PI M5: AN ADDITIONAL ALPHA-1-ANTITRYPSIN PI M SUBTYPE REVEALED BY IEF WITH IMMOBILIZED PH GRADIENTS

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Introduction

Alpha-1-antitrypsin (α_1AT) is an important protease inhibitor (PI) capable of inhibiting a wide variety of serine proteases. Since the first description of genetic variation in 1963 (1) more than 60 genetic variants of this protease inhibitor have been observed (2). For classification of PI variants isoelectric focusing (IEF) has been employed since ten years. IEF has been particularly useful in identifying subtypes of the most common variant PI M (3-9).

For generating stable pH gradients a modification of the IEF method has been developed (10). Immobilized pH gradients (IPG) represent an entirely new concept in electrofocusing. Unlike Ampholine carrier ampholytes, the Immobilines are not amphoteric, so they cannot migrate electrophoretical to an equilibrium position in IEF. Since in IPG the pH drift is completely eliminated it is possible to produce extremely narrow pH ranges (0.01 pH-unit/cm) in polyacrylamide gels by mixing Immobilines with different pK values.

The application of this technique to the PI system has permitted the distinction of ten common PI M subtypes (11). In this report, an additional subtype will be described.

Materials and Methods

For classification of PI phenotypes fresh sera or sera stored at -20 °C were used. Two different IEF-methods were employed:

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1. IEF experiments with ampholytes were carried out on thin-layer polyacrylamide gels with the pH-range of 4.2 - 4.9 according to Weidinger et al. (12).
2. IEF with an immobilized pH gradient was performed with a pH-range of 4.45 - 4.75 (11).

Results and Discussion

Classification of PI phenotypes is routinely carried out by IEF with ampholytes. Figure 1 shows several PI types as obtained by IEF with Pharmalytes (pH 4.2 - 4.9). Separation with this pH-range reveals the different PI m-regions called m4, m6, and m8 (13). The phenotypes of

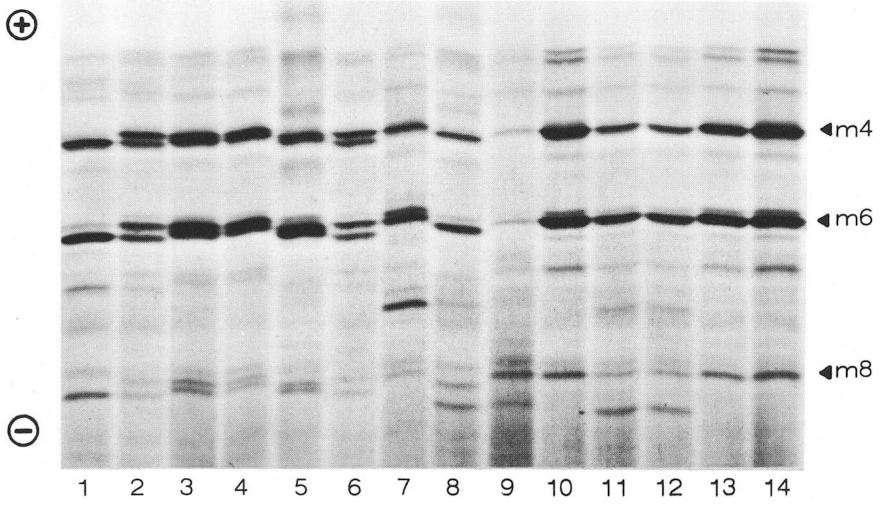

Fig. 1. Demonstration of PI phenotypes as analyzed by IEF with carrier ampholytes (pH-range 4.2-4.9). From left to right: (1) M2, (2) M1M2, (3) M1M4, (4) M1M3, (5) M2M3, (6) M1M2, (7) M1 S, (8) M3 Z, (9) M1 Z, (10) M1, (11) M1 Zaug, (12) M1 Zaug, (13) M1, and (14) M1

two persons with the deficient allele PI*Z augsburg (PI*Zaug) are also illustrated and compared with the common PI Z. Although the six common PI M subtypes (M1, M1M2, M2, M1M3, M2M3 and M3) can readily and reliably classified, the differentiation of M3 and M4 by conventional IEF

has been a problem.

