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ICROPROCEDURE TO DETERMINE THE POLYMORPHIC FORMS OF CID α_1-GLYCOPROTEIN IN PLASMA

PPLICATION TO DEPRESSIVE PATIENTS TREATED WITH MITRIPTYLINE

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UMMARY

The polymorphic forms of α_1-acid glycoprotein have been determined by isoelectric ocusing of small samples of whole plasma, without prior isolation of the protein. The results btained by this technique confirm the microheterogeneity of this glycoprotein, which is ot due to artefacts. Densitometric measurements of the polymorphic forms of this protein, hich binds antidepressive drugs, have been performed in twelve depressive patients.

NTRODUCTION

The general biological and clinical significance of glycoproteins has been eviewed extensively [1, 2]. In psychopharmacology, some research has been ocused on the α_1-acid glycoprotein. Indeed, the most commonly used anti-epressive drugs are bound in blood to this protein [3].

α_1-Acid glycoprotein (AAG) is heterogeneous in the native state [4]. On arch gel electrophoresis, the native protein exhibits four different patterns, ith 5, 6, 7 or 8 bands [5]; the same patterns are observed on polyacrylamide el isoelectric focusing (PAGIF) [6]. This heterogeneity is due to different nkages of sialic acids to galactose residues of the oligosaccharide side-chain nd to different loci of the oligosaccharide chains linked to the protein [4]. hese different linkages modify the p*I* of the protein and produce the poly-orphic forms of AAG. This polymorphism appears to be genetically ansmitted and different races exhibit different percentages of the poly-orphic forms [7]. Until now AAG had to be isolated from large volumes of

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plasma prior to PAGE or PAGIF analysis; moreover, it is only recently that the polymorphic forms have been quantitated [6].

The present paper describes a simple method for the determination of polymorphic forms by direct analysis in only 20—50 μl of total plasma, without prior isolation of AAG. Results on the distribution and the quantitation of polymorphic forms in depressive patients are presented.

MATERIAL AND METHODS

Acrylamide (twice crystallised), N,N'-methylene bisacrylamide (twice crystallised) and Coomassie Brilliant Blue R-250 were from Serva (Heidelberg, G.F.R.). Ampholines were purchased from LKB (Bromma, Sweden). All other chemicals were of analytical grade and obtained from E. Merck (Darmstadt, G.F.R.).

Polyacrylamide gel slabs with a pH gradient of 2.5—4.2 are prepared according to ref. 8. PAGIF is performed using an LKB Multiphor, with a constant power of 15 W and a run time of 3 h.

A 20—50 μl volume of heparinised blood is applied at the cathodic end of the gel using small pieces of filter paper, which are removed after 1 h of focusing.

At the end of the analysis the pH gradient is measured across the gel using a surface electrode (Metrohm, Herisau, G.F.R.) at 15°C.

The gels are fixed, stained and preserved according to ref. 8, with a modification to the staining period which is lengthened to 1 h instead of 10 min.

Densitometric measurements are performed using a Beckman Densitometer R-112.

Subjects

The development of the method has been carried out with plasma obtained from the Swiss Red Cross (Lausanne, Switzerland) and with blood samples from healthy subjects. Twelve depressive patients treated for three weeks with 150 mg of Laroxyl® (amitriptyline) participated in a clinical study. The pharmacological treatment started on day 1 after the first blood sampling.

Preliminary experiments have been performed according to those proposed by Giannazza and Righetti [9] in order to detect an eventual presence of artefacts.

RESULTS AND COMMENTS

Artefacts

Four tests were performed to detect the possible presence of artefacts: (1) PAGIF with and without prefocusing; (2) PAGIF with different Ampholine concentrations; (3) PAGIF in 8 *M* urea; and (4) two-dimensional PAGIF.

Identical results are obtained when PAGIF is performed, with the same sample, according to tests 1—3, demonstrating the absence of artefacts. Two examples illustrate experiment 1 (Fig. 1a and b) and experiment 2 (Fig. 2a and b).

