Classification of alphal antitrypsin phenotypes by high-resolution two-dimensional electrophoresis (2-D PAGE)

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INTRODUCTION

The ability of two-dimensional gel electrophoresis (2-D PAGE) to visualize soluble proteins is well documented (Anderson and Anderson 1977). By this tecnique, exploiting charge/mass differences to separate polypeptides, many polymorphic serum proteins were exactly located in 2-D PAGE maps of human serum (Anderson and Anderson 1979). The main advantages of this procedure are: first, a better molecular characterization of mutants is obtainable, secondly a simultaneous detection of several proteins is possible in the same slab gel;finally, the monitoring of non-conventional phenotypes,as the null variants (which would otherwise be difficult to detect) could be obtained (Asakawa et al. 1985).
Alphal antitrypsin (PI) has a high electrophoretic variability, due to aminoacidic substitutions along the proteic backbone and to three different carbohydrate side chains as prosthetic groups (Vaughan et al. 1982).
In this paper we sought to improve the detection of PI molecular variants by a 2-D PAGE procedure. M, S and Z electrophoretic types were easily discriminated. A modified procedure, based on the use of ultranarrow pH gradients in the IEF dimension together with SDS PAGE electrophoresis suitable for the Mr of PI, was finally employed, with a view to separate M subtypes.

MATERIALS AND METHODS

Sera were obtained from healthy donors and typed for PI. Prior to the first dimension step, 9 M urea and 0.5 to 1% 2-mercaptoethanol were added to the samples. Immunoprecipitates were obtained from sera of self-assessed homozygous products additioned with specific antiserum to PI, by centrifugating after each addition and finally discarding the supernatant. Immunoprecipitate pellets were solubilized in 9M urea/2-ME immediately prior to electrophoresis. Isoelectric focusing ran on ultrathin (0.3 mm thick) polyacrylamide (T=5%) gels (containing 5%

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ampholytes and 9M urea) adhered to GelBond plastic film. Gradients of nominal pH 4-6 and 4.2-4.9 were in turn used, with a voltage of 7000 V/hs (final voltage 1500 V/1h). After fixing (10% W/V sulfosalicylic acid), staining (0.5% Coomassie R250) and destaining (10:10:80 ethanol:acetic acid:water) gels were dehydrated, cut in strips and frozen. Prior to the second dimension, strips were equilibrated for 10 min. in 0.25% M Tris/Hcl buffer pH 6.8 containing 2.1% SDS and 10% glycerol, then embedded onto the second dimension gel's edge and sealed with 1% W/V agarose. Second dimension gradient gels gave best results with a 12.5-13.5% T. Electrophoresis (25 mA/gel, maximum voltage 500 V) was continued until 45 min. after the Coomassie dye front had left the gels (about 5 h for 10-20% gradients, 3 h for the narrower's); proteins were then visualized using a silver stain procedure.

RESULTS

We identified PI components by a comparison of immunoprecipitates patterns with 2-D PAGE images of native serum: five major anodic spots were identified, and two minor on their cathodic side, with an apparent Mr slightly decreasing towards the cathode. A range of pH 4-6 was employed to separate M, S and Z subtypes; it became apparent that intensities of spots would progressively fade from M to S to Z, with slightly changed distances. Z patterns possess especially strong spots n° 4 and 5, while S patterns have selectively intense spots n° 3, 4, 5; heterozygous MS duplicate spots n° 6 and 7, whereas other components partially merged into adjacent M spots. Since the three allele produce one-to-one corresponding trains of spots, the discrimination of MS an MZ heterozygous is quite simple. This procedure did not show any mass variability between M1, M2 and M3 allele product, whose trains of spots were yet different along the pH axis. Finally, the application of an ultranarrow gradient of pH along the first dimension resulted in an improved 2-D separation of M subtypes (see discussion).

