Agarose Isoelectric Focusing for Classification of Plasminogen - Variants

H.-J. Leifheit, J. Howe, A.G. Gathof

Blood Transfusion Service of the Bavarian Red Cross, Munich, FRG

Introduction

Plasminogen (PLG) is the inactive precursor of plasmin. It is a plasmaprotein of the ß-fraction, the quantity in serum varies from 6 - 25 mg/dl. PLG has a molecular weight of 91.000 (Summaria et al. 1976), the part of carbohydrate is 1.5 % (Hayes, Castellino 1979).

The genetic polymorphism has been demonstrated by different electrophoretic methods and immunological or functional detection techniques. It was first described by Hobart (1979) and Raum et al. (1980). The nomenclature followed the proposal of a recent international workshop (Skoda et al. 1986). The common alleles are PLG*A and PLG*B. The variants were classified into three groups: the acidic A- variants, the basic B-variants and the intermediate M-variants.

In this paper the PLG-polymorphism was examined by isoelectric focusing on agarose gels with subsequent immunofixation (Leifheit et al. 1987). We examined a sample of 1344 individuals. We attempted to demonstrate the high resolving power of our classification procedure, in particular, for the separation of the rare variants.

Material and methods

Before electrophoretic separation the sera from 1344 healthy unrelated blood donors from the Blood Transfusion Service of the Bavarian Red Cross were treated with neuraminidase (CPN, Boehringer).

Enzyme treatment:	0.04 U/50 µl serum for 2 hrs at 37 °C
Gel composition:	0.8 % agarose IEF (Pharmacia) 10 % sorbitol dissolved in 18.6 ml dest. water ampholites (LKB) 0.7 ml pH 3.5 - 9.5 0.7 ml pH 5.0 - 8.0
Electrode solution:	anode: 0.25 m acidic acid cathode: 0.25 m NaOH
Application:	8 µl sample on the anodal side of the gel

Advances in Forensic Haemogenetics 2
Edited by W.R. Mayr

Separation:	cooling temperature: 8 °C 30 min prefocusing, setting 8 W 30 min salt run, setting 0.8 W 75 min setting 1.200 V, 50 mA, 8 W
Immunofixation:	90 min at 37 °C with 1 : 3 diluted PLG-antiserum (Atlantic Antibody)
Pressing:	20 min with filterpaper
Washing:	over night in saline
Drying:	with a heating plate
Staining:	30 min with 0.5 % Coomassie brillant blue solution

Results and discussion

Figure 1 presents the PLG-phenotypes as obtained by IEF on agarose gels followed by immunofixation. The alleles PLG*A and PLG*B determine the three common phenotypes. In addition rare phenotypes are illustrated. The A1, A2 and A3 are arranged in steps. A3 is combined with the common types A and B; A2 and A1 is shown with B. Another variant was observed in the cooridore between A1 and A designated provisionally A.90. In Fig. 1 this variant is demonstrated with both common types (AA.90 is the mother of BA.90). Also the M2- and M4- variants are combined with A and B. The B1-phenotype is slightly displaced to the cathode. The banding pattern of B3 shows a displacement of two bands below the cathodal B-band. All rare phenotypes were confirmed by family investigations.

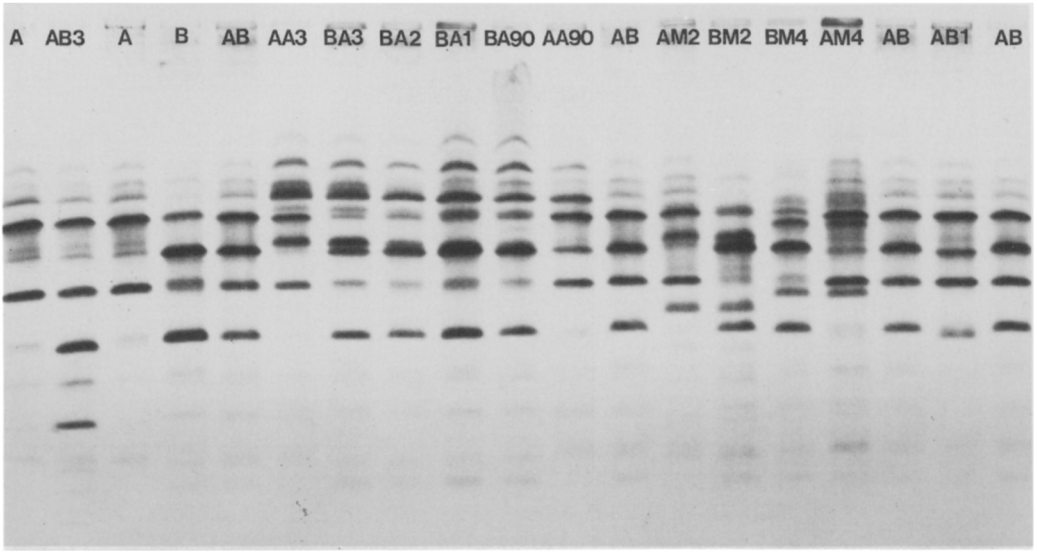

Fig. 1: Isoelectric focusing on agarose gels and subsequent immunofixation of PLG phenotypes. Anode on top.