The results of two-dimensional PAGIF (experiment 4) (Fig. 3) clearly show

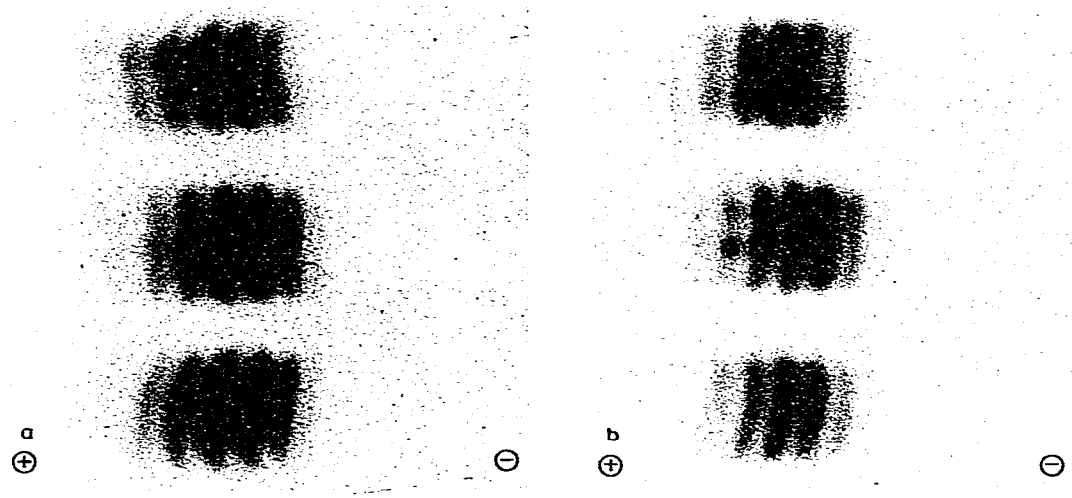

Fig. 1. Test for artefacts. Comparison of two PAGIF analyses of the same plasma sample, without prefocusing (a) and with prefocusing for 1 h (b). Conditions: 3 h focusing at 10°C, or 1 h prefocusing and 2 h focusing, with a constant power of 15 W.

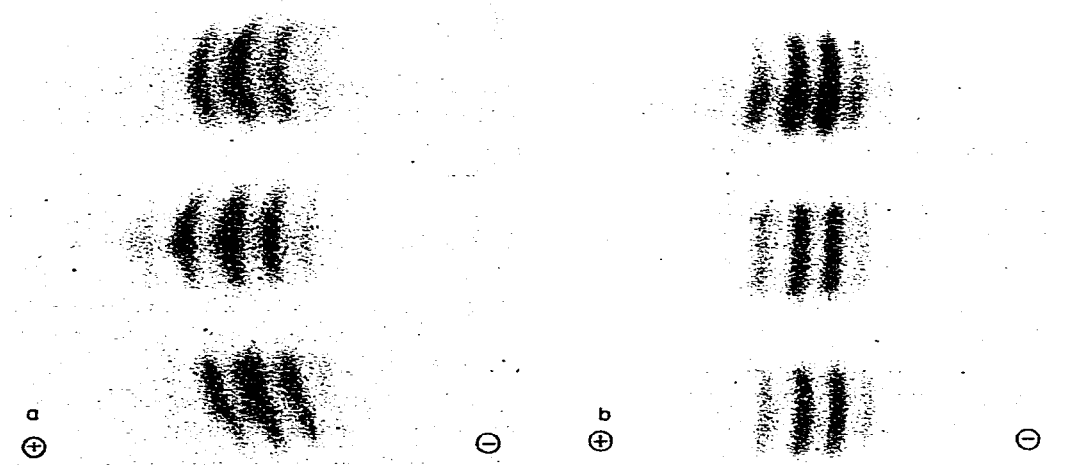

Fig. 2. Test for artefacts. The same plasma sample is focused with two different Ampholine concentrations: 5% Ampholine (a) and 2.5% Ampholine (b). Conditions: 3 h focusing at 10°C, with a constant power of 15 W.

the absence of artefacts. If artefacts were present, some bands in the second dimension would not be aligned diagonnally with the others (cf. ref. 9).

pH gradients

Experimental gradients are usually 0.1—0.8 unit higher than the theoretical ones. This difference is not constant along the whole length of the gel slab; there might be differences of 0.1—0.3 unit between the gradients measured at the top and those measured at the bottom of the gel (Fig. 4). Therefore, in all