Recently, IEF with immobilized pH gradients (IPG) has been employed for the analysis of PI variants (14). By using a special IPG an improved resolution of PI M subtypes was obtained (11). This modified procedure

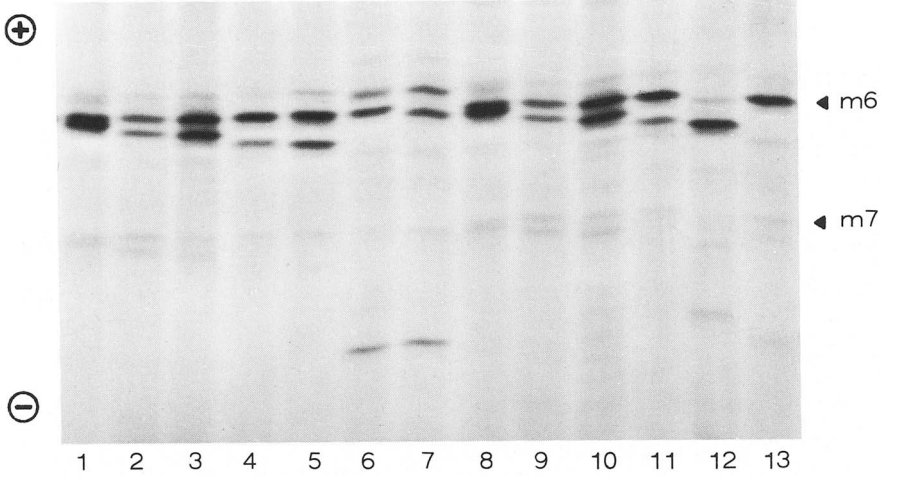

Fig. 2. Demonstration of PI M phenotypes as analyzed by IEF with immobilized pH gradients (pH-range 4.45 - 4.75). From left to right: (1) M1M5, (2) M1M3, (3) M1M4, (4) M1M7, (5) M1M2, (6) M1 S, (7) M5 S, (8) M1M5, (9) M1M3, (10) M1M4, (11) M1M7, (12) M2 Z, and (13) M1 Zaug

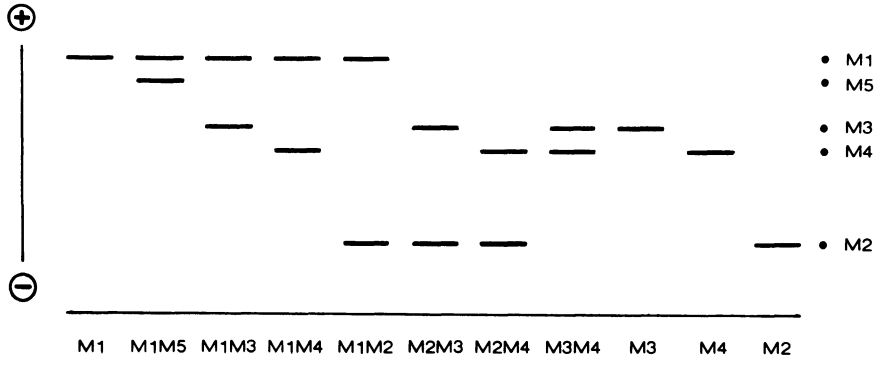

Fig. 3. Schematic representation of PI M subtypes

permitted the distinction between M3 and M4, and quite recently, also

between M1 and M5.

The newly observed subtype, Pl M5, is located between M1 and M3. It was noted in a mother and her child who had the phenotypes M1M5 and M5S, respectively (Fig. 2 No.8 and No.7). The defendant of this case of disputed paternity had the type Pl M1. He was excluded in the Pl system as well as in five other blood group systems. The distance between M1 and M5 bands is approximately 0.006 pH-units only. If a gradient as narrow as 0.3 pH-units is employed M1M5 can be noted as a double band pattern.

Figure 2 shows a further new subtype, previously called Pl M7. The allele products of Pl*M7 are located between M2 and M4. The inheritance was confirmed by family study.

Figure 3 gives a simplified schematic representation of different Pl M subtypes, including the new type M1M5. The number of subtypes has now reached seven; their sequence from anode to the cathode is in the order: M6, M1, M5, M3, M4, M7, and M2.

In conclusion, we feel that IEF with immobilized pH gradients offers certain advantages over several procedures which have been used for Pl subtyping. The possibility to produce very narrow stable pH gradients ensure high resolution and provide, thus, a powerful tool for the study of genetic variability.

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