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PARTIAL AMINO ACID SEQUENCE OF TWO FORMS OF HUMAN POST - Y - GLOBULIN

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SUMMARY

We have purified two different electrophoretic forms of Post-Y - globulin defined by electrophoresis as "native slow" form and "fast" form respectively. Amino acid sequences of the first fifty-two residues of the "native slow" form and twenty-nine of the "fast" form were determined. The sequence shows that the "fast" form lacks the first nine amino acids of the "native slow" form.

This observation is consistent with the existence of a "native slow" form that is degraded into other more acidic forms of Post-Y -globulin by loss of basic amino acids.

INTRODUCTION

Post- γ-globulin (Pγ), "Low molecular weight" protein of about 12.000 daltons, was first described in humans as a constant protein component of cerebrospinal fluid (1), and in urine from patients with tubular disorders. It has also been found in several fluids of normal humans : saliva, serum, urine, ascitic and seminal fluids, (2,3,4). Pγ occurs in several electrophoretic forms, especially after storage, although CEJKA (5), did not observe any variability in the mobility of urinary Pγ . "Slow, intermediate and fast"forms, showing complete antigenic identity have been described (6). These 3 forms were shown to have different NH_2 terminal amino acids but no difference in molecular weight was detected. The various forms of Pγ could be degraded upon storage into faster electrophoretic forms, such that the slowest form could become sequentially, the "slow" form, then the "intermediate" and the "fast" form.

We have isolated and purified a "fast" form as well as a new "slow" form which is slightly slower (more cathodal) than the "slow" form described previously (6), and which will be refered to below as the "native slow" form from now on. The first results of the sequence determination of these two forms are presented and discussed.

Post γ globulin : Pγ.
Sodium Dodecylsulfate : S.D.S.
High Performance chromatography : HPLC.

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MATERIAL AND METHODS

Preparation of Pγ .

Pγ was obtained from urine of a patient with a congenital tubulopathy. NaN_3 was used as preservative. As previously described (6), the P γ was purified through Biogel P100 followed by ion exchange chromatography on DEAE-Sephadex A50 (Tris-HCl buffer 0.05M pH 8.8). The purity was determined by electrophoresis in 5% polyacrylamide-agarose gel (7), immunoelectrophoresis (8), isoelectric focusing (9) and 7% S.D.S. polyacrylamide gel electrophoresis (PAGE) (10).

Oxidation, reduction and alkylation.

Disulfide bonds were oxidized with performic acid (11) or reduced with dithiothreitol ; in the latter case, the half cystinyl residues were alkylated with iodoacetamide (12).

Amino acid sequence determination.

The total amino acid content was determined after 24h and 72h hydrolysis of native and oxidized protein in 6N HCl. The amino acid analysis was performed on a BECKMAN 121M analyser.

Direct sequence analysis was obtained with the BECKMAN 890C automatic sequencer using 1M or 0.1M Quadrol with polybrene, according to Klapper et al (13). PTH derivatives were identified by High Performance Liquid Chromatography (HPLC) according to the following conditions : a 5 minute exponential gradient of methanol-acetate (gradient curve no 10) was initiated with the analysis (WATERS ASSOCIATES Liquid chromatograph); initial concentrations : 25% methanol, 75% 0.01M sodium acetate pH 4.5. ; final concentrations : 45% methanol, 55% sodium acetate. The overall run time was 16 minutes. An aliquot of each PTH derivative was hydrolyzed with HI (14), and the corresponding amino acid residue was identified on the amino acid analyzer.

RESULTS

Pγ was identified via a rabbit anti-Pγ serum, prepared and tested for monospecificity as previously described (6). The purity of the Pγ preparation was tested by gel immunoprecipitation, using a horse antiserum against total human serum. In addition, it was checked that no reaction occured against antiβ 2-microglobulin, anti lysozyme, anti κ and anti λ chains, nor against anti-immunoglobulin sera. The Pγ preparation showed one single band revealed by isoelectric focusing, or polyacrylamide-agarose or SDS polyacrylamide gel electrophoresis.

The native form migrated in one single band at the most cathodal end. The "fast" form appeared as a single band very distinct from the native form. The "slow and intermediate" forms appear in fig.1, at two positions slightly faster than that described above as the "native" form. Therefore we propose to refer to the native Pγ as the "native slow" form.

