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A NEW IMMUNOGLOBULIN MARKER

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In an examination of 16 human immunoglobulin λ -light chains, one was found to have amino acid substitutions which have not previously been reported for the constant region of the λ -chains. It involved in two positions: an arginine instead of lysine at position 130, and a leucine for glutamine at position 195 (Sh numbering(1)). The structural variation is tentatively designated as "Is" marker.

In contrast to the k-chains, the constant region of human λ -chains have been found to carry considerable heterogeneity as exemplified by Oz(2), Mz(3), Kern(4), and Mcg(5). All of these are believed to be isotypes rather than allotype as have been defined for the k-chains(6).

In connection with a basic question how the great variability of antibody sequence is generated and how many structure genes to be assumed to account for antibody diversity, we have made a series of studies on the primary structure of Bence Jones proteins of different types as well as light chains and of heavy chains of monoclonal immunoglobulins(7-12). Through these analyses we have demonstrated a new λ -chain subgroup V_λ VI(9), two subsubgroups V_λ I-1 and V_λ I-2(10), subgroups V_λ II(11), V_λ IV(12), and two cases of heavy chain subgroup V HIII (13-14). These data gave additional supports for the hypothesis which has been proposed in 1973 by one of the authors (T.S.) that much of the variability of immunoglobulins can be accounted for by assuming a relatively small number, for example $\sim 10^2$, of germ line genes(7-8). This communication describes the amino acid sequence of the constant region of a human λ -chain in which a new type of structural variation is demonstrated.

MATERIALS AND METHODS

Bence Jones protein(NIG-68) and its fragments were isolated from urine of a patient with multiple myeloma associated with Fanconi syndrome in the following manners: 1) fractionation with 65% saturation of ammonium sulfate, 2) DEAE-Sepharose CL-6B column chromatography (5mM Na-phosphate-0.025% NaN_3, pH 8.4, 0-0.5M NaCl gradient, 4°C), 3) gel chromatography with Sephadex G-75 (0.1M NH_4HCO_3-8M urea, pH 8.3, 4°C). The methods of isolation, purification and characterization with immunoelectrophoresis, SDS-disc electrophoresis,

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amino acid analysis of proteins and peptides, complete reduction and aminoethylation, and N- and C-terminal analyses have been described(7-11).

Purified and aminoethylated constant region fragment(24mg in 0.1M NH_4HCO_3) was digested with 0.3mg Of TPCK-trypsin(2x crystallized, Worthington) and was treated in the way as described(9-11). The digest was chromatographed on a column of DEAE-Sephadex A-25(1.5 x 42cm). The column was eluted at room temperature first with 360ml of 10mM NH_4HCO_3-10% 1-propanol, and then by a linear gradient of an increasing NH_4HCO_3 concentration(0-0.6M) containing 10% 1-propanol. Peptides were further purified, if necessary, by gel filtration with Bio-Gel P-6 column(1.5 x 98cm) in 0.1M NH_4HCO_3 at room temperature or by reverse phase column chromatography using a column(0.6 x 25cm) of Nucleosil C8(5μ, Macherey-Nagel, Duren) under the reported conditions(10).

Sequence analysis was carried out by manual Edman degradation method and PTH-amino acids were identified by high pressure liquid chromatography as reported(9-11). Digestion with carboxypeptidases A and/or B were performed in 0.2M N-ethylmorphorine-acetic acid(pH 8.0) by the method of Ambler(12). Digestion with acid carboxypeptidase was carried out in 0.2M formic acid-pyridine buffer of pH 2.5. Aliquots of the reaction mixture were taken at intervals and subjected to amino acid analysis under the standard condition.

RESULTS AND DISCUSSION

When crude urinary proteins fractionated with 65% saturation of ammonium sulfate were chromatographed on a column of DEAE-Sepharose CL-6B, two major peakes were obtained(Fig. 1A, Fr1, 2). The first peak was shown to contain two major components whose molecular weights were, after SDS-disc electrophoresis , shown to be approximately 24,000 and 13,000, respectively. These components reacted only with anti λ-antiserum, and the former was found to

