Selective Staining of $\alpha_1$-Antitrypsin ($\alpha_1$-Protease Inhibitor) with Schiff's Reagent after Separation from Serum by Analytical Isoelectrofocusing in Polyacrylamide Gel

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After electrofocusing serum from human and various animals in the pH range 4.0–6.0, only the isoproteins of antitrypsin stain with Schiff's reagent. As an additional advantage, this staining provides information about the relative contents of the Schiff-positive carbohydrates of various antitrypsins.

For several years, the method of acid starch-gel electrophoresis, followed by crossed immunoelectrophoresis in agarose, was the common procedure for typing serum antitrypsin $\alpha_1$. Lately several authors have introduced various modifications of analytical isoelectrofocusing (3–6) to the same end. In the latter case, the gel is usually treated with Coomassie Brilliant Blue, resulting in the staining not only of antitrypsin, but also of other serum components within the focusing range.

A selective staining of antitrypsin would appreciably facilitate the interpretation of the results of electrofocusing, particularly in cases in which the isoproteins of antitrypsin are interspersed with other serum components. On the basis of these considerations we compared results obtained by staining electrofocused serum aliquots with Coomassie Blue on the one hand and Schiff's reagent on the other.

Materials and Methods

Our conditions of electrofocusing in polyacrylamide gel were described earlier (7).

Gels were stained with Coomassie Brilliant Blue at 60 °C for 15 min in a mixture of methanol/water (1/2 by vol), containing, per liter, 1.1 g of dye, 32.5 g of sulfosalicylic acid, and 110 g of trichloroacetic acid. The gel was then destained overnight in water/ethanol/glacial acetic acid (8/3/1 by vol) at room temperature.

At every step of staining with basic fuchsin (Schiff's reagent) there was gentle shaking of the gel in a tray at room temperature. After electrofocusing, the gel was immersed into a solution of trichloroacetic acid (125 g/liter). Within a few minutes, precipitation of the proteins in the gel was detected by the appearance of numerous opaque bands. After an additional 5 to 10 min the above solution was replaced with periodic acid (5 g/liter) and the gel was kept in the latter for 30 min. In the next step, the gel was incubated with a 5 g/liter solution of sodium arsenite in acetic acid/water (5/95 by vol). Within a few minutes it gradually became dark brown, but after an additional 20 to 30 min it became colorless again. Two consecutive treatments with a 1 g/liter solution of sodium arsenite in acetic acid/water (5/95 by vol) followed, each lasting for about 10 min, and then the excess arsenite was removed by 15-min incubation in acetic acid/water (5/95 by vol). After this step the gel was immersed in a solution of basic fuchsin, prepared according to Kodousek (8). Within 5 min, red bands started to appear in the positions of the isoproteins of antitrypsin, reaching full intensity in 1 to 2 h. Failure to transfer the gel into the destaining solution at this stage results in a dark-red background that obscures the protein bands. We destained with a 1 g/liter solution of sodium metabisulfite in dilute (10 mmol/liter) hydrochloric acid. If it is desired to store the gel for an extended period, changing the destaining solution after about 30 min is advisable.

Results

Serum aliquots from humans and various animals were electrofocused in duplicates in the pH range of 4.0–6.0 and subsequently one half of the gel was stained with Coomassie Blue (Figure 1A), the other half with Schiff's reagent (Figure 1B). Many protein bands became visible in the gel after staining with the former (Figure 1A), but no proteins other than the isoproteins of antitrypsin appeared in gel B. As antitrypsin appeared to be the only Schiff-positive protein in the given pH range, staining the gel with this reagent is tantamount to selectively staining antitrypsin. A further advantage of this staining is that it provides information about the relative sialic acid contents of different samples. For instance, by Coomassie Blue staining the concentration of antitrypsin in chicken serum (sample 4A) would be judged to be about the same as the rest of the aliquots, but in the absence of sialyl residues the same bands were barely discernible by fuchsin staining (sample 4B). Although not of direct concern in this work, this observation indicates that the microheterogeneity of chicken antitrypsin, similarly to the case with human antitrypsin (9, 10), is caused by a factor(s) other than an uneven siylation of the isoproteins.

In the past few years the powerful method of immunofixation, subsequent to separation of antitrypsin from serum has been introduced for phenotyping (e.g., refs. 11 and 12). Although the selectivity of immunofixation cannot conceivably be surpassed by any known technique, it does not provide information about the carbohydrates of the isoproteins. In view of this and of the observations above, we recommend the application of fuchsin staining along with the other methods for the examination of electrofocused serum.

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