STRUCTURAL STUDIES OF ANOTHER HUMAN IgG3 MYELOMA PROTEIN (Kam) CARRYING THE ALLOTYPIC MARKERS Gm(s,t) AND ITS ALTERRATION INDUCED BY CHEMICAL MODIFICATION.

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INTRODUCTION

Immunoglobulins of the IgG class can be divided into four isotypes, IgG1, IgG2, IgG3 and IgG4, on the basis of the amino acid differences in the heavy chain constant region, of variation in the position of attachment of light to heavy chain, and of the number and arrangement of inter-heavy chain disulfide bridges (1,2). These structural differences are reflected in unique antigenic determinants that allow their serologic recognition and in a series of allotypic determinants of gamma markers (Gm) that reflect genetically controlled polymorphisms (1-3).

A great number of genetic markers so far discovered in man are located in the constant region of the molecule. Fifteen allotypes have so far been described for the IgG3 subclass constant domain (4). G3m(s) and G3m(t) allotypes were first described in 1966(5), and they are now generally recognized as the characteristic amino acid markers of Mongoloid populations (5,6). Rechts et al.,(7) and van Loghem et al.,(8) described the differences in amino acid sequences found in the binding of IgG3 and other proteins to Staphylococcal protein A; they also described the differences in the residues found at position 435-436 in the CH3 domain between G3m(s) and G3m(u) proteins, using the same myeloma protein, Goe, which had the allotypic markers G3m(b0,b3,b5,s,t,v). The authors described the amino acid substitutions determining G3m(s) and G3m(t) specificities, which characterize Mongoloid populations, by sequence analysis of the Fc region of a myeloma protein (Jir) (9). By comparing the amino acid sequences of the IgG3 and the other IgG subclasses analysed to date, it was established that G3m(s) was an isoallotype specified by an amino acid substitution at position 435; i.e., whereas the subclasses IgG1, IgG2, and IgG4 had histidine in common, G3m(s-) had arginine in this position. This was also confirmed by the observation that the Fc fragment bound to protein A (10). It was also established that the amino acid at position 379 of G3m(t-) IgG3 and the other subclasses was valine, whereas methionine in this position was specific for G3m(t+).

The authors obtained an another monoclonal IgG3 protein (called Kam) derived from a Japanese female with essential cryoglobulinemia treated by plasmapheresis. By sequence analysis of the pFc' fragment of the protein Kam we reaffirmed that G3m(s) was an isoallotype specified by histidine instead of arginine at position 435 and G3m(t) specific substitution was methionine in stead of valine at position 379, as described previously (9). This paper also concerns the results of chemical modification to investigate the structural environment around the epitopes.

MATERIALS AND METHODS

Purification of myeloma protein (Kam). IgG3 protein was isolated from the serum of the myeloma patient (Kam) by precipitation in cold saline.

Gm typing. The classical hemagglutination inhibition test on microflocculation slide was used for allotype determination.

Preparation of the IgG3-pFc' fragment. IgG3(Kam) (10 mg/ml) dissolved in 0.1 M sodium acetate buffer (pH 4.5) was incubated with pepsin (Worthington, Freehold, NJ) at an E/S ratio 1;100 (w/w) for 1 hr at 37 ^{o}C. The reaction was stopped by addition of 0.1 g of Tris per 1 ml of the mixture.

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The digest was applied to a Shimpack DIOL-150 column (7.9x50 cm, particle size 5 um) equilibrated with 10 mM phosphate buffer (pH 7.0) containing 0.2 M sodium sulfate.

After peptic digestion of this protein in acidic solution, the pFc' fragment (CH3 domain) was purified by gel permeation chromatography on high-performance liquid chromatography (HPLC-GPC). The tryptic peptides of completely reduced and carboxymethylated pFc' fragment (Kam) were separated by reversed-phase chromatography on fast protein liquid chromatography (FPLC-RPC). The chromatogram and the amino acid composition of peaks were were identical to those of pFc' (Jir). Peptide PT8 and 12, which had a substitution specific to G3m(t) or G3m(s), were partially sequenced by manual Edman degradation and carboxypeptidase treatment.

Chemical modification. 0.5 mg of myeloma protein (Kam) was employed for chemical modification. Each modified sample was dialized against buffered saline for the serial titration of Gm typing. Amidation of aspartic acid or glutamic acid on the surface of myeloma protein (Kam) was carried out in 0.5 ml of 1 M glycineamide-HCl buffer (pH 4.75) with 9.6 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide for 1 hr at room temperature (10). Methylthio group of methionine was reacted to monoiodoacetic acid under an acidic condition (11). The protein was dissolved in 0.5 ml of diluted HCl (pH 2.5) and added to 3 mg of monoiodoacetic acid. The mixture was stood for 4 hrs at 37°C. For iodination of hydroxy group of tyrosine, the protein in 0.5 ml of 0.1 M carbonate buffer (pH 9.5) was iodonated with 20 µl of 0.1 M I/0.2 M KI for 5 hrs at 4°C (12). For trinitrophenylation of ε-amino group of lysine, the protein was dissolved in 0.5 ml of 1 M borate buffer (pH 9.2), and then 50 µl of 0.02 M sodium 2,4,6-trinitrobenzene-1-sulfonate (TNBS) was added and the mixture was kept for 4 hrs at room temperature (13). Histidine residues were photoxidized with 10 µl of 0.5 % methylenblue in 0.5 ml of 0.1 M phosphate buffer (pH 7.3) under light (14).

