Molecular Inclusions. Adsorption of Macromolecules on Porous Glass Membranes

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Abstract: Our original intent was to separate macromolecules according to molecular size by dialysis through glass membranes constructed with controlled pore sizes and distributions. Proteins were rapidly lost from solution upon exposure to these membranes. Kinetic studies indicated that the initial adsorption was isoelectric-point dependent while subsequent losses were inversely proportional to the molecular weight of the protein. The three apparent forces involved in the reaction of proteins with porous glass are ionic amine silanol bonding, hydrogen bonding, and diffusion.

The ability to control closely pore sizes within a formed body of glass appeared to offer a procedure for separating macromolecules through a dimensionally stable membrane in a variety of solvents. Our initial intent was to use Corning Code 7930 porous glass as a dialysis membrane to separate proteins on the basis of molecular weight. Early experiments indicated that considerable quantities of protein were rapidly lost from

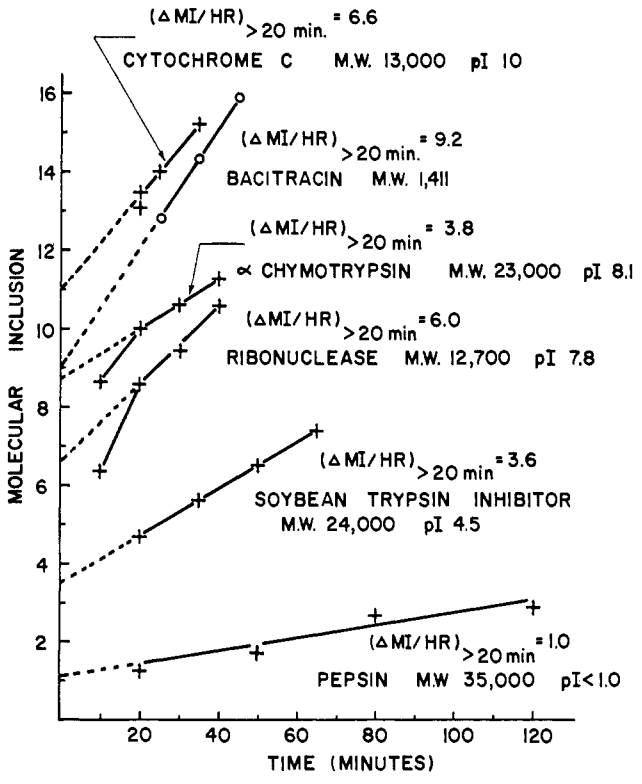

Figure 1. Molecular inclusion kinetics, 0.5–1°.

the solution contained by membrane test tubes. However, little or no protein appeared after several days in the buffer outside of the membrane. Attempts to extract the protein from these membranes with a variety of solutions differing in pH and in ionic strengths resulted in very limited recoveries of protein. In order to understand the mechanism by which these proteins were lost to the membranes a series of carefully controlled kinetic experiments were performed.

Experimental Section

Test tubes made of Corning Code 7930 porous glass, 10 mm i.d., 12 mm o.d., 11.5–13.0 cm long, pore diameter 75 Å, were equilibrated in 0.1 *M* phosphate buffer, pH 7.0. The tubes were drained for 2 min before use. Individual tubes were used for the determination of each point.

Five milliliters of solution, precooled to 1°, containing 0.5 mg/ml of protein in 0.1 *M* phosphate buffer, pH 7.0, was delivered to the tube at time zero (t_0). The tube was immersed in a cylinder containing a quantity of precooled 0.1 *M* phosphate buffer, pH 7.0, such that the meniscus of the cylinder matched the height of the meniscus in the tube after a stirrer had been immersed in the protein solution. The cylinder was jacketed in an ice bath. During the period of exposure to the porous glass tube, the protein solution was continually stirred with a Corning LM-2 mixer, vibrating stirrer. At the completion of the timed interval (t_f), the protein solution was immediately transferred to a cuvette, and the optical density was measured at 280 mμ.

The per cent loss of protein from solution to the membrane [molecular inclusion (MI)] was calculated by comparing the optical density of the reacted protein solution (A_f) at time (t_f) with that of the optical density of the original solution (A_0) at (t_0) as

$$\mathrm{MI} = [1 - (A_f/A_0)]100$$

Results

Protein was removed from solution too rapidly during the first 20 min of exposure to the porous glass (Figure 1) to obtain reliable quantitative information. After the initial reaction, the rate of inclusion of protein in the membrane was considerably diminished and the determinations became reproducible. Molecular inclusion appears to be linear with respect to time for approximately 90 min after the initial 20 min of reaction.

When the rate of molecular inclusion after 20 min, $[\Delta\mathrm{MI/hr}]_{t>20\ \mathrm{min}}$, is plotted against the molecular weight of the protein (Figure 2), an inverse relationship becomes apparent. The fact that the rate after 20 min is molecular weight dependent seems to indicate that this phase of molecular inclusion is diffusion controlled.

