The Anomalous Electrophoretic Behavior of Certain Adsorbed Protein Derivatives (344450)

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The assumption has often been made that the electrophoretic properties of proteins adsorbed on colloids are the same as those of the proteins in solution. In most cases (1, 2), this assumption was demonstrated by the fact that determinations of the electrophoretic properties of certain proteins and their isoelectric points were the same whether the moving boundary method or microelectrophoresis was used. It was the purpose of the present study to demonstrate that interactions of the protein with the colloid surface may lead to anomalous electrophoretic behavior as a result of orientation of the protein molecules. Such a possibility has been suggested (2–4), but evidence for such orientation has been lacking. Most microelectrophoresis experiments are conducted in such a manner that multilayers of protein are formed on the colloid particle surface which mask any orientation that may be present on the initial monolayer. Few studies have been undertaken to compare the amount of a protein bound by colloidal particles with the microelectrophoretic behavior of the particles (5). Further, orientation as evidenced by anomalous electrophoretic behavior (vide infra) is a function of both the protein and the colloidal surface and can only be demonstrated under appropriate experimental conditions.

In the course of investigations into the behavior of colloids to be injected into rats, a model system was devised consisting of monodisperse polystyrene latex (PSL) with surface characteristics controlled by adsorption of various macromolecules, particularly amino acid derivatives of gelatin. The adsorption of these various materials was followed by microelectrophoresis, and the changes in electrophoretic mobility in turn were found to influence the organ distribution of the colloids upon intravenous injection (6, 7). Microelectrophoresis was carried out at various concentrations of the material under consideration, and the curves, when compared to directly determined adsorption isotherms, were shown under certain conditions to exhibit anomalous behavior.

Methods and Materials. Microelectrophoresis was carried out in an apparatus similar to that described by Bangham *et al.* (8). This consists of a cylindrical cell suspended in a water bath at 25 ± 0.1°. Particles in the stationary layer were directly observed through a microscope linked to a closed-circuit television system. All electrophoresis measurements represent the mean of at least 10 measurements timed in both directions. Unless otherwise mentioned, all measurements were made in 0.0145 *M* NaCl. Poly-L-lysyl gelatin (PLG) was prepared according to the method of Sela and Arnon (9) and, after labeling with ^{131}I (10), was exhaustively dialyzed and then lyophilized. Adsorption isotherms were determined by adding a suspension containing 5.5 mg of PSL to a series of cellulose nitrate (0.5 × 2.5 in.) centrifuge tubes containing PLG solutions in a suitable range of concentrations. The total volume in each tube was 5.0 ml and the final salt concentration 0.0145 *M* NaCl. After equilibration for 30 min at 37°, the tubes were centrifuged at 35,000 *g* for 15 min and washed several times with 0.0145 *M* NaCl solution until the PSL pellet showed no further change in counts. Microelectrophoresis was also carried out on suspensions of the washed PSL in 0.0145 *M* NaCl.

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Emulsions of mineral oil (Drakeol-6VR, kindly supplied by the Pennsylvania Refining Co., Butler, Pa.) were prepared by sonification of 0.5 ml of oil and 50.0 ml of distilled water in a beaker. Aliquots of this emulsion were added to PLG solutions in 0.0145 *M* NaCl for electrophoresis.

For injection into rats ^{125}I-labeled PSL (6) was suspended in a range of concentrations of PLG in 0.145 *M* NaCl. This material was filtered by membrane filtration (0.45 μ diam) (Millipore Corporation, Bedford, Mass.) to remove excess PLG and resuspended in 0.145 *M* NaCl with gentle sonification. Injections were carried out in Nembutal anesthetized rats, and distributions and rate of clearance from the blood were determined as previously described (6). The results were expressed as the percentage of the injected dose accumulated in the spleen after 15 min.

Sheep red blood cells (SRBC, Baltimore Biological Laboratory, Baltimore, Md.) were washed three times with 0.145 *M* NaCl with a packed cells/saline ratio of 1:20.

Polystyrene latex of diameter 1.099 and 1.305 μ was kindly provided by Dr. J. Vanderhoff of the Dow Chemical Co., Midland, Michigan.

Results. Figure 1 shows the adsorption of PLG on PSL as a function of the concentration of PLG. Curve A represents the amount bound by the original PSL precipitate after centrifuging once, while curve B shows the amount bound after five washes with 0.0145 *M* NaCl. Further washes produced no further reduction in mobility. Note that the uptake as measured by electrophoresis (curve C) differs from the adsorption isotherm (curve B) by showing a distinct maximum in positive mobility and then decreasing again to a constant value. It is this maximum in the mobility which is of interest since it presumably reflects a particular orientation of the PLG molecules upon adsorption.