RESULTS

Isolation of the monoclonal IgG3 protein (Kam). Protein Kam was separated from serum of the patient with myeloma by cryoprecipitation. The cryoprecipitate ability of this separated protein was less than that of IgG3 (Jir). The IgG3 Kam was identified as a lambda type IgG3 carrying G3m (b0,b3,b5,s,t) allotypes by immunodiffusion and Gm typing.

Isolation of the pFc' (Kam) fragment. The pFc' (Kam) fragment was isolated by gel-permeation chromatography (GPC) on a Hitachi HPLC apparatus (type 638). No differences between Jir and Kam could be detected in the first 7 amino-terminal residues of carboxymethylated pFc' fragment by a manual Edman degradation.

Sequence analysis of the Gm(s,t) specific peptides. The tryptic peptides from carboxymethylated pFc' (Kam) fragment were separated by reverse-phase chromatography. The chromatogram was identical to that of pFc' (Jir). The pooled fraction was carried out rechromatography under a acidic elution system. Amino acid composition of peaks was shown in table. Except for PT1 (Lys) and PT3 (Thr-Lys), 10 peptides were obtained on these chromatography. The two peptides might be eluted in the first peak on rechromatography. Peptide PT12 carrying G3m(s) specific residue was partially sequenced by manual Edman degradation and carboxypeptidase treatment; Trp-Gln- - - -Asx-His-Tyr-Thr-Glx-Lys. Similarly, peptide PT8 carrying G3m(t) was determined; Gly-Phe-Tyr-Pro-Ser-Asp-Ile-Ala-Met- - - -Glx-Asx-Tyr-Lys.

Gm activities after chemical modification or protein A binding. Myeloma IgG3 (Kam) and protein A (Sigma) were mixed at a molar ratio 1:2, and then the mixture was passed through a Protein A-Sepharose CL-4B (Pharmacia) column in order to remove the non-bound IgG3. No effect of a IgG3-protein A interaction resulted in G3m(s) and G3m(t) activities. Amidation and trinitrophenylation had not influence on the both Gm activities. After carboxymethylation, the activities were completely missing. These results were obtained on controlled and modified proteins. Iodination of tyrosine allowed to lose the Gm activities, while photoxidation of histidine influenced on G3m(s) activity at x12 dilution level.

DISCUSSION

Myeloma protein Kam was purified by cold precipitation in the same way as myeloma protein Jir (9). However, the property of cryoglobulin faded with purification; the purified protein had not precipitated at 4°C in concentration 30 mg/ml. The peptide mapping of Kam was identical to that of Jir on FPLC chromatograms. This protein had methionine at position 379 and histidine at 435 by partial sequence analysis of tryptic peptides PT8 and PT12. This results are consistent with that of the myeloma protein Jir. The IgG3 Kam carrying Gm(s,t) reacted with protein A.

After chemical modification or protein A binding, G3m(s) and G3m(t) activities were examined to inspect the chemical enviroments around their epitopes. G3m(s) and (t) activities were not influenced by IgG3-protein A interactions. Only G3m(s) activity was detected on the pFc' fragment. These results suggested that epitope of G3m(s) marker localized on only CH3 domain, and that the formation of G3m(t) epitope was necessary for a CH2-CH3 domain interaction. The epitopes of G1m(f) and G1m(z) markers were found to be located on the Fd fragment and required the presence of both L and H chains (15). The chemical modification of aspartic acid, glutamic acid, lysine, histidine residues of protein Kam had no effect on both G3m(s,t) activities, while iodination of tyrosine resulted in complete inhibition of those activities. In the case of carboxymethylation, the protein must lose the activities because of the breakage of secondary structure in the acidic solution. From these results, we speculate that the epitopes of G3m(s) allotype is present on the last C-terminal beta-strand of CH3 domain, which is formed by antiparallel beta-pleated sheet (16), and that epitope of G3m(t) is present on the portion including the 3rd, 4th beta-strand and turn structure between them, which have tyrosine at position 391. And the CH2 domain was closely associated with this G3m (t) epitope.

Prealbumin from a patient with heredofamilial amyloid polyneuropathy had a substitution of methionine for valine at position 30 (16). This substitution resulted in the amyloid fibril formation. As a domain structure of immunoglobulin, prealbumin is composed of eight beta-strands with forming the two antiparallel beta-sheet (18). Cryoprecipitation and amyloid fibril formation, which are aggregation of protein molecules, are thought to be attributable to some abnormalities on the surface of protein molecule. The substitution, which is methionine instead of valine, may be responsible for these phenomena. IgG3 protein Jir carrying G3m(t) was characteristic of strong cryoprecipitation. Nishimura (19) suggested that one of the aggregation sites reside on the Fc portion of Protein Jir. However, purified protein Kam and protein Goe (20) carrying the same markers have no properties of cryoprecipitation. It is a matter of interest to clarify the mechanisms and relationship between the amino acid substitution and the molecular abnormalities.

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