Fig. 1. Binding isotherms of four container surfaces. Data for six proteins are displayed: alcohol dehydrogenase (+), β-amylase (△), lactate dehydrogenase (○), α-chymotrypsinogen A (□), thyroglobulin (◆), and immunoglobulin G (▽). Each data point is an average of three determinations.

solution was added to each vial, yielding a surface/volume ratio of 2.4 cm^2 ml^{-1}. Solutions were not mixed or inverted to limit surface exposure to the solution contact region. Adsorption was allowed to proceed for only 24 h to minimize any effect of changes in protein stability on the

Fig. 2. Extent of protein binding to containers. The maximum amount of protein adsorbed at an initial concentration of 20 μg/ml to the surfaces of eight containers after 24 h at 4°C is represented. The amount bound was determined by averaging three measurements of protein concentration from each vial and calculating the amount bound from the difference in protein in solution before and after incubation. The proteins (with molecular mass and p*I* as indicated) examined are as follows: A, acidic fibroblast growth factor (15.9 kDa, 7.0); B, alcohol dehydrogenase (141 kDa, 5.4); C, apoferritin (443 kDa, 4.3); D, aprotinin (6.5 kDa, 10.5); E, β-amylase (200 kDa, 4.8); F, bovine serum albumin (66 kDa, 4.9); G, α-chymotrypsinogen A (25 kDa, 9.1); H, conalbumin (80 kDa, 5.6); I, cytochrome *c* (12.4 kDa, 10.3); J, immunoglobulin G (150 kDa, 7.5); K, L-lactic dehydrogenase (228 kDa, 8.3); L, lysozyme (14.3 kDa, 10); M, thyroglobulin (669 kDa, 4.5); N, covalent conjugate of transforming growth factor α with a 40 kDa fragment of *Pseudomonas* exotoxin (45 kDa, 5.0). Purcoat® is a trademark of the West Company.


Untreated Glass
Siliconed Glass
Sulfur-treated Glass
Purcoat Glass
Polyester + 0.3%
Polypropylene
Nylon
Polyester 5x0
A B C D E F G H I J K L M N
maximum bound (ug/cm2)
protein

results. The time dependence of the binding of several of the proteins was examined and binding was complete within 1 h. The isotherms thus appear to represent a steady state (and probably equilibrium) process over the time course of the measurements. The amount of adsorbed protein was determined by sampling three aliquots of each vial through the flow cell of a SpectraFOCUS detector (Spectra-Physics, Inc.) and recording the absorbance of the peptide bond at 215 nm with the detector and sampling line at ambient temperature. Bound protein was calculated from the difference between initial protein concentration and that present in solution after the 24 h incubation. Protein concentrations were determined from individual standard curves for each protein. Standards were prepared immediately prior to analysis at a lower surface/volume ratio of 1.5 cm^2 ml^{-1} in polypropylene and measured immediately to minimize adsorption. Standards were also remeasured after all the vials were analyzed and generally were superimposeable. No correction was made for protein adsorption to the inner surfaces of the flow cell, but the flow cell was washed between proteins to ensure no residual protein was retained in the flow cell. Four point direct binding isotherms were employed to estimate the amount of protein on a surface (see Fig. 1).

Typical binding isotherms for six proteins in four containers are illustrated in Fig. 1. The complete binding isotherms for 20 proteins as well as plots of protein molecular mass and p*I* vs various binding parameters of surface adsorption are available upon request from the authors. Most of the 20 proteins examined manifest evidence of eventual saturation on all eight containers tested like the examples shown in Fig. 1. In many cases, proteins appear to saturate at approx. 5 μg/ml of protein, a level similar to that previously observed for several proteins on different surfaces (Elgersma et al., 1990; Luey et al., 1991). In a few instances, however, saturation is clearly not achieved at the highest protein concentration tested (20 μg/ml). Nevertheless, even in the worse cases, only 10–15% of the protein is adsorbed. The degree of binding appears to be primarily a property of the individual proteins themselves since proteins such as lactate dehydrogenase which display increased surface interaction do so on all surfaces examined. The properties of the surface itself clearly do influence binding as illustrated by the very low affinity of several proteins for untreated glass and the enhanced interaction of BSA for the same material.

The maximum amount of protein bound for 14 proteins in eight different containers is summarized in Fig. 2. Inspection of the binding isotherms from which the data in Fig. 2 was obtained as well as similar experiments from seven other proteins reveals a number of generalities. No correlation was found between molecular mass or the isoelectric point of the proteins and their interactions with the containers under these experimental conditions. In addition, the difference in surface interaction between the proteins was much greater than the variation in container surface type. Nevertheless, some types of containers do, on average, appear to manifest lower protein surface adsorption than others. Overall, siliconed and untreated glass appear to bind less protein than the other materials examined, while sulfur-treated glass and polyester containers bind certain proteins in somewhat increased amounts.

In summary, and somewhat surprisingly, protein adsorption to container surfaces does not appear to be a major problem above a 5–20 μg/ml protein range with many if not most proteins over the 4°C, 24 h incubation period examined. Since proteins sometimes manifest slow structural changes on surfaces over longer periods (Andrade, 1985), further studies are required to establish the utility of these containers for long-term storage. Nevertheless, untreated and treated glasses generally appear to bind the least protein, but all of the surfaces tested appear to have acceptable adsorption characteristics. It is clear, however, that proteins need to be individually evaluated in this regard. Not evaluated in this study was the adsorption to vial stoppers, which could potentially contribute significantly to protein loss. In situations where protein adsorption is significant, the inclusion of high concentrations of an inert protein (e.g., serum albumin) to saturate the container surface or the presence of compounds to reduce surface interactions such as

surfactants, carbohydrates, or amino acids can be employed to reduce the problem (Suelter and DeLuca, 1983; Wang and Hanson, 1988).

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