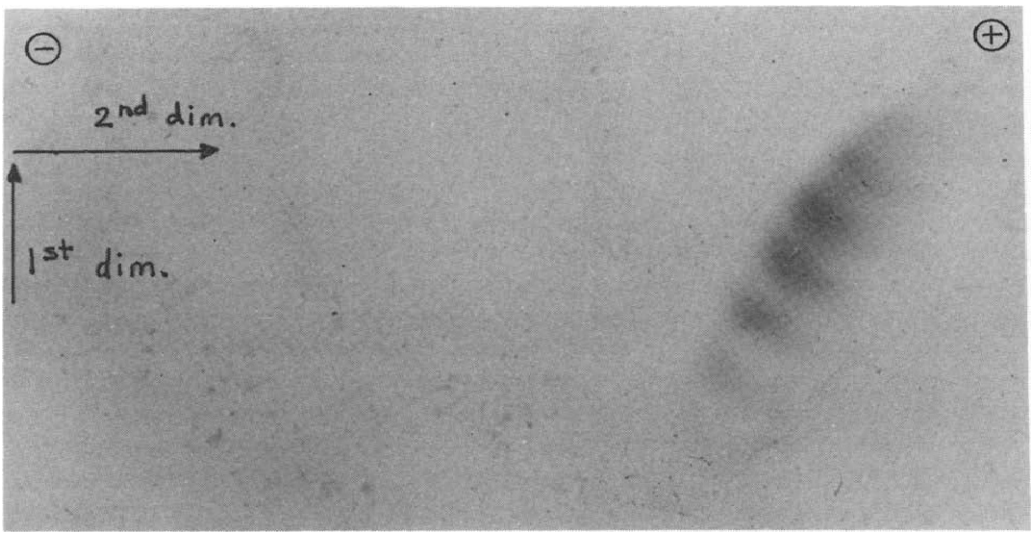

Fig. 3. Test for artefacts. Polymorphic forms of AAG after two-dimensional PAGIF. Conditions: 2 h focusing in the first dimension and 2 h in the second at 10° C, with a constant power of 15 W.

subsequent experiments, at least three sets of pH measurements will be performed, usually at 5, 12 and 20 cm from the top of the gel. For each set, ten pH measurements are performed every cm across the gel.

Polymorphic forms of AAG

Polymorphic forms have been determined in twelve depressive patients in a three-week study.

Fasting blood samples were drawn at days 1, 8, 15 and 22, and the total plasma samples were analysed by PAGIF. For each patient, the p*I* values and the relative intensity of the individual bands were determined.

The patients can be classified in three groups from the number of bands of the polymorphic patterns: group 1 — two subjects with 6 bands; group II — four subjects with 7 bands; group III — six subjects with 8 bands. An example of each of the three phenotypes is presented in Fig. 4.

In each patient, the number of bands and the p*I* of the individual bands are constant for all four determinations. In Fig. 5 the average p*I* values of polymorphic forms for the twelve subjects are shown. It seems from Fig. 5 that it is not possible to decide which bands are identical interindividually. For this reason, no means of the p*I* values were calculated within the group.

The average relative intensities of the bands for the twelve subjects are represented in Table I; as for the p*I*, the intensities are constant for the four analyses. This is consistent with the assumption that the polymorphism of AAG is genetically determined, and that the polymorphic forms are synthesised in the same ratios.

