The Interaction of Nonionic Detergents with Protein in Paper Electrophoresis

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Addition of polyoxyethylene nonionic detergents to the buffer in paper electrophoresis results in alteration of the rate of migration and shape of protein peaks. At low detergent concentrations, disruption of paper-protein binding leads to faster migration and sharper peaks. At high detergent concentrations, interaction of detergent with protein leads to slower migration and broader peaks. On glass-fiber paper, which binds protein less strongly than ordinary cellulose filter paper, only slowing of migration occurs upon addition of detergent to the buffer. Electrophoresis of polysaccharides using buffer-containing detergent results only in a slight increase in migration.

While reactions of anionic and cationic detergents with protein are well known (1), nonionic detergents have long been thought not to interact with proteins. Recently, evidence of the interaction of certain polyoxyethylene detergents with protein has been produced. Such detergents have been used in the stabilization of mitochondrial enzymes (2) and separation of microsomal protein (3). Triton X-100* has been shown to prevent the adsorption of proteins on ion exchange resins (4). The physical properties of the protein-detergent complex (5) and the effect of detergent on the titration curve and absorption spectrum of an azomercurial dye attached to protein (6) are consistent with the notion that the nonionic detergent results in a greater solvation of the protein molecule. It has been reported that paper electrophoresis of serum using buffer containing a nonionic detergent (7-9) results in better separation and sharper peaks.

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and in patterns which resemble more closely those obtained upon moving-boundary electrophoresis of serum. The mechanism of these effects is unknown. Because of our interest in detergent-protein interactions, we explored the action of nonionic detergents in paper electrophoresis in extenso.

**Materials and Methods**

Paper electrophoresis was carried out on washed Whatman No. 1 paper in a hanging-strip electrophoresis cell* at a voltage gradient of 3.5 v/cm. for 16 hours. In some experiments, electrophoresis was performed on Whatman No. GF/B glass-fiber paper for 8 hours, with 8 μg. of serum or of a 5% aqueous solution of a crystalline protein applied to the paper. The crystalline proteins studied were bovine serum albumin, β-lactoglobulin and ribonuclease† Barbital buffer, \( \Gamma/2 = 0.075, \) pH 8.6, was used, which at times also contained varying concentrations of p-t, t-octylphenoxypolyoxyethylene ethanol of 5-, 10-, 16- or 20-ethylene oxide units—Triton X-45, X-100, X-165, and X-205 respectively—or Sterox SE,‡ a similar thioether detergent. To study the effect of detergent on the migration of protein, two cells with the same number of paper strips were connected in parallel; one cell contained barbital buffer in distilled water, the other cell contained detergent in addition. After electrophoresis, paper strips were fixed in methanol and the protein stained with bromphenol blue (10) and scanned in a Spinco Analytrol densitometer. In other experiments, electrophoresis was carried out on 8 μg. of a 3% solution of heparin or chondroitin sulfate§ at a voltage gradient of 3.5 v/cm. for 6 hours. Color was developed in the latter experiments by spraying the paper strips with toluidine blue (11).

**Results and Discussion**

The effect of increasing concentrations of the nonionic detergent, Triton X-100, on the electrophoretic pattern of normal human serum is shown in Fig. 1. As the detergent concentration is increased, there is faster migration of all components and some sharpening of the peaks. For albumin the greatest rate of migration and the tallest and sharpest peak occurs at a detergent concentration of 0.1%. At

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*Spinco-Durrum.
†Supplied by Pentex, Inc., Kankakee, Ill.
‡Monsanto Chemical Company, St. Louis, Mo.
§California Corporation for Biochemical Research, Los Angeles, Calif.
this concentration, however, the \( \alpha_1 \) and \( \alpha_2 \) globulins have merged into a single peak. As the detergent concentration is increased further, the migration of the albumin decreases and the peak becomes broader and lower. The area under the albumin peak increases with increasing detergent concentration (Table 1). At higher detergent concentrations the \( \alpha \) globulins merge into the albumin peak. At a concentration of 10% Triton X-100 the electrophoretic pattern is grossly distorted.

The mechanism of these effects was elucidated by observing the behavior of single crystalline proteins. In moving-boundary electrophoresis, where paper–protein interaction is avoided, the crystalline proteins showed an appreciable drop of specific mobility at

<table>
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<tr>
<th>Triton X-100 (%)</th>
<th>Albumin</th>
<th>( \alpha )-globulin</th>
<th>( \beta )-globulin</th>
<th>( \gamma )-globulin</th>
</tr>
</thead>
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<tr>
<td>0.0</td>
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<td>6.4</td>
<td>11.8</td>
<td>12.5</td>
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<td>6.1</td>
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<td>4.8</td>
<td>9.4</td>
<td>12.6</td>
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<tr>
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<td>4.7</td>
<td>9.4</td>
<td>11.6</td>
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<tr>
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<tr>
<td>10.0</td>
<td>68.8</td>
<td>....</td>
<td>20.1</td>
<td>17.6</td>
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</table>
detergent concentrations of 0.005-0.04% with a further, though less marked, drop in specific mobility for higher detergent concentrations (5).

In Fig. 2, the ratio of the migration of protein upon paper electrophoresis in buffer containing detergent to the migration in buffer in water for three crystalline proteins is shown for various detergent concentrations. At low detergent concentrations the bovine serum albumin and \(\beta\)-lactoglobulin migrated farther, reaching their highest values at about 0.1\% detergent for bovine serum albumin and at 0.03\% for \(\beta\)-lactoglobulin. As the detergent concentration is further increased, the migration decreases.

These findings are interpreted to be the result of two separate actions of the detergent: a disruption of the binding between the paper and protein, and an association of detergent with protein, forming complexes with smaller specific mobilities. The increased solvation of the protein molecules (5, 6), changing their hydrodynamic properties, results in the decreased mobility. This larger shell of water also presumably "insulates" the proteins from attractive forces of the paper. At low detergent concentrations the disrup-
**Fig. 3.** Comparison of the migration of three crystalline proteins upon electrophoresis on glass-fiber paper, using barbital buffer ($M_b$) and buffer containing various concentrations of Triton X-100 ($M_s$). Values are means of four experiments.

**Fig. 4.** Comparison of the migration of two polysaccharides upon electrophoresis on Whatman No. 1 paper, using barbital buffer ($M_b$) and buffer containing various concentrations of Triton X-100 ($M_s$). Values are means of four experiments.
tion of the binding forces is more important, resulting in more rapid migration of protein. At high detergent concentrations, the complexes formed by association of detergent with protein results in slower migration.

In order to obtain supporting evidence for these conjectures, electrophoresis was carried out on glass-fiber paper. If the disruption of protein-paper binding was important, the increase in migration at low detergent concentrations should not be as prominent on glass-fiber paper, which binds protein less strongly than ordinary cellulose filter paper. In fact, only slowing of migration was observed on glass-fiber paper. The increased rate of migration at low detergent concentrations, noted on paper electrophoresis, was not observed on the glass-fiber paper (Fig. 3).

When nonprotein material, e.g., chondroitin sulfate or heparin, was subjected to electrophoresis, addition of detergent to the buffer resulted only in a slightly increased rate of migration (Fig. 4). This is consistent with the notion that the decrease in migration depends upon the association of detergent with protein. The slight increase in migration is due, presumably, to the disruption of polysaccharide-paper binding by detergent.

Similar results were obtained with the other detergents.

References