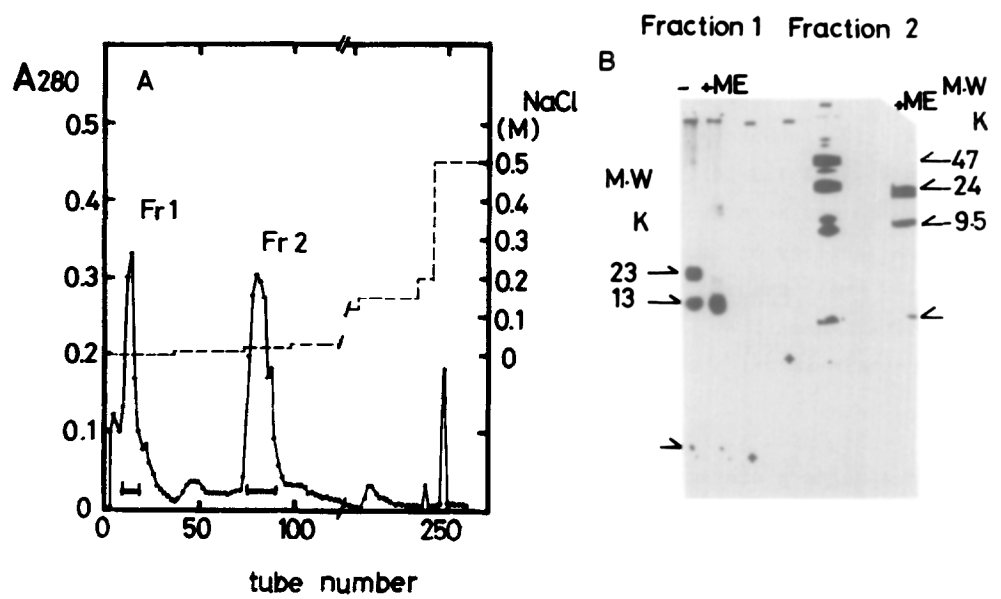

Fig. 1. Purification of Bence Jones protein NIG-68 and its spontaneous V_λ and C_λ fragments on a column of DEAE-Sepharose CL-6B. A, elution profile; B, SDS-disc electrophoresis before (-ME) and after(+ME) the reduction with 2-mercaptoethanol.

give a single band with Mw 13,000 after the reduction with 2-mercaptorthanol. Thus, it was considered to be a dimer of the latter, and has subsequently been assigned to be the constant region of the λ-chain by amino acid composition and the complete sequence analysis. From the second peak, the three components with different molecular weights of 47,000, 24,000 and 10,000 respectively, were obtained following the further chromatography with Sephadex G-75(super - fine) in the presence of 8M urea(Fig. 2). After the reduction, the one with Mw 47,000 gave a single homogeneous band with Mw 24,000, suggesting that it was a dimer form of the latter. The component was later identified to be a whole λ-chain. The component with Mw 24,000(Fr 2-2, Fig.2B) did not change its molecular weight before and after the reduction, and was subsequently identified to be a monomer of the whole Bence Jones protein based on the data of the amino acid composition, serological characterization, N-terminal sequence and C-terminal analyses.

Although, the remaining one with Mw 10,000 did not react with any antisera including an anti λ-chain, it had the N-terminal sequence of Tyr-Asp-Leu-Thr-Gln-Ala-Pro-Ser-Leu-Ser-Val-, which is identical with that of the whole Bence Jones protein NIG-68. Digestion of the component with acid carboxypeptidase gave a sequence -Thr-Val-Leu, which is identical with that of residues 105-107 of the variable region of the whole λ-chain. These findings, together with the amino acid composition, suggests that the component corresponds with

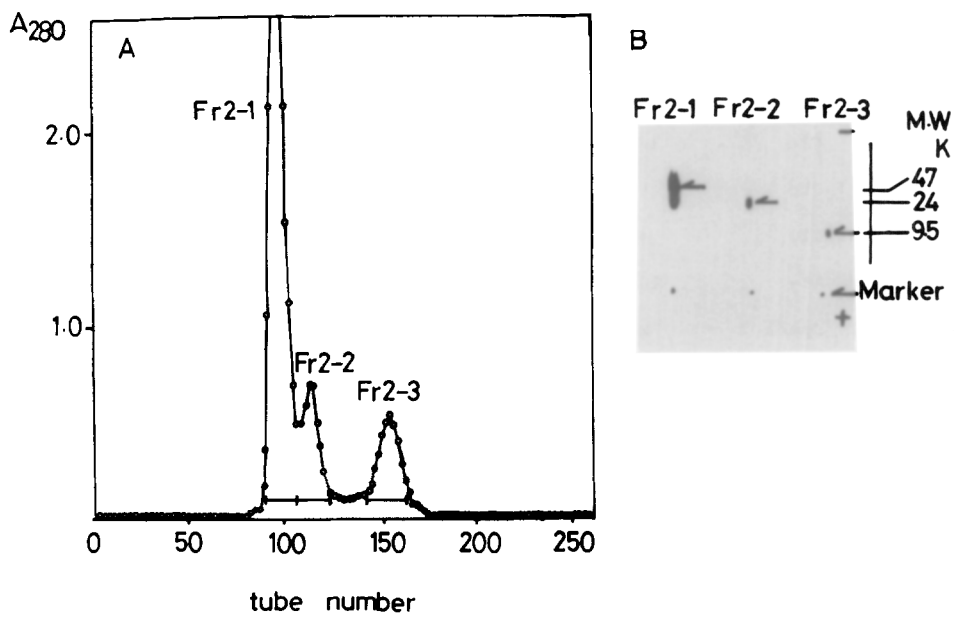

Fig. 2. Further purification of Fraction 2 of Fig. 1 by gel filtration. A, elution profile; B, SDS-disc electrophoresis.

the entire variable region of the whole Bence Jones protein NIG-68. A detail of the sequence analysis of this component will be described elesewhere.