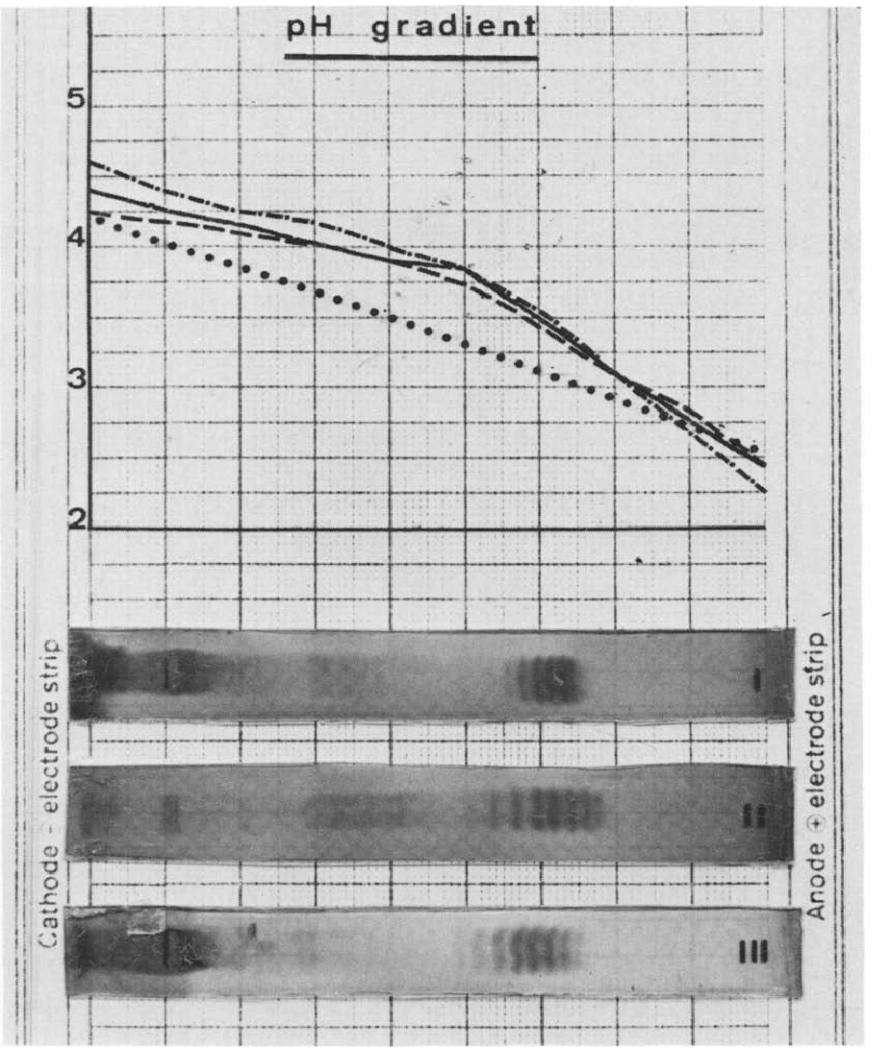

Fig. 4. Experimental pH gradient and polymorphic forms of AAG. Above: the pH is measured every cm with a surface electrode, from the top of the gel: ——, gradient at 5 cm; — · —, gradient at 12 cm; — — —, gradient at 20 cm, · · ·, theoretical gradient. Below: comparison of the three polymorphic forms of AAG: I = pattern with 6 bands; II = pattern with 7 bands; III = pattern with 8 bands.

CONCLUSION

PAGIF has proved to be a very valuable technique for the determination of polymorphic forms of AAG in total plasma samples. It is shown that AAG needs not to be isolated from plasma prior to focusing; its very acidic p*I* allows its complete separation from other plasma proteins on a gel with a pH gradient of 2.5—4.2.

With home-made gels the formation of the pH gradient is highly reproducible. Moreover, the deviation from theoretical gradient never exceeds