The distribution of the common PLG phenotypes, the PLG variants and the allele frequencies in our population are exhibited in table 2. The allele frequencies of the common alleles are in accordance with the results of other authors (Hobart 1979, Raum et al. 1980, Kühnl und Spielmann 1982, Mauff et al. 1983, Dykes 1983, Weidinger et al. 1985). The variants show a slightly higher frequency. The PLG type A3 was observed fairly often. It is expected that also the homozygote type PLG A3 will be occasionally encountered in our population.

Table 1: Distribution of PLG-phenotypes and allele frequencies in Southern Germany

Phenotypes	observed		expected		allele frequencies
	n	%	n	%	
PLG A	680	50.59	687.1	51.12	PLG*A = 0.7150
AB	541	40.26	527.0	39.21	
B	94	6.99	101.0	7.52	PLG*B = 0.2742
AA.90	1				
AA1	3				
AA3	10				
AM2	4				
AM4	1				
AB1	1				
AB3	1				
		2.61	28.7	2.14	PLG*Var = 0.0108
BA1	1				
BA2	1				
BA3	3				
BM2	1				
BM4	1				
Total	1344	100.0	1343.8	99.99	

$Chi^2 = 0.9342$

References

Cortivo P, Caenazzo L, Crestani C, Scorretti C, Benciolini P, Ponaro E (1986) The polymorphism of plasminogen (PLG) by ultra-thin-layer isoelectric focusing, Distribution in the Veneto population (Italy). Z Rechtsmed 96 (4), p 275.

Dykes DD (1983) Distribution of plasminogen allotypes in eight populations of the Western Hemisphere. Electrophoresis 4, p 417.

Eiberg H, Mohr J, Nielsen (1981) Genetics and linkage relations of plasminogen. Clin Genet 19, p 500.

Hayes ML, Castellino FJ (1979) Carbohydrate of the Human Plasminogen Variants I, II, III. J. Biol. Chem. 254, p 8786.

Hitzeroth HW, Skoda U, du Toit E, Mauff G (1986) The plasminogen polymorphism in South African Negro populations: genetics and anthropogenetics. Hum Genet 74, p 341.

Hobart MJ (1979) Genetic polymorphism of human plasminogen. Ann Hum Genet 42, p 419.

Hummel K, Jäger H, Krüger HJ (1983) Bestimmung der Phänotypenhäufigkeiten im Plasminogen (PLG)-System bei Einwohnern der Stadt Mexiko. Ärztl Lab 29, p 198.

Kühnl P, Spielmann W (1982) Erfahrungen mit neueren Blutgruppensystemen für die Vaterschaftsbegutachtung: FXIIIA und FXIIIB, PLGN, AMY2, GAA, GDH, PGM1-Thermostabilitätsvarianten. Ärztl Lab 28, p 367.

Leifheit H-J, Gathof AG, Cleve H (1987) Plasminogen (PLG) - Typisierung mittels isoelektrischer Fokussierung auf Agarose-Gelen und Immunfixation. Ärztl Lab 33, p 10.

Mauff G, Erfurdt U, Pulverer G (1981) The application of human plasminogen (PLG) polymorphism to paternity testing. Lecture 10th Int Cong Forensic Hemo, p 531.

Nakamura S, Abe K (1982) Genetic polymorphism of human plasminogen variants and relationship between plasminogen phenotypes and their biological activities. Hum Genet 60, p 57.

Nishigaki T, Omoto K (1982) Genetic polymorphism of human plasminogen in Japanese: Correspondence of alleles thus far reported in Japanese and difference of activity among phenotypes. Jpn J Hum Genet 27, p 341.

Nishimukai H, Shinmyozu K, Tamaki Y (1986) Polymorphism of plasminogen in healthy individuals and patients with cerebral infarction. Hum Hered 36, p 137.

Raum D, Marcus D, Alper CA (1979) Genetic control of human plasminogen (PLG). Clin Res 27, p 458.

Raum D, Marcus D, Alper CA (1980) Genetic polymorphism of human plasminogen. Am J Hum Genet 32, p 681.

Skoda U, Bertrams J, Dykes D, Eiberg H, Hobart M, Hummel K, Kühnl P, Mauff C, Nakamura S, Nishimukai H, Raum D, Tokunaga K, Weidinger S (1986) Proposal for the Nomenclature of Human Plasminogen (PLG) Polymorphism. Vox Sang 51, p 244.

Summaria L, Spitz F, Arzadon L, Boreisha IG, Robbins KC (1976) Isolation and Characterization of the Affinity Chromatography Forms of Human Glu- and Lys-Plasminogens and Plasmins. J Biol Chem 251, p 3693.

Weidinger S, Schwarzfischer F, Müller H, Cleve H (1985) Plasminogen (PLG): A Useful Genetic Marker for Paternity Examinations. Z Rechtsmed 94, p 165.