Polymorphism of AHS-Glycoprotein: Comparison of different IEF and detection techniques

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INTRODUCTION

The human plasma protein alpha-2-HS-glycoprotein (A2HS, Schultze et al. 1962) was first described separately by Heremans (1960) and Schmid and Bürgi (1961). A2HS consists of one polypeptide chain with a molecular weight of 49000 D. The content of carbohydrate amounts to 13.4% (Schmid/Bürgi 1961) and of sialic acids to 4.1% (Putnam 1975). Lewis/Andre 1978).
A genetic polymophism of this protein was reported by Cox and Andrews (1983) with the existence of three alleles (A2HS*1,2 and 3) at an autosomal locus. In 1984 Umetsu et al. and Weidinger reported the presence of further rare alleles. Eiberg et al. assigned in 1983 the A2HS locus to human chromosom no.3. Our aim was to present a routine method, giving a clear separation of all different gene products and to give results on family and population studies of Southwestern Germany.

MATERIALS AND METHODS

Blood was obtained from healthy donors and their families. EDTA-plasma was stored at -30 deg.C until used. The following different techniques were examined. IEF was always run in 260x125x 0.5 mm polyacrylamide gels.

1. IEF with immobilines in the pH-range 4-5, using native samples, detected by immunofixation (Klett 1985).

Polyacrylamide gel: 2.5 ml 29.1% Acrylamide (LKB)/ 0.9% N,N'-Methylenbisacrylamide (LKB), 0.751 ml immobilines pK 4.6 (LKB), 0.371 ml immobilines pK 9.3 (LKB), 9.278 ml A. bidest., 2.1 ml 87% glycerol (Merck), 0.130 ml 10% TEMED (Serva), 0.060 ml 10% APS (Serva). Polymerization at 50 deg.C for 60'.
Focusing: Ultraphor with 10 deg.C temp. of cooling water, anolyte: 0.025 M asparaginic acid, catholyte: 0.025 M glutamic acid, 15 ul of undiluted plasma were applied on 1.0x0.5 cm filter papers (LKB) 2 cm from the cathodal end for 6 hrs, running conditions: 5000 V,
25 mA, 5 W for 6 hrs and 2500 V, 25 mA, 5 W for further 14 hrs.
Detection: Immunofixation with anti-human alpha-2-HS-glycoprotein (Behring, 1:1 in physiol. saline) in CAF (Schleicher/Schüll),

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print contact 1 h at 37 deg.C, CAF washed overnight, stained with 0.115% Coomassie Brilliant Blue G 250 (Serva) in destainig solution (25% ethanol, 8% glacial acetic acid in A. bidest.).

2. IEF with carrier ampholytes in the pH-range 4-6 in 2.5 M urea, using native samples, detected by Western-Blotting.

Polyacrylamide gel: 2.7 ml 28% Acrylamide (LKB), 1.2 ml 2% N,N'-Methylenbisacrylamide (LKB), 0.2 ml ampholytes ph 3.5 - 10, 0.8 ml ampholytes ph 4-6, 2.5 ml A. bidest., 2.5 M urea, 0.1 ml 3% TEMED (Serva), 1.0 ml 5% APS (Serva). Polymerization overnight at room temperature.
Focusing: Ultraphor with 10 deg.C temp. of cooling water, anolyte: 1M H PO , catholyte: 1M NaOH; 10 ul of undiluted plasma were placed on the gel surface with 0.5x0.5 filter papers (LKB) 1.5 cm from the cathodal end for 45', prefocusing without samples with 1500 V, 25 mA, 3 W for 30', with 1500 V, 25 mA, 5 W for 15', focusing 3 hrs.
Detection: Western-Blotting and EIA, passive transfer on Nitrocellulose Membrane NCM (Schleicher/Schüll, 0.45 um) for 40' at room temp., NCM washed in 0.05% Tween 20 (Merck) in PBS and blocked with 5% Albumin (Sigma) in PBS, reaction with the 1.antibody (rabbit anti-human alpha-2-HS-glycoprotein (Behring), 1:200 in 1% Albumin) and the 2. antibody (anti-rabbit immunoglobulin conjugated peroxidase (ATAB), 1:1000 in 1% Albumin) each for 1h at room temperature, visualization was performed by O-Toluidin dependent POD-reaction.

