IMPROVED DIAGNOSIS OF ANTITRYPSIN SUBTYPES BY ULTRANARROW IMMOBILIZED pH GRADIENTS

Vincenzo L.Pascali(*), Gabriella Conte(*), Pietro Auconi (+) and Angel Carracedo (°)
(*)Istituto di Medicina Legale,Università Cattolica del S.Cuore, Roma, and (+) Dip.Pediatria, Ospedale,Arezzo,Italy;(°) Departamento del Medicina Legal,Santiago de Compostela,Spain

The ability to discriminate isotypes whose spectra lie very close one to another is one of the today's compelling tasks to every workers applying their efforts on the electrophoresis of the genetic markers of blood. This statement applies well to acid glycoprotein systems of human serum, whose pherograms are growing overcrowded.
As a consequence, the adoption of high resolution electrophoretic methods is presently a must, and the technique of IEF on ultranarrow pH gradients (IPGs) promises to be providential for this purpose (1).
Like other groups of Forensic Serology area, our laboratory unit is planning to introduce IPGs in research programs and in routine paternity casework. As a first system with which to refine our skilfulness with IPGs we choose Antitrypsin(PI) ,that for at least one good reason. PI resolution is not quite easy with carrier ampholytes (CA). The resolving power of CA is barely reproducible from run to run, and whenever accurate Volt/hs coefficient be applied, uneven batch-to-batch outcome will ensue. The efficiency of IPGs in improving PI separation has been repeatedly stressed by the group of Munich (2)(3). We have recently summarized a method for PI separation by IPGs on a paper in press (4). Here we shall outline our procedure,with some further improvements, and deal with current problems of introducing IPGs in the routine.

Materials and Methods

Ultrathin layers IPGs were performed either as described previously (4) or as advised in Ref.(5). Samples of serum previously typed for PI in Santiago and Rome labs by CAIEF were first tested on broad IPG pH 4.1-5.1 (buffering/titrating amounts as in (5)). Twofold (or more) deeper intervals were then derived from such gradient by a simple linear interpolation. Intervals of pH 4.35-4.85 (dense solution:0.368 ml pK 4.6 and 0.150 ml pK 9.3 Immobiline; light solution:0.390 pK4.6 and 0.270 ml pK9.3) and pH 4.40-4.80 (dense solution: 0.370 ml pK4.6 and 0.160 pK9.3; light solution: 0.389 ml pK 4.6 and 0.258 ml pK 9.3) were especially experimented.

Results and Discussion

The routine implementation of the above described intervals

Advances in Forensic Haemogenetics 1
Edited by B. Brinkmann and K. Henningsen

suggested us some comments.
1)On a methodological standpoint, the strategy to interpolate shallow gradients from larger is a convenient shortcut to Henderson-Hasselbach equation. Precalculated broad gradients on which to rely are numerous and interpolated gradients have accurate endpoints and satisfactory buffering properties. All our gels contained sucrose instead of Glycerol as density medium. That would allow lessticky gels and prevent water exudation. Moreover sucrose additioned gels would impart counteract the side broadening of band patterns, provided that the preliminar gel washing be avoided. In order to optimize the polyacrylamide-Immobiline bond we found useful to replace water with a Tris/Gly buffer(50 mM, pH 8.3), and to increase the amounts of TEMED and Ammonium Persulfate, as suggested by Righetti et al.(6).
2) Large IPGs of one point of pH do not notably improve PI resolution yet given by properly drifted CA gradients. But, quite obviously, they are undefinitely stabler and more reproducible than CA gradients. Shallower intervals provide a far more powerful way to separate PI, which,this way,splits easily all M mutants, including the currently mistyped M4. But shallow IPGs are not shortcoming-free. First they must overrun many more hours(about 15) and thereby a side broadening occurs. Even worse, resolution increases at the expenses of band sharpness (the shallower interval the fainter its pattern). We cannot presently avoid thinking of that as a general mishap of ultranarrow IPGs, whose removal needed proband sera amounts to be drastically increased. We could estimate that 30 to 40 µl serum still mantain the PIM pattern within the range of Coomassie stain sensitivity. Neverthless the problem is not solved for the Z heterozygous whose faint proteic activity still risks mistyping. Therefore a two step procedure should be adopted whenever the MZ diagnosis is the case: firstlarge CA IEF, then narrow IPGs for M subtyping.
3)We shall not leave this topic without hinting the most cumbersome problem which hinders the full introduction of IPGs in the routine, say the high instability in solution. Whoever dealt sufficiently long with IPGs must have been deeply disappointed by at least one expired batch of expensive chemicals. Our personal experience was made on an Immobiline pK 9.3 which had most likely lost its ability to bind the polyacrylamide by still keeping titrating properties.
In summary, our current use of IPGs is in solving some special problems of stressed resolution, such as in PIM4 typing. We do expect for the future that such a powerful tool be mended from its mistrustful features: lowest conductivity and strong defocusing effect of narrow gradients, chemical insta

bility in gels and in solution.

References

1) Bjellqvist P, Ek K, Righetti P. G, Gianazza E, Görg A, Westermeier R, and Postel W. J. Biochem. Biophys Methods, 6:317-339 (1982)
2) Gorg A, Postel W, Weser J, Weidinger S, Patutschnick W, and Cleve H. Electrophoresis ,4:153-157 (1983)
3) Weidinger S, and Cleve H. Electrophoresis,5:223-226 (1984)
4) Pascali V L, and Conte G.Electrophoresis,(1985)in press
5) LKB Application Note N.324 (1984)
6) Righetti PG, Ek K, Bjellqvist B, J.Chromatogr.291:31-42 (1984)

VLP and AC thankfully acknowledge Prf.E.Villanueva and Prof. A.Fiori, who encouraged a collaboration of the two lab.staffs during the Vth Course of Instrumental Techniques applied to Forensic Sciences (University of Granada, Feb.1985).

Fig.1. Resolution of Antitrypsin subtypes on a shallow immobilized pH gradient (interval of pH 4.35-4.85, anode on top). From left: M2, M1M2, M1, M1, M1M3, M1, M1M3, M1M3, M1M2, M1, M1M2, M2M3