Amino acid sequence of an amyloidogenic Bence Jones protein in myeloma-associated systemic amyloidosis

Hiroshi Tonoike, Fuyuki Kametani, Akihiko Hoshi, Tomotaka Shinoda* and Takashi Isobe+

Department of Chemistry, Tokyo Metropolitan University, Setagaya-ku, Tokyo 158 and +*Department of Medicine, Kobe University, Chuo-ku, Kobe 650, Japan*

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The complete amino acid sequence of an amyloidogenic Bence Jones protein(NIG-84) from an individual with myeloma-associated systemic amyloidosis has been determined. The protein, with a blocked N-terminus, represents a complete light chain consisting of 217 residues and it has a structural feature characteristic of the Vλ II subgroup. In addition to a two-residue insertion at positions 28 and 29, it has an additional rare insertion of alanine at position 100. NIG-84 is an example of the first complete sequence presented for the amyloidogenic Bence Jones protein of the Vλ II subgroup.

Amyloidosis *Bence Jones protein* *Primary structure* *Subgroup* *Amyloidogenicity*

1. INTRODUCTION

It is now accepted that immunoglobulin light chain fragments (AL), or less commonly whole chains, consist of the major part of the amyloid fibrils in primary and myeloma-associated systemic amyloidoses, as well as in some forms of localized amyloidosis [1,2]. However, because of the limited and rather incomplete sequence data, it is not yet possible to elucidate the 'amyloidogenicity' of such proteins in relation to their primary structure, though AL proteins of certain subgroups have been reported to be more prone to association with the amyloid process [3–6]. Since only a fraction of the immunoglobulin light chains seems to be involved in amyloidogenesis, their sequence characteristics may be an essential clue to understanding of amyloid fibril formation in the disease.

This paper presents the complete amino acid sequence of the amyloidogenic λ Bence Jones Protein (BJP) NIG-84 in myeloma-associated systemic amyloidosis and shows a unique structural feature which may give some insights into the amyloidogenicity of the light chains.

* To whom correspondence should be addressed

2. MATERIALS AND METHODS

2.1. *Purification of NIG-84*

Protein NIG-84 was isolated from the urine of a patient with multiple myeloma-associated amyloidosis as follows: (i) fractionation with 65% saturation of $(NH_4)_2SO_4$; (ii) DEAE-Sephadex A-50 column chromatography [8 × 11 cm, in 10 mM Tris-HCl, pH 8.5, with a linear gradient in NaCl (0–0.3 M)]; (iii) gel filtration on a column of Sephadex G-100 (2.6 × 70 cm, in 0.1 M Tris-HCl, pH 8.1, containing 0.15 M NaCl); (iv) reverse-phase high-performance liquid chromatography (HPLC) with Hitachi Gel 3013-O (0.4 × 25 cm, in 20% CH_3CN, 0.1% trifluoroacetic acid (TFA), pH 2.5, with a linear gradient in an increasing concentration of CH_3CN (20–75%), 0.1% TFA, pH 2.5 [6,7]).

2.2. *Trypsin digestion and purification of the peptides*

Following complete reduction with 2-mercaptoethanol and aminoethylation with ethylenei-

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mine [3,7], NIG-84 (25 mg in 10 ml of 20 mM NH_4HCO_3) was digested with 1.25 mg TPCK-trypsin (2 × crystallized, Worthington) and treated as in [6–8]. The digest was chromatographed first by cation-exchange HPLC with Hitachi Gel 3013-C (0.4 × 25 cm, in 0.5% CH_3CN, 25% isopropanol, 4 mM $NH_4CH_3SO_4$, pH 6.4). The column was eluted at a flow rate of 0.5 ml/min at 70°C with a linear gradient in an increasing concentration of the initial buffer (to 50% CH_3CN, 25% isopropanol, 0.4 M $NH_4CH_3SO_4$, pH 6.4). Peptides were further purified, if needed, by reverse-phase HPLC as described [6,8].

2.3. *Sequence analysis*

Sequence analysis of the peptides (15–60 nmol) was carried out by manual Edman degradation and the resulting PTH-amino acids were identified by HPLC under the reported conditions [3,6–8]. Digestions with carboxypeptidase A and/or B, and hydrazinolysis were performed as in [3,7]. The nomenclature of the peptides of the protein followed that adopted for other λ chains [3,9].

3. RESULTS AND DISCUSSION

The complete amino acid sequence of protein NIG-84 is shown in fig.1. The sequence was deduced by the analysis of 20 distinct tryptic peptides purified as above. It began with a blocked N-terminus and consisted of 217 residues, indicating that it was a complete light chain form. Of the 217 residues, the V region consisted of the first 112 residues, the remainder being the C region. When the sequence of the V region was compared with those of the reported λ chains, it was most homologous with that of the V λ II subgroup (72–86%), thus it was tentatively classified into this subgroup. This was the first specimen among the amyloidogenic λ BJPs whose sequence was identified as V λ II subgroup.

