VARIANT OF A HUMAN IMMUNOGLOBULIN: "ALPHA CHAIN DISEASE" PROTEIN AIT

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SUMMARY

Protein AIT is a human alpha chain disease protein belonging to the IgA1 subclass. It has a molecular weight of 34,200 instead of 55,000 for intact α chain. It contains an internal deletion which comprises about the entire Fd fragment. Normal synthesis resumes at a valine residue in the middle of the heavy chain where a series of short, unusual duplicated fragments containing carbohydrate are present. From there on the molecule appears to have an intact Fc fragment. Although the origin of these immunoglobulin chain variants is not understood, it is remarkable to observe that an homologous position is involved in defective α and γ genes (1).

INTRODUCTION

Structural studies of variants of human immunoglobulin heavy chains provided valuable information concerning the mechanisms controlling the synthesis of normal heavy chains. The majority of these studies were performed on variants of immunoglobulin heavy chain belonging to the γ chain class: γ Heavy Chain Disease (HCD) proteins (1). Recently (2) we have studied a variant of human IgA heavy chain (α HCD protein DEF) and it was shown that this protein contained an internal large gap. Normal synthesis resumed at a valine residue in the middle of the heavy chain, at the beginning of a section containing the disulfide bonds joining both α chains and we postulated that it would be a site equivalent to position 216 in γ chain variants (1).

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In this paper we present similar studies carried out on a second variant of IgA heavy chain: a HCD protein AIT, which indicate that the above assumption is correct.

MATERIALS AND METHODS

A three-step schedule was adopted for the purification of the protein (3). Enzyme digestion and separation of peptides: Protein AIT (375 mg) was digested with pepsin (Worthington, twice crystallized) at an enzyme/substrate ratio of 1:50 (w/w) in 5% formic acid for 15 hours at 37°. The digest was freeze-dried and was further digested with L(1-tosylamide—2-phenyl) ethyl chloromethyl ketone-treated trypsin (Worthington) in 0.2M ammonium bicarbonate (pH 8.3) for 15 hours at 37°, at an enzyme/substrate ratio of 1:50 (w/w). The digest was fractionated by filtration on a Sephadex G-50 column (3.5 x 180 cm) equilibrated in 1M acetic acid at room temperature. Fractions (6 ml) were collected at a flow rate of 25 ml/hr. The eluate was monitored at 280 nm. Partial reduction and radioactive alkylation were described previously (4). The radioactive peptides were isolated by filtration on a Sephadex G-25 column (2.2 x 25 cm). Purification, amino acid analysis and determination of amino acid sequences were done as previously reported (4).

RESULTS AND DISCUSSION

Figure 1 shows the elution pattern obtained by fractionation of a peptic-tryptic digest of protein AIT on a column of Sephadex G-50. A peptide, containing the interheavy-heavy disulfide bridges (hinge peptide), was localized in the first peak after partial reduction and alkylation with (^{14}C) iodoacetic acid, followed by fractionation on Sephadex G-25 and paper electrophoresis. The amino acid composition of all radioactive peptides isolated from this fraction are shown in Table I. The partial sequence of

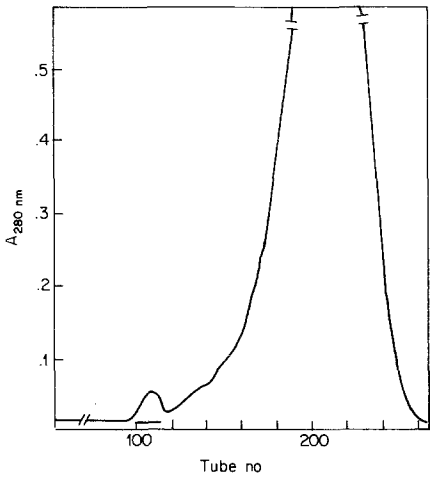

Fig. 1: Gel filtration of a peptic-tryptic digest of protein AIT (375 mg) on Sephadex G-50 (3.5 x 180 cm) in 1M acetic acid. Fractions of 6 ml were collected at a flow rate of 25 ml/hr. The pooled fraction is indicated on the figure.