Table 1 summarizes the amino acid compositions of the purified protein components from the urine. This includes the whole λ-chain, a fragment equivalent to the variable region, and the one corresponding to the constant region which is the sample used for the present sequence analysis. The sum of the compositions of the latter two fragments is in good agreement with that of the whole λ-chain, NIG-68.

The covalent structure deduced by the sequence analyses of the tryptic peptides prepared from the completly reduced and aminoethylated C_λ fragment is shown in Fig. 3. The sequence is characteristic of that of the constant region of the λ-chain. It had 106 residues and began with glycine which is corresponding with the residue 108 of the λ-chain(Sh numbering(1)). Although the specimen was negative for all the reported λ-chain isotypes such as Oz, Kern, Mz, and Mcg, it had rare amino acid replacements at two positions: an

Table 1. Amino acid composition of NIG-68 and its spontaneous Fragments

	Fr. 1	Fr. 2-1	Fr. 2-2	Fr. 2-3
Lys	6.7	10.9	11.1	3.4
His	2.3	3.9	3.0	0.7
Arg	2.1	9.1	8.6	7.7
Asp	5.6	17.9	16.6	10.9
Thr	11.1	19.5	20.4	9.7
Ser	17.2	26.1	25.2	11.9
Glu	12.2	21.8	22.4	10.9
Pro	9.5	15.1	16.1	6.5
Gly	4.3	15.6	15.2	8.9
Ala	10.8	15.7	16.0	6.4
Cys/2	2.3	4.3	4.5	1.4
Val	9.2	13.7	13.5	5.8
Met	0.1	1.2	1.2	1.2
Ile	1.1	5.3	5.5	3.9
Leu	7.3	14.6	14.1	8.3
Tyr	4.3	8.2	8.7	5.
Phe	2.2	5.3	4.9	3.2
Trp	1.7	3.5	3.7	1.5

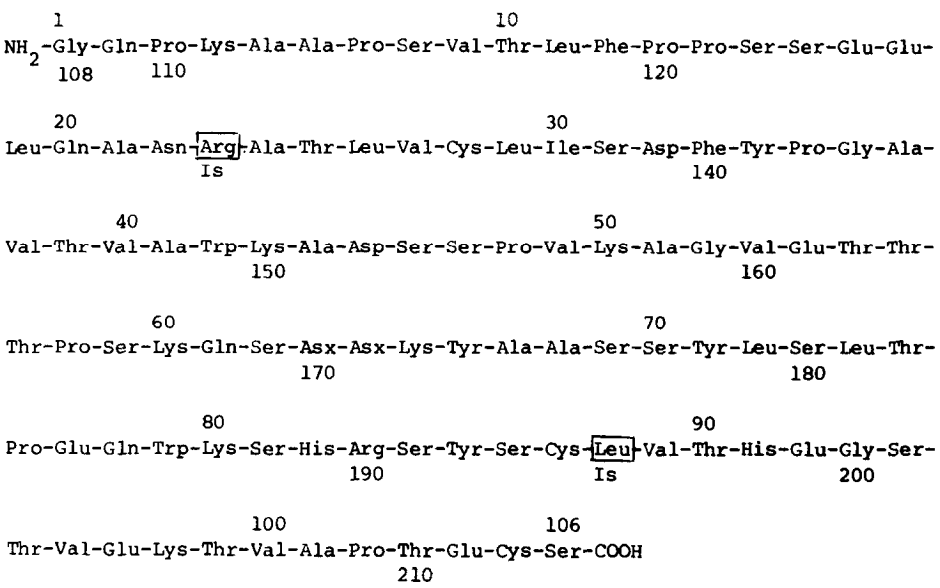

Fig. 3. Amino acid sequence of the C fragment and the positions of amino acid replacements associated with the "Is" marker. The numbers under the sequence correspond to the residue positions given for the human -light chains after Sh numbering(1).

arginine for lysine at position 130(Sh numbering) and a leucine for glutamine at position 195(Sh numbering), which were not previously descrived for the constant region of the λ-chains. Except these two replacements, the entire sequence of the specimen was shown to be identical with that of the corresponding region of protein Sh including the positions of amides(1). Since the variation is an unreported one among the λ- chains, we will designate it as "Is" marker.

Although the sequence for the marker is established, its biological and functional significances are not understood at present. Whether or not it is allotype is also remained to be clearified . An atempt to solve the problem is now underway using an antiserum against to the marker.

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