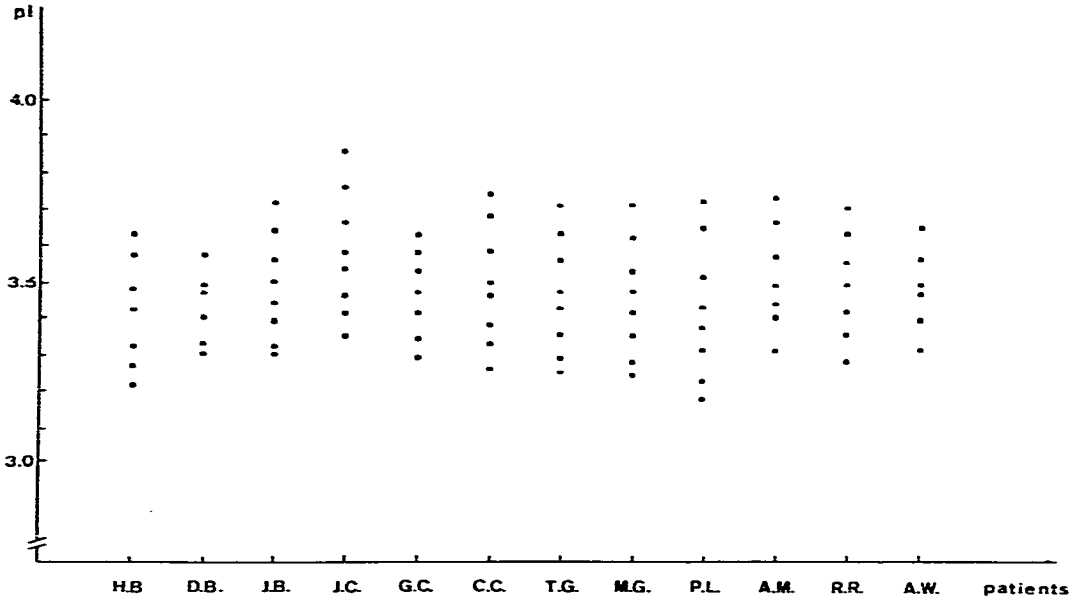

Fig. 5. Average p*I* values of the polymorphic forms of AAG in twelve depressive patients.

TABLE I

AVERAGE RELATIVE INTENSITIES (%) OF POLYMORPHIC FORMS OF AAG IN TWELVE DEPRESSIVE PATIENTS

Patient	No. of bands							
	1	2	3	4	5	6	7	8
H.B.	4.2	6.9	11.3	19.9	24.5	20.1	14.5	
D.B.	3.6	8.4	15.3	25.3	27.5	19.9		
J.B.	2.7	4.6	8.8	18.2	21.4	24.7	14.3	5.8
J.C.	3.8	5.0	10.2	16.9	20.5	23.3	14.4	6.1
G.C.	3.0	5.9	11.5	23.1	27.1	17.8	11.8	
C.C.	4.2	5.0	7.8	19.8	23.9	24.9	11.5	3.5
T.G.	2.8	6.7	11.8	16.9	25.0	20.2	11.2	5.1
M.G.	3.6	6.5	9.8	17.2	24.9	20.3	11.0	6.7
P.L.	1.1	3.4	8.3	15.2	21.8	23.6	17.0	
A.M.	1.3	4.5	11.6	21.0	25.3	23.0	15.1	
R.R.	3.6	9.0	19.8	30.7	21.6	11.2	4.1	
A.W.	3.3	9.2	19.0	30.6	26.0	29.0		

0.8 pH unit, i.e. 15%, which is satisfactory for home-made gels. Experimental pH gradients are always more basic than in theory, except at the anode where they are more acidic; the maximum anodic deviation is 0.34 pH unit, i.e. 16%. Other experiments would be necessary in order to determine if this deviation is due to cathodic drift [10] or if it is due to an incomplete formation of the pH gradient, which would not be achieved in 3 h.

The experimental pH gradients are not linear along the whole gel; it is there-

fore necessary to perform at least three series of pH measurements, one at the top, one at the middle and one at the bottom of the gel.

Tests for artefacts show that none are formed during PAGIF of total plasma samples.

The determination of polymorphic forms in twelve patients during a 3-week period reveals no intraindividual differences in the number and in the relative concentrations of polymorphic forms. For each subject, the polymorphic pattern and the relative intensities of bands are conserved, even when total AAG plasma levels rise [12]. This observation confirms that the polymorphic forms of AAG are genetically determined.

The present results complete earlier findings, where qualitative analysis gave evidence of at least seven polymorphic forms with quantitative evaluation [11]. It is difficult to compare our densitometric data with those of Berger et al. [6], as comparative measurements of the p*I* values of the corresponding bands are missing. Until a method exists for a more complete chemical characterization of the bands, assignment of a number to the bands is rather arbitrary.

With regard to the binding of the antidepressive drug amitriptyline to AAG, nothing is known about the possibility that one polymorphic form would bind this drug more than another. If this were the case, interindividual differences could occur in the binding of antidepressive drugs as the number of bands vary between subjects.

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