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glycopeptide PT 1 (hinge peptide), as determined by the dansyl-Edman procedure (4), is:

Asp-Lys-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Pro (Ser,Cys,Cys,His,Pro,Arg).

Peptide PT 2 (Table I) appears to be the same that was found to form a disulfide bridge with the "hinge" peptide of a normal a1 chain (5), as well as in a1 chain variant DEF (2), with a sequence:

Asp-Leu-Cys-Gly-Cys-Tyr-Ser-Val-Ser.

Figure 2 shows a comparison of the sequence of the "hinge" region of a normal a1 heavy chain with those of heavy chain disease protein DEF and AIT. As was the case with protein DEF, the "hinge" region of protein AIT shows no homology at its N-terminal end, while starting at the Val residue the sequence is identical with that from a normal a1 chain. The duplicated fragments as

Table I. Amino acid composition of S-carboxymethylcysteine containing peptides obtained from the first peak (Fig. 1) of a peptic-tryptic digest of protein AIT.

Peptide	Cys	Asp	Thr	Ser	Pro	Gly	Val	Leu	Tyr	Lys	His	Arg	CHO
PT 1	1.17	1.0	3.96	4.90	10.56		1.18			1.09	1.13	1.17	+
PT 2	1.36	1.0		1.88		1.21	0.69	1.09	0.89				

well as the carbohydrate moiety (6) which are a common feature of αl chains have been preserved in protein AIT. However, the sequence Asp-Lys is not present in the constant region of Fd, and since no other features were detected from Fdc, it was concluded that they belong to the V region. Attempts to determine its amino-terminal sequence with an automatic sequencer have been unsuccessful due to heterogeneity (N-terminal residues were Val and Ile (7)). This difficulty, together with the failure to show any idiotypic antigenic specificity (7), does not allow us to establish how much of the Fdv fragment is present in protein AIT. However, molecular weight determinations (34,200 instead of 55,000 for intact α chain (8), and the presence of the entire Fc fragment (7), suggest that the V region is very short.

These results indicate that protein AIT is synthesized as an internally deleted αl chain followed by post-synthetic, amino-terminal proteolysis. The deletion comprises almost the entire Fd fragment and normal synthesis restarts at a Val residue at the beginning of the region containing the interheavy disulfide bridges.

Figure 3 shows comparison of the protein AIT with deletions observed in another human α HCD protein, several human γ HCD proteins and a spontaneous

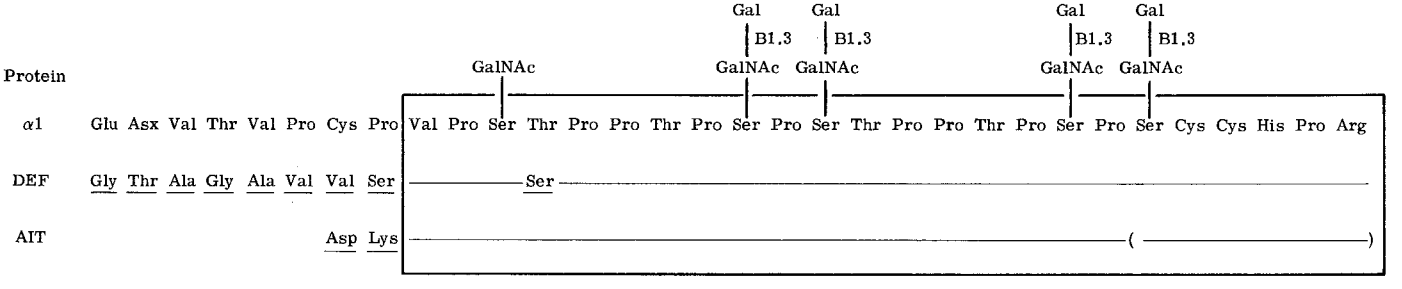

Fig. 2: Comparison of the "hinge region" of α1 Ig heavy chain and α HCD Proteins DEF and AIT. Identical residues are in the box, non-homologous residues are underlined. Line in the box indicates a sequence identical to that shown in top peptide. The sequence of α1 chain and of carbohydrate is taken from references 11 and 6 respectively. Protein DEF, ref. (2). Note duplications of two short sequences: Pro-Ser-Pro-Ser and Thr-Pro-Pro-Thr as well as carbohydrate.