3. IEF with Pharmalyte in the pH-range 5-6 in 2.5 M urea, using desialyzed samples, detected by Western-Blotting.

This method, previously described by Umetsu et al. (1986), was modified by us concerning the following parameters. We changed the sample preparation, the running conditions, the anolyte solution and omitted glycerol for preparing the gel.
Polyacrylamide gel composition was the same as described in method 2 with the exception of the carrier ampholytes: 1 ml Pharmalyte pH 5-6, electrolyte solutions, running conditions and detection were as described above (method 2).
Enzyme treatement: 10 ul plasma with 20 ul neuraminidase (1mg/ml, Boehringer) were incubated at 37 deg.C in a moist chamber about 24 hrs, 9 ul of each sample were applied with 0.5x0.5 cm filter papers (LKB) at a distance of 2 cm from the cathode for 45'.

RESULTS AND DISCUSSION

Comparison of the different techniques shows, that separation of native samples by immobilines and by carrier ampholytes reveals series of A2HS bands. Detection of the different gene products seems to be best in the cathodal part of the proteinogram. The additional banding-patterns, designated by a star, are conforma-

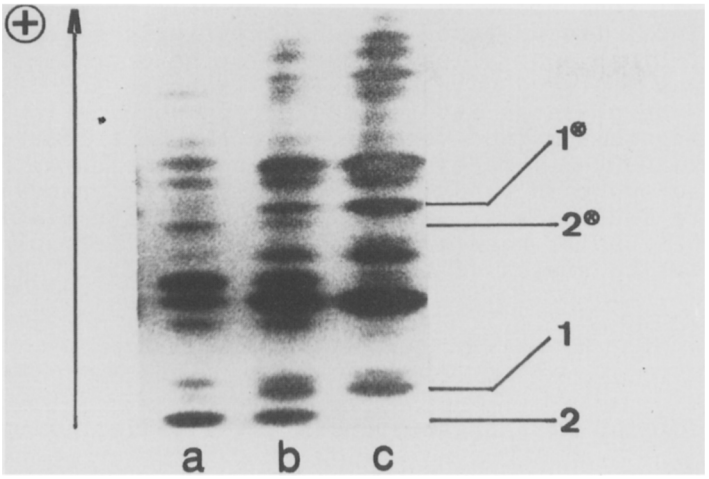

Fig.1. A2HS profiles in native samples, analyzed by IEF with Immobilines in pH 4-5, detected by immunofixation:
a = A2HS : 2
b = A2HS : 2-1
c = A2HS : 1

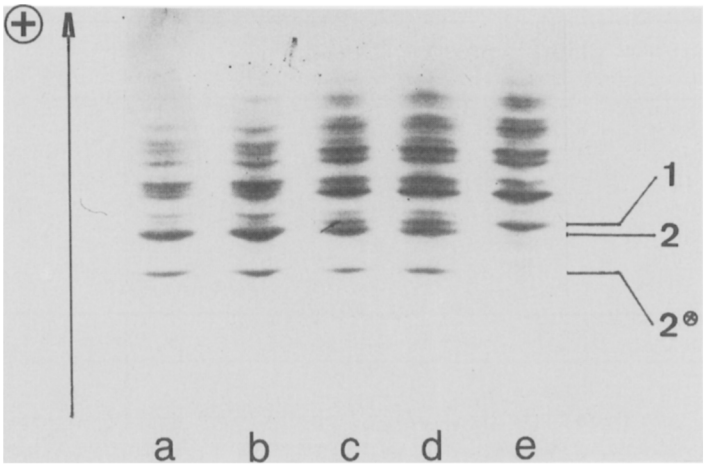

Fig.2. A2HS profiles in native samples, analyzed by IEF in 2.5 M urea with carrier ampholytes in pH 4-6, detected by Western-Blotting and EIA :
a,b = A2HS : 2
c,d = A2HS : 2-1
e = A2HS : 1

