Isoelectric focusing of Orosomucoid in agarose gels

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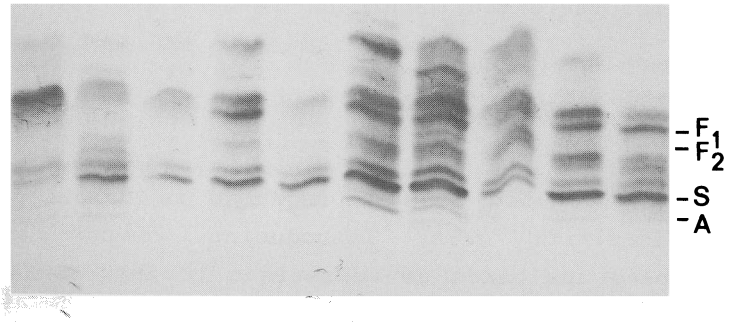
A genetically determined polymorphism of the plasmaprotein Orosomucoid has been originally demonstrated by Schmid et al., 1965. Isoelectric focusing of this protein in agarose was first published by Thymann and Eiberg in 1985. Meanwhile many investigators seemed to prefer polyacrylamide gels (Weidinger 1987, Wimmer et al., 1987) because of better separation. Agarose is in comparison a nontoxic technique. In our study we experimented with the immunoblot for detection of the samples.

Material and methods

We examined 468 samples of healthy, unrelated blood donors in southern Bavaria. The samples were treated with neuraminidase (CPN, Boehringer, Mannheim) 0.04U/50µl serum for 18h at 37°C. Gels were composed of 0.8% agarose (Pharmacia),10% sorbitol, 18.6ml aqua dest, 0.7ml ampholyte, pH 4.2-4.9 (Pharmalyte), 0.7ml ampholyte, ph 4.5-5.4 (Pharmalyte). As electrode solutions we used for the anode 0.25m acidic acid, for the cathode 0.25m NaOH. 8µl of sample (diluted 1:4) were applied cathodally. Separation was performed at 8°C, 30min prefocusing (setting 8W), 30min salt run (0.8W), 90min or more (8W).
Immunoblotting was done for 45min with a 0.45µm NC-membrane. For blocking and washing (3 x 10min) 5% skim milk powder in 0.1m PBS, pH 7.3, was used. The first antibody, a 1: 500 dilution of acid-α_1-Glykoprotein (ORM) from goat (AHS) was applied for 14h at room temperature, followed by 3 x 10min washing.
As second antibody a 1: 500 dilution of anti-goat from rabbit (AHS) was used for 2h at room temperature. For visualisation 30mg 4-chlor-1-naphtol in 10 ml ethanol and 20µl H_2O_2 in 50ml 0.1m PBS pH 7.3, were used.

Results

The separation of the different phenotypes is shown in 2 gels. Table 1 gives the the statistical analysis of our data.



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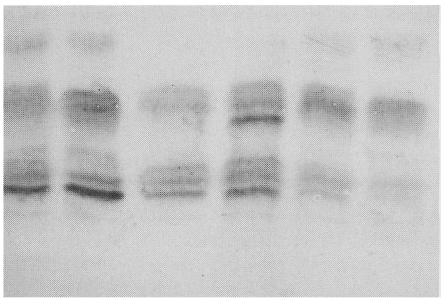

Table 1

phenotype	observed n	observed (%)	expected n	expected (%)
F_1	188	40.17	196	41.77
F_1 S	220	47.01	205	43.78
S	46	9.83	53	11.47
F_1 F_2	9	1.92	9	1.87
F_2 S	5	1.07	5	0.98
	468	100.00	468	99.87

$Chi^2 = 2,3486$; $p < 0,05$

Based on these results we calculated the allele frequencies: ORM1*F_1 = 0.6463, ORM1*S = 0.3387 and ORM1*F_2 = 0.0145. The theoretical chance for the exclusion of non-fathers was calculated to be 18.07%.

Discussion

The separation of Orosomucoid (ORM) in agarose is comparable to conventional polyacrylamid gels. Introducing immunoblot for detection has sharpend the bands in comparison to immunofixation. Also the costs for antibodies were reduced. Detection of weak

minor bands in places of bands from other phenotypes is a disadvantage of immunoblotting. In the begin of this series, differentiation between the phenotypes F_1 and F_2 was difficult in some cases. Better resolution through immunoblotting has improved this differentiation). Extended focusing times sharpend bands, but the minor bands also became more intense.
In contrast to other studies in southern Germany (Weidinger, 1987) our allel frequency for F_2 is a little lower. This might have technical reasons as mentioned above. Further studies with our improved method might solve this question.

Literature

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