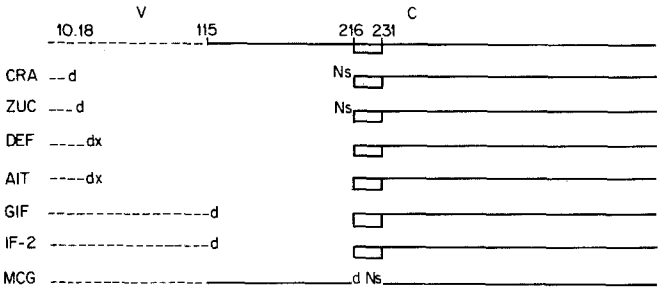

Fig. 3: Correlation between a normal heavy chain and some defective human and mouse immunoglobulin heavy chains. Internal deletions are present in all. The position of deletions are indicated by the amino acid residue number above the line. —— indicates constant (C) region fragments; ▭: hinge region; -- variable (V) region fragments; d: indicates the site where the deletion starts; (dx, not precisely delineated); NS: site where normal synthesis resumes. Although not represented the two fragments of each variant are joined - CRA and MCG belong to (γ1) human heavy chain subclass, GIF (γ2), ZUC (γ3), DEF and AIT (α1). IF-2 (γ1) mouse heavy chain (1, 2, 9, 13).

mutant isolated from tissue cultures of mouse MOPC 21 plasma cells: IF-2 (9). Residue 216 (γ1 numbering, (10)) of the four human γ chain subclasses is glutamic acid. The homologous position in human α chain and mouse γ1 is valine (2, 9).

These studies have demonstrated that i) deletions end or start at position 216, ii) 216 is the beginning of a segment with an unusual amino acid sequence with very little homology with the rest of the molecule and iii) such a segment can undergo tandem duplications (11, 12). Because of these findings it was suggested that the stretch of DNA coding for position 216 could be a site for specific recombination (11). Whether the site is related to those involved in variable (V) and constant (C) genes integration remains to be shown.

REFERENCES

1. Frangione, B. and Franklin, E.C. (1973), Sem.Hematol., 10, 53-64.

2. Wolfenstein-Todel, C., Mihaesco, E. and Frangione, B. (1974), Proc.Nat. Acad.Sci. (USA), 71, 974-978.
3. Seligmann, M., Mihaesco, E., Hurez, D., Mihaesco, C., Preud'homme,J-L. and Rambaud, J.C. (1969), J.Clin.Invest., 48, 2374-2389.
4. Wolfenstein, C., Frangione, B., Mihaesco, E. and Franklin, E.C. (1971), Biochemistry, 10, 4140-4145.
5. Wolfenstein-Todel, C., Prelli, F., Frangione, B. and Franklin, E.C. (1973), Biochemistry, 12, 5195-5197.
6. Baenziger, J. and Kornfeld, S. (1974), J.Biol.Chem., 249, 7270-7281.
7. Seligmann, M., Mihaesco, E. and Frangione, B. (1971), Ann.N.Y.Acad.Sci., 190, 487-500.
8. Dorrington, K.J., Mihaesco, E. and Seligmann, M. (1970), Biochim.Biophys. Acta, 221, 647-649.
9. Milstein, C., Adetugbo, K., Cowan, N.J. and Secher, D.S. (1974), In Progress in Immunology, 2 (edit. by Brent, L., and Holborow, E.J.), 157-168, Holland, Amsterdam.
10. Edelman, G.M., Cunningham, B.A., Gall, W.E., Gottlieb, P.B., Rutishauser, H. and Waxdal, M.J. (1969), Proc.Nat.Acad.Sci. (USA), 63, 78-85
11. Frangione, B. and Wolfenstein-Todel, C. (1972), Proc.Nat.Acad.Sci. (USA), 69, 3673-3676.
12. Adlersberg, J., Franklin, E.C. and Frangione, B. (In Press), Proc.Nat.Acad. Sci. (USA).
13. Fett, J.W., Deutsch, H.F. and Smithies, O. (1973), Immunochem., 10, 115-118.