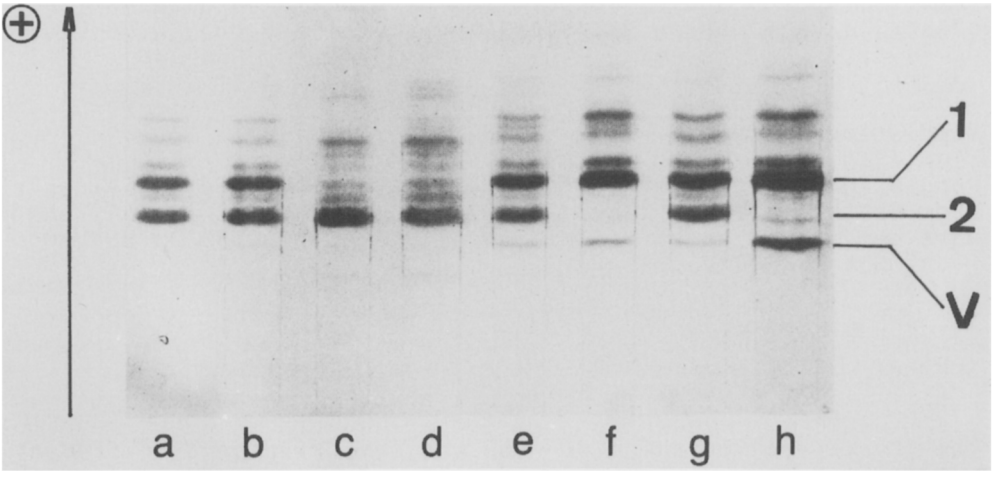

Fig.3. A2HS profiles in desialyzed samples, analyzed by IEF in 2.5M urea with Pharmalyte in pH 5-6, detected by Western-Blotting and EIA : a,b,e,g=A2HS:2-1; c,d=A2HS:2; f=A2HS:1; h=A2HS:1-V

tions of the original gene products. Method 1 as well as method 2 reveal series of bands, the distance between the single bands being fairly small. Clear separation of the major gene products a2hs*1 and a2hs*2 was improved using desialyzed samples and IEF with 2.5 M urea in the pH-range 5-6 (method 3). In combination with Western-Blotting this method seems to give the best results. Homozygotes A2HS 1 and A2HS 2 each show a single main band with different IEP. These two main bands can consequently be observed also in heterozygote individuals. So the results of desialyzation demonstrate that the complex banding patterns, seem to be caused by different sialyzation status of the A2HS gene products.

Formal genetics

Tab.1. Segregation of the A2HS phenotypes in 124 families (Klett 1985).

mating type	number of fam.	ch.	phenotypes of children 1	2-1	2	4-1	4-2	x^2	df
1x1	25	84	84						
1x2-1	39	108	44	64				3.704	1
1x2	14	40		40					
2x1-2x1	22	66	22	32	12			3.091	2
2x2-1	20	57		33	24			1.421	1
2x2	2	7			7				
4-1x2-1	2	6	2	1		1	2	0.667	3
Total	124	368	152	170	43	1	2	8.883	7

Tab.1 presents the segregation analyzes, performed in 124 families including 368 children from Southwestern Germany. The results, showed above, support the hypothesis "3 alleles (a2hs*1, a2hs*2, a2hs*4) at an autosomal locus" (x^2 = 8.883, 0.70-P-0.80, df=7).

Population genetics

Population studies were conducted on 249 unrelated individuals from Southwestern Germany. The allele frequencies in this sample were determined to be : A2HS*1=0.631, A2HS*2=0.363, A2HS*V=0.006 (V=A2HS*4 + A2HS*Var.).

SUMMARY

The genetic polymorphism of A2HS was investigated by different methods. The technique, using desialyzed plasma, Pharmalytes in the pH-range 5-6 and 2.5 M urea, followed by Western-Blotting,

gives efficient and clear separation.
The formal genetic data confirm an autosomal codominant transmission of the alleles. The frequencies in Southwestern Germany are:
A2HS*1=0.631, A2HS*2=0.363, A2HS*V=0.006.

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