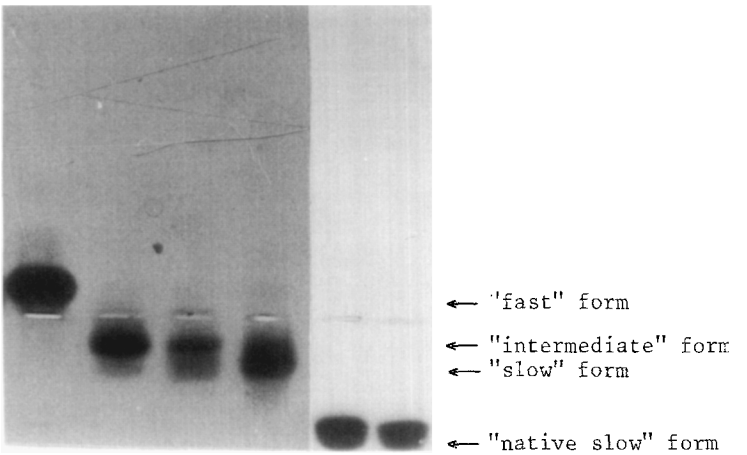

Fig.1 - Electrophoresis in 5% polyacrylamide-agarose gel of the four forms of Pγ.

1 - Amino acid composition.

As shown in table 1 the amino acid composition of the "native slow" form is a mean of three hydrolysis results at 24h and two hydrolyses at 72h. The values for Ser and Thr were obtained by extrapolation to zero time.

The amino acid composition of the "fast" form is the average of the results obtained from three 24h hydrolyses. In this case, 3% and 10% corrections (15) for threonine and serine residues respectively were made to correct for destruction during acid hydrolysis. The half cystine residues were determined as cysteic acid from the oxidized protein.

2 - NH_2 terminal amino acid sequence determination.

The two forms of Pγ were subjected to automatic Edman degradation, with the following results :

a - the "fast" form : 300 nmoles of oxidized protein were used without polybrene resin. The terminal amino acid was a Leucine and 24 residues were identified (see table 2).

b - the "native slow" form : 50 nmoles of reduced and carboxymethylated "native slow" Pγ were loaded into the sequencer, in the presence of polybrene. The first residue gave the PTH derivative, which eluted on HPLC at a position very close to tyrosine, but the back hydrolysis to free the amino acid did not yield a tyrosine. Since in our system as in other systems

TABLE I : Amino acid composition of the "native form" and "fast" form of Pγ mol/100mol amino acid.

Amino Acid	Pγ "native slow"	Pγ "fast"	Difference
Lysine	6.26	5.43	-0.83
Histidine	2.71	2.52	-0.19
Arginine	6.22	4.56	-1.66
Aspartic Acid	11.0 (1)	11.53 (2)	+0.53
Threonine	6.07	6.48	+0.39
Serine	6.72 (1)	6.10 (3)	-0.62
Glutamic Acid	10.42	11.63	+1.21
Proline	5.62	4.50	-1.12
Glycine	7.32	6.75	-0.57
Alanine	8.90	9.38	+0.48
Half Cystine	2.8 (4)	3.64 (4)	+0.84
Valine	7.85	7.92	+0.07
Methionine	3.38	2.93	-0.45
Isoleucine	1.02	1.63	+0.61
Leucine	6.71	7.11	+0.40
Tyrosine	2.92	3.14	+0.22
Phenylalanine	3.98	4.45	+0.47

(1) Values obtained by linear extrapolation to zero time.
(2) 3% correction was made to correct for destruction during acid hydrolysis (15).
(3) 10% correction was made to correct for destruction during acid hydrolysis (15).
(4) Analysis of the oxidized derivative : cysteic acid.

(16.17) the seryl residue occasionally gives a PTH derivative that behaves as the PTH - tyrosine, we propose that Ser is the NH_2-terminus. Residue no 2 was not determined. From residue 3 onward, 43 positions were identified, with a repetitive yield of 97%. (see table 2).

DISCUSSION

Comparison of the sequences of the"native slow" and "fast" forms of P γ demonstrated that the latter is probably derived from the former by a simple loss of an eight-residue peptide. We have previously determined the NH_2 terminal amino acid of an "intermediate" form of Pγ and the first two NH_2 terminal amino acids of the "slow" form obtained from another patient (6). The "slow" form was apparently derived from the "native slow" form by a loss of 4 amino acids. The presence of an arginyl residue at the NH_2 terminal of the "intermediate" form is suggestive that this form starts at residue no 8 of the native protein (see table 3).

TABLE 2

	1 5 10 15 20
"native slow" form	(Ser)-(X)-Pro-Gly-Lys-Pro-Pro-Arg-Leu-Val-Gly-Gly-Pro- X -Asp-Ala- X -Val-Glu-Glu- X -Gly-Val-
"fast" form	Leu-Val-Gly-Gly-Pro-Met-Asp-Ala-Ser-Val-Glu-Glu- X -Gly-Val-
Resulting sequence of the P_γ	
"native slow" form	(Ser)-(X)-Pro-Gly-Lys-Pro-Pro-Arg-Leu-Val-Gly-Gly-Pro-Met-Asp-Ala-Ser-Val-Glu-Glu- X -Gly-Val-