Discussion

The linear portion of the molecular inclusion *vs.* time plots (Figure 1) were extrapolated back to zero time. An approximation of the magnitude of the initial reaction may be obtained by reading the extrapolated intercept at the ordinate. A rough correlation exists between the extent of the initial reaction and the isoelectric pH of the protein. The initial reaction appears to be greater for the proteins with higher isoelectric points. The higher isoelectric pH proteins contain

relatively more reactive basic groups. The predominant basic group in proteins is $-NH_3^+$; therefore, this suggests that the initial reaction in molecular inclusion involves the formation of ionic bonds between the amine groups of the protein and the dissociated silanol (SiO^-) groups of the porous glass. Support for this hypothesis may be derived from the studies of reactions of soluble silicic acid with heptadecylamine and with proteins performed by Holt and Bowcott.[1]

The protein–glass bond formed during the molecular inclusion process is so strong that the protein cannot be released subsequently by strong acids, ammonium hydroxide, or by a variety of ionic-strength buffer solutions. This indicates that the ionic amine silicate cannot be the sole mechanism involved in the molecular inclusion process.

Weldes'[2] investigations of the interactions of alkali-metal silicates with amino acids, peptides, and proteins suggested that hydrogen bonding might be responsible for the adherence of the protein to the glass. However, attempts to remove the included protein with urea were unsuccessful, thus indicating that hydrogen bonding was not the sole mechanism. However, when the tubes were extracted with urea in either dilute or concentrated acid solutions, the protein was rapidly and quantitatively released from the glass. The acid probably protonated the silanol group, thus releasing the protein amine, while urea broke the hydrogen bonds formed between the protein and glass surface.

The three apparent forces involved in the reaction of proteins with porous glass are ionic amine silanol bonding, hydrogen bonding, and diffusion.

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(1) P. Holt and J. Bowcott, *Biochem. J.*, 57, 471 (1954).
(2) H. Weldes, *Adhesives Age*, **10**, 32 (1967).

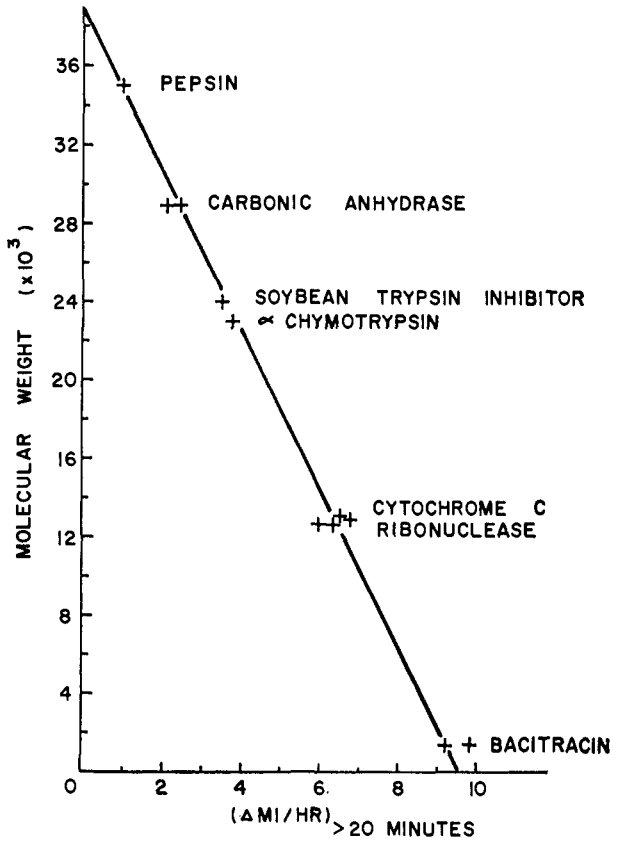


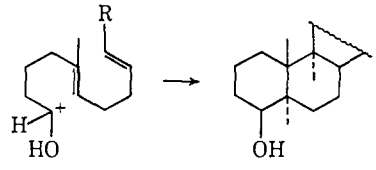
Figure 2. Molecular weight *vs.* molecular inclusion rate after 20 min.

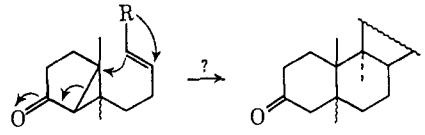
Communications to the Editor

Six-Membered Rings *via* Olefin Participation in the Opening of Acylcyclopropanes

Sir:

Impressive success has recently been achieved by Johnson and his collaborators in the synthesis of polycyclic systems with natural steroid stereochemistry *via* cyclization of properly constituted acyclic polyenes in which the initiating cation is the conjugate acid of an aldehyde.[1] We have been interested for some time in the possibility of initiating such cyclization *via* acid-catalyzed opening of cyclopropyl ketones which, if successful, would result in the direct formation of polycyclic systems with a keto group at the position





(C_3) where it is normally found in natural steroids. The use of cyclopropyl ketones such as I was especially interesting to us because of their expected easy accessibility *via* the internal diazo ketone insertion method which we introduced some years ago.[2] We report

(1) For a recent review, *cf.* W. S. Johnson, *Accounts Chem. Res.*, **1**, 1 (1968).
(2) G. Stork and J. Ficini, *J. Am. Chem. Soc.*, **83**, 4678 (1961).