DISCUSSION

Fig. 1 and Fig. 2 illustrate two different stages of 2-D PAGE experiments.
Using carrier ampholytes in the range of pH 4-6, M, S and Z phenotypes are readily identified (Fig. 1). However no considerable differences could be detected between PIM subtypes.

In a second set of experiments, ultranarrow-range carrier ampholytes (pH 4.2-4.9) were used, spanning just around the isoelectric points of the PI isoforms in the first dimension.
The application of this high-resolution 2-D PAGE protocol (ultranarrow IEF gradient and narrow T% SDS PAGE) resulted in some improvements of the patterns of Alphal antitrypsin, evidently due to the use of ultranarrow pH gradients in the first dimension. Distances between the spots were enlarged and some minor components of Antitrypsin microheterogeneity were revealed. Conversely, there were only minor advantages by the use of narrow polyacrylamide gradient gels in the second dimension.
As shown in Fig. 2, additional components of microheterogeneity became apparent in the heterozygous patterns, along the pH axis. Conversely, no further components of microheterogeneity appeared along the Mr axis, complying with the hypothesis that M variants are devoid of mass variability.
The meaning of these differences is difficult to evaluate. Uncertainties arise from the fact that each spot may not be granted as the product of a single M allele, presumably because of some overlapping of different allele products during the SDS PAGE dimension.
On the other hand, by assuming each pattern as a whole, a diagnosis of subtype is possible.

CONCLUSIONS

From these experiments we conclude that:
a) Unlike PIM, PIS and PIZ, M subtypes are devoid of significant mass hererogeneity (i.e. they possess almost the same rate of carbohydrate moyety).
b) Differences between M subtypes may be assumed to be only due to charge variability.
c) Comparisons between 2-D PAGE patterns with convective isoelectric dimension (CA IEF) are somewhat unadequate to prevent different spots to merge one into another during the second dimension. This considerably impair the resolution of the technique.
d)The technique of 2-D PAGE has been recently proposed for the simultaneous typing of several genetic markers in father/mother/child trios (Asakawa et al. 1985). However, owing to the substantial loss of informativeness in each system, its adoption in the routine of paternity testings is not realistic. As shown by the case of PI system, a share of charge variability is lost during the second dimension. Improvements in 2-D PAGE protocols are still needed. They will hopefully derive from the application of immobilized pH gradients in the IEF dimension.

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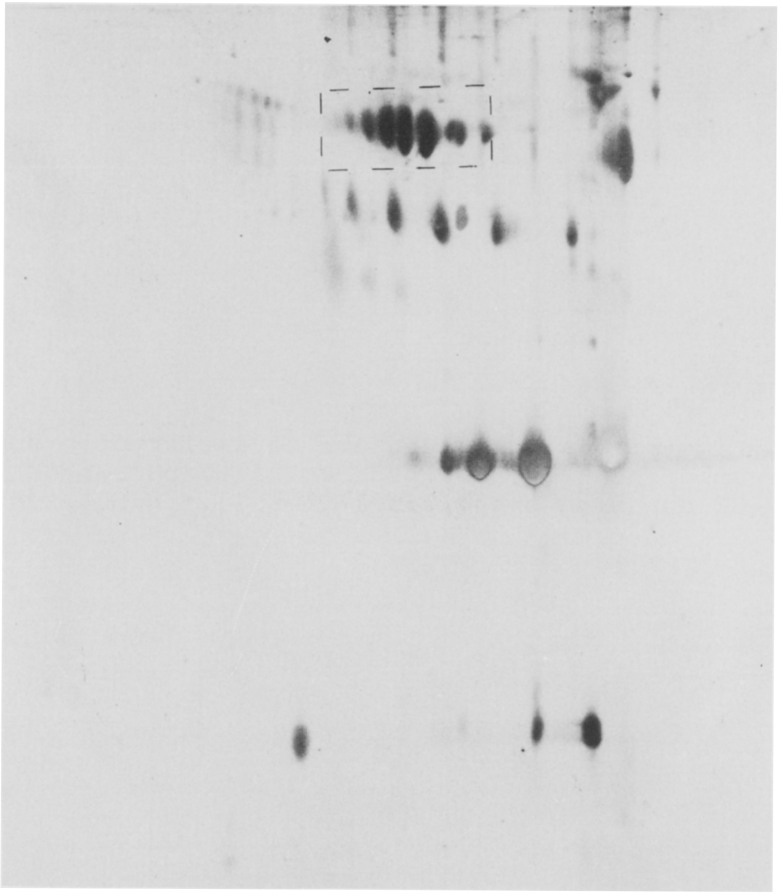