	25 30 35 40 45
"native slow" form	Arg-Arg-Ala-Leu-Asp-Phe-Ala- X -Gly-Glu-Tyr-AsN-Lys-Ala- X -AsN-Asp- X -Tyr- X - X -Arg-Ala-Leu-
"fast" form	Arg-Arg-Ala-Leu-Asp-Phe-Ala-Val-Gly- X - X -AsN- X -Ala-
Resulting sequence of the P_γ	
"native slow" form	Arg-Arg-Ala-Leu-Asp-Phe-Ala-Val-Gly-Glu-Tyr-AsN-Lys-Ala- X -AsN-Asp- X -Tyr- X - X-Arg-Ala-Leu

	50
"native slow" form	GlN-Val- X - X -Ala
"fast" form	
Resulting sequence of the P_γ	
"native slow" form	GlN-Val- X - X -Ala

NH_2 terminal amino acid sequence determination of "native slow" and "fast" forms of P_γ.

(1)numbering is for the "native slow" form.
X : amino acid not determined.

TABLE 3 : NH_2 terminal amino acid sequence comparison of four forms of P_γ

	1	2	3	4	5	6	7	8	9	10
"native slow" form	: (Ser)-	(-)-	Pro-	Gly-	Lys-	Pro-	Pro-	Arg-	Leu-	Val
"slow" form (1)					Lys-	Pro-				
"intermediate" form(1)(2)								Arg-		
"fast" form									Leu-	Val-

(1) results obtained previously (6).
(2) hypothetic position of NH_2 terminal amino acid.

Each degradation product is characterized by a loss of basic amino acids Lys_5, Arg_8. These data may account for the different electrophoretic forms, the loss of basic amino acids being responsible for the increase of electrophoretic mobility. These results suggest a high sensitivity of this protein to proteolysis. A contaminant amino peptidase, normally present in urine (18), may, perhaps, explain the modification of the P_γ during storage. However, this modification was also described for P_γ in cerebrospinal fluid, where an aminopeptidase activity has not yet been described. Previously (6) no proteolytic or esterase activity could be detected in any sample of P_γ using synthetic substrates or bovine serum albumin, but further tests must be used to check other enzymatic specificities.

Whatever the mechanism, the present results bring unequivocal evidence for the heterogeneity of the various forms of P_γ which can be derived from the native form.

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REFERENCES

1 - CLAUSEN, J. (1961) Proc. Soc. Exp. Biol. Med. 107 170-172.

2 - MANUEL, Y., LATERRE, EC. (1970) in "Proteins in normal and pathological urine" p153 (MANUEL, Y., REVILLARD, J.P., BETHUEL,H. Eds) Basel NEW YORK Karger.

3 - MACPHERSON, C.F. and COSGROVE, JBR (1961) Can. J. Biochem. Physiol. 39 1567-1574.

4 - COLLE, A., GUINET, R., LECLERCQ, M., MANUEL, Y. (1976) Clin. Chim. Acta. 67 93-97.

5 - CEJKA, J and FLEISCHMANN, L.E. (1973) Arch. Bioch. Biophys. 157 168-176.

6 - TONNELLE, C., COLLE, A., LECLERCQ, M., SIRE, J. and MANUEL, Y. (1977) Bioch. Biophys. Acta 490 35-43.

7 - URIEL, J. (1966) Bull. Soc. Chim. Biol. 48 969-982.

8 - SCHEIDEGGER, J.J. (1955) Int. Arch. Allergy 7 103-110.

9 - AWDEH, Z.L., WILLIAMSON, A.R. and ASKONAS, B.A. (1968) Nature Lond. 219 66-67.

10 - WEBER, K and OSBORN, M. (1969) J. Biol; Chem. 244 4406-4412.

11 - HIRS, C.H.W. (1967) Methods in Enzymology. 11 P.197.

12 - WAXDAL, M.J., KONIGSBERG, W.H., HENLEY, W.L. and EDELMAN, G.M. (1968) Biochem. 7 1959-1966.

13 - KLAPPER, D.G., WILDE, Ch.E. and CAPRA, J.D. (1978) Anal.Biochem. 85 126-131.

14 - SMITHIES, O., GIBSON, D., FANNING, E.M., GOODFLEISCH, R.M., GILMAN, J.G. and BALLANTYNE, D.F. (1971) Biochem. 10 4912-4921.

15 - DOWNS, F. and PIGMAN, W. (1969) Int. J. Protein Res. 1 181-184.

16 - HUNKAPILLER,M.W. and HOOD, L.E. (1978) Biochem 17 2124-2133.

17 - BRANDT, D.C. and JATON, J.C. (1978) J. Immunol. 121 1194-1198.

18 - ONO, T., ETO,K. and ARAKAWA,K. (1978) Clin. Chim. Acta 19, 257-265.