Genetic Polymorphism of Urinary Pepsinogen (PGA) Detected by Immunoblotting

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INTRODUCTION

Pepsinogen is classified into two immunochemically distinct groups, PGA and PGC. PGA is detected in serum and urine as uropepsinogen, and while PGC is present in serum and seminal fluid. Genetic models of PGA have been proposed by several investigators (Samloff and Townes 1970; Taggart et al. 1979; Korsnes and Gedde-Dahl 1980; Frants et al. 1984), who have used all the proteolytic properties of acid-activated PGA for the detection of the isozymogens on agar or polyacrylamide gels. However, the complexities of these techniques have yielded a number of conflicting results, leading to various different genetic models.

In the present work, we describe a new technique utilizing polyacrylamide gel isoelectric focusing (IEF) followed by immunoblotting with anti-PGA antibody, which provides a better band resolution, and propose that a number of the uropepsinogen isozymogens with different pI values can be explained by the presence of one autosomal locus with two alleles (Kishi and Yasuda 1987).

MATERIALS AND METHODS

Purification of PGA for Immunization

PGA for immunization was purified from human urine mainly using the method described by Minamiura et al. (1984). Rabbit antisera against purified PGA were obtained as described previously (Kishi and Iseki 1983). A total of 4 mg of the purified PGA was injected into a rabbit.

Detection of PGA Isozymogens on IEF gels by Immunoblotting

The urine samples for IEF were concentrated by ultrafiltration, dialyzed against 0.1% glycine and then lyophilized according to the method described in our previous paper (Kishi et al. 1985). Five-microliter aliquots of a 0.5-1.0% solution of

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

freeze-dried urinary sample, corresponding to about 100 times the concentration of the original urine, were used for IEF analysis. After IEF, PGA isozymogens on IEF gels were detected by immunoblotting using anti-PGA antibody (Yasuda et al. 1987).

RESULTS

Phenotypic Variation and Nomenclature of PGA Isozymogens

The detection method involving IEF followed by blotting transfer to a Durapore (Millipore) strip and immune binding showed that PGA was generally separable into five fractions on the gel between pI 3 and 4, as shown in Fig. 1, and these fractions were named I to V in order of decreasing anodal mobility.

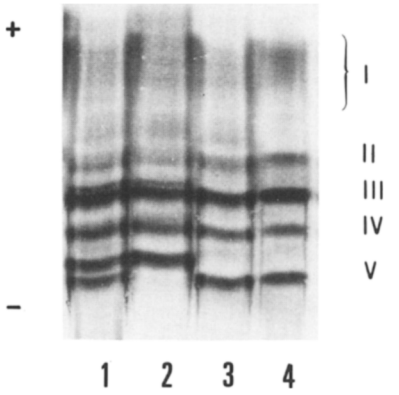

Fig. 1. Isoelectric focusing of urinary samples in polyacrylamide gel and visualization of uropepsinogen (PGA) by immune blotting with specific antibody. Samples were (1) FS, (2) F, (3) S, and (4) S.

Several subjects showed an additional band between IV and V, being nearer to V, as shown in Fig. 1. At this point it is convenient to introduce a new form of nomenclature: this band will be termed the PGA V F band, indicating that it is a fast-moving band, while the normal V band will be termed PGA V S, a slow-moving band. The F band was found to be present in 34 out of 258 Japanese subjects, while the S band was absent in 2 out of the 34 subjects having the intense F band (Table 1). Phenotype FS, containing both F and S bands, corresponds to a phenotype which is a mixture of two types, F and S. The distinct nature of the three discrete phenotypes in the V fraction remained apparent; the F band without the S band exhibited a higher intensity than the F band with S. Similarly, the S band without F had a higher intensity than S with F.

Table 1. Distribution of phenotypes and allele frequencies of human PGA V among unrelated Japanese

Phenotypes	Observed number	Observed frequency	Expected number	χ^2
S	224	0.868	223.2	0.0029
FS	32	0.124	33.5	0.0672
F	2	0.008	1.3	0.3769
Total	258	1.000	258.0	0.4470

Allele frequencies: PGA V*S=0.930, PGA V*F=0.070; d.f.=1; $0.7>p>0.5$

Table 2. Segregation of PGA V phenotypes

Mating	Number of families	Number of children	Children's phenotypes F	FS	S
F x FS	1	2		2	
FS x S	3	5		3	2
S x S	34	52			52
FS x N.T.[a]	1	3		2	1
S x N.T.[a]	1	3			3
Total	40	65	0	7	58

[a]Not tested because one parent was unavailable for this study.

Inheritance of PGA V F and PGA V S Bands

The family materials were matings with three PGA V-typed children. The inheritance of the isozymogen pattern was investigated by listing the numbers and phenotype categories of the offspring observed for each different type with regard to the presence or absence of each PGA band. Three of the theoretical six types of mating were observed among the three phenotypes.

S behaves as a Mendelian dominant, this being strongly supported by the absence of F among the 52 offspring of 34 S x S matings. The F band is likewise a clear Mendelian dominant, as shown in Table 2, since in an F x FS mating, each of the two types of children have received only one of the corresponding characteristics from each parent, PGA V*F from the father and PGA V*S from the mother, and therefore have no intense S band. We may conclude from the above observation that the presence of an F or S band is governed by at least one pair of alleles and by a structural gene dose effect.

DISCUSSION

Up to now, two major views have been advanced to explain the genetics of PGA: a locus system (Samloff and Townes 1970; Weitkamp and Townes 1975; Taggart et al. 1979; Frants et al. 1984) and a multilocus system (Korsnes and Gedde-Dahl 1980). Our study has provided evidence that one component of PGA, fraction V, is polymorphic with two alleles, F and S, at a single locus. Similar observation have not been reported by others previously, as the present results were obtained using a new combination technique involving both IEF and immunoblotting with PGA-specific antibody, which facilitated high resolution of the individual urinary pepsinogen isozymogens. According to our classification method based on the differences in pI values, rather than on the intensity levels of isozymogens, phenotype S is clearly distinguishable from F on the gel. Moreover, instead of a proteolytic activity test for the isozymogen, an immunoblot technique with specific anti-PGA antibody was used in this study. This immunological detection method facilitated discrimination of PGA isozymogens from other proteolytic enzymes like PGC and slow-moving protease (SMP).

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