To examine the possibility that this presumed orientation could be due to the nature of the particle surface, the electrophoretic mobility of various particles in the same range of concentration of PLG solutions was determined. Figure 2 shows the results of some typical experiments using mineral oil

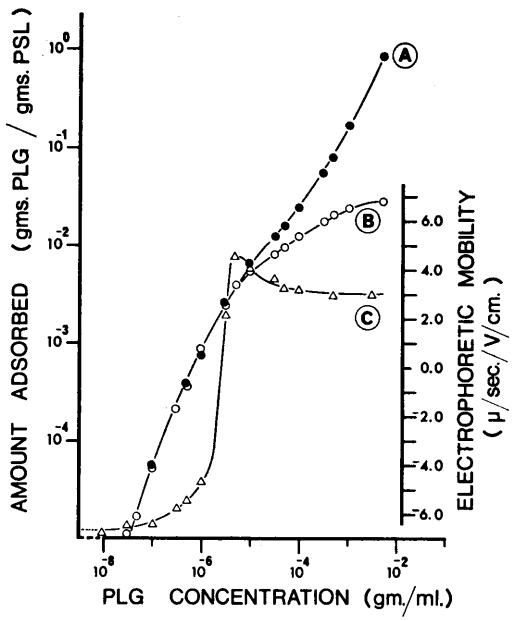

FIG. 1. Adsorption and electrophoresis of PLG on PSL (diam 1.305 rμ) as a function of PLG concentration; (●), amount of PLG bound by PSL after centrifuging; (○), amount of PLG bound by PSL after centrifuging and exhaustive washing; (Δ), electrophoretic mobility of the washed PSL above. Both adsorption and electrophoresis in 0.0145 *M* NaCl at a pH of 5.5–6.0.

and washed SRBC. The mineral oil curve shows an even greater maximum in positive mobility while the red cells show none at all. It is also apparent that the orientation may be observed with particles suspended in PLG solutions or with particles suspended in PLG solutions and then filtered, washed, and resuspended in salt solutions (see Fig. 1).

Figure 3 shows the results of a typical intravenous clearance experiment, in which the percentage of the injected PSL accumulated in the spleen 15 min after injection is plotted as a function of the coating concentration of PLG, together with the electrophoretic mobility of the injected particles. As shown, the sequestration of PLG particles in the spleen is in fact reflected in the electrophoretic characteristics of the particles.

Discussion. By comparing the electrophoretic uptake with the adsorption isotherm, it was observed that the maximum mobility occurs at or about the concentration neces-

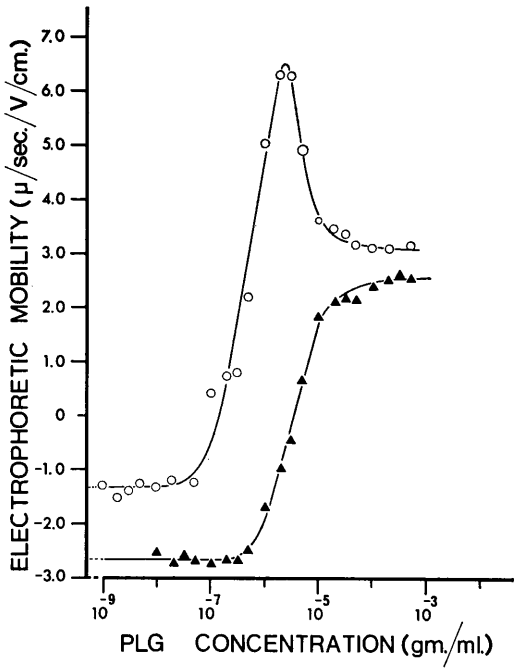

FIG. 2. Electrophoretic mobility of mineral oil droplets and sheep red cells as a function of ambient PLG concentration; (○), mineral oil droplets in 0.0145 *M* NaCl; (▲), washed sheep red blood cells in 0.0145 *M* NaCl/5% sucrose.

sary to form a "monolayer," or perhaps better at the concentration necessary to saturate the surface, since it is difficult to visualize precisely what is meant by a monolayer when dealing with proteins (11, 12). At the point where curves A and B of Fig. 1 diverge, or the peak of the electrophoretic curve, the coverage is approximately 1.0 mg of PLG/m^2 of PSL, very similar to the coverage determined by several workers (13) for a protein "monolayer" at the air/water interface. As further PLG was added, the mobility decreased again to a constant value as a multilayer was built up and presumably the orienting effect of the substrate surface damped out. A similar effect was noted by Matijevic and Ottewill (14), who showed a periodic change in electrophoretic mobility of silver halide sols as a function of added cationic detergent. This was ascribed to successive layers of detergent having opposite orientations on a charge basis which was "damped out" at higher concentrations of detergent. The adsorption isotherm is not Langmuirian, under the experimental conditions used, in that a true asymptotic value of coverage is not reached, presumably because the PLG–PLG interaction is stronger or perhaps more slowly reversible than the PLG–H_2O interaction.

In view of the fact that adsorption of PLG on SRBC shows no anomalous behavior and on mineral oil shows a larger positive mobility than on PSL, it is interesting to speculate on how the nature of the substrate affects the orientation of the adsorbing polymer. In this connection it is of interest that PLG may be easily washed from the red cell surface, and indeed it is this reversible behavior which presumably leads to a typical Langmuir isotherm. However, it is very difficult to wash further PLG from the PLG-saturated PSL surface once the loosely bound material is removed.

Thus after curve B was reached (Fig. 1),

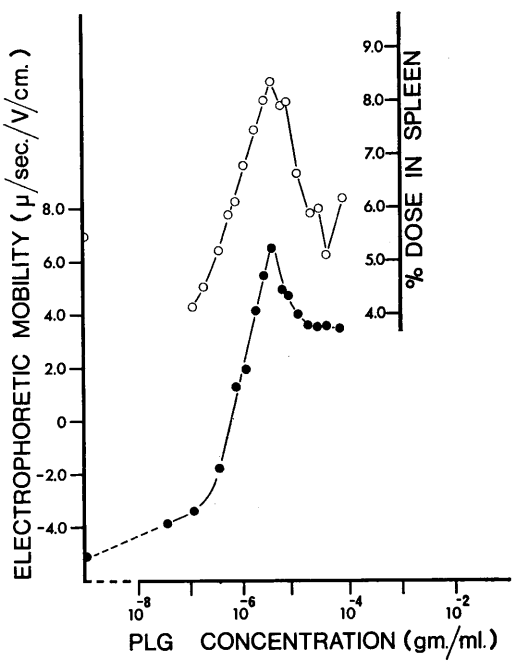

FIG. 3. Electrophoretic mobility and amount taken up by the spleen 15 min after injection as a function of PLG concentration; (●), mobility of PSL particles; (○), spleen uptake of 0.73 mg of PSL/100 g of rat. Both electrophoresis and spleen uptake in 0.145 *M* NaCl. Diameter of PSL 1.099 μ.

further washing with 0.0145 *M* NaCl at 37° produced no further reduction in mobility at any concentration of PLG. Further, since adsorption on SRBC reaches a plateau, this implies that more than a monolayer cannot be adsorbed. Hence, orientation would not be observed since, unless a multilayer is adsorbed with subsequent "damping" and reduction of mobility after a peak mobility is reached, the peak would be unobserved.

If one considers the adsorption in terms of electrostatic interactions, then obtaining a positive maximum in electrophoretic mobility is the reverse of what might be expected, since the surface of PSL has a high negative zeta potential (15). Thus the basic groups of adsorbing PLG molecules might be expected to be concentrated near the PSL surface, leaving a preponderance of acidic groups in the electrophoretic plane of shear. It is known, however, that proteins bind strongly to hydrophobic surfaces (16); therefore, it is proposed that the PLG is adsorbed at the PSL surface in such a manner that many of the acidic groups of the protein are adjacent to the largely hydrophobic surface, leaving a preponderance of positive groups in the electrophoretic plane of shear. This argument seems to be given some value by the observation tht mineral oil, which is known to preferentially adsorb anions (17), shows the largest positive mobility peak. Interestingly, mineral oil was originally used at the suggestion of Dr. H. A. Abramson because of the fluid nature of the interface, which should permit easier orientation, and indeed this may account for the exaggerated effect when mineral oil emulsions are used.

A result of this work which should not be overlooked is that it emphasizes again a point made first by Abramson (2) that the properties of proteins adsorbed upon particle surfaces can only be interpreted properly if adequate coverage of the surface is assumed. However, it is now clear that not only must sufficient protein be present to obscure any charge contribution by the underlying surface, but in the case of basic proteins the concentration must be high enough to exceed the region where orientation could give rise to excessively high mobilities and of course erroneous isoelectric points. Thus any results reported where such precautions were not observed must be carefully evaluated.

In view of the importance of interfaces in biology, both intracellular and extracellular, it is interesting to speculate on the biological significance of such orientations. It was shown previously that the distribution of an intravenously administered colloid is influenced by the surface character of the injected colloid. Electrophoretically positive colloids showed an increase in splenic accumulation over negative colloids (6). Thus the fact that the spleen can recognize far more subtle differences in surface characteristic (see Fig. 3), as shown by how precisely the spleen uptake mirrors the anomalous adsorption behavior, takes an added interest. Investigation of other possible biological effects such as the influence upon antigenicity or upon the rate of enzyme reactions is contemplated.

Summary. Determination of the electrophoretic mobility of PSL particles in increasing concentration of PLG showed an unexpectedly high positive mobility at a coverage corresponding to one monolayer. This elevated mobility was tentatively ascribed to a particular orientation of the adsorbing PLG molecules due to interaction with the PSL surface. Other surfaces examined suggest that the effect is produced by hydrophobic surfaces. The mobility changes were shown to be correlated with changes in the organ distribution of PSL particles injected into rats.

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