Fig. 1. Two-dimensional pattern of acidic seroproteins (IEF in the range of pH 4-6, SDS PAGE 10-20% T; anode is on the left, higher Mr components uppermost; PI train of spots is indicated). Heterozygous MS is easily recognized.

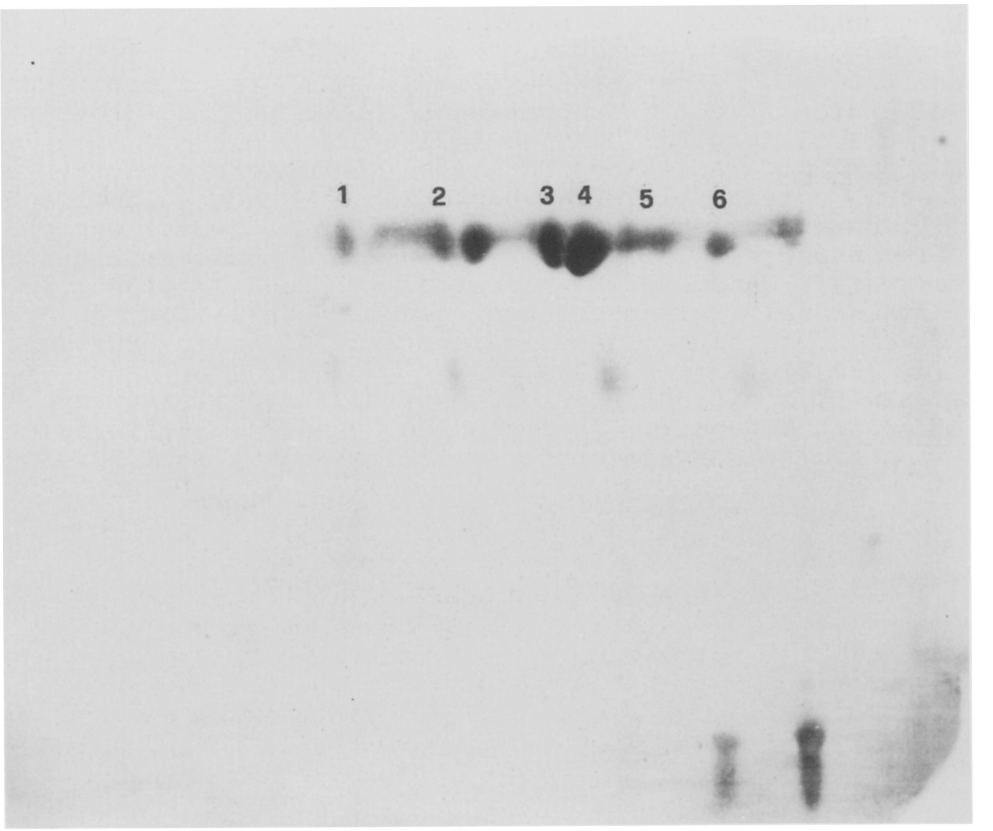

Fig.2. Two-dimensional pattern of antitrypsin spots by a high-resolution 2-D PAGE protocol (IEF pH range 4.2-4.9; SDS PAGE spanning over 12.3-13.5 % T). M2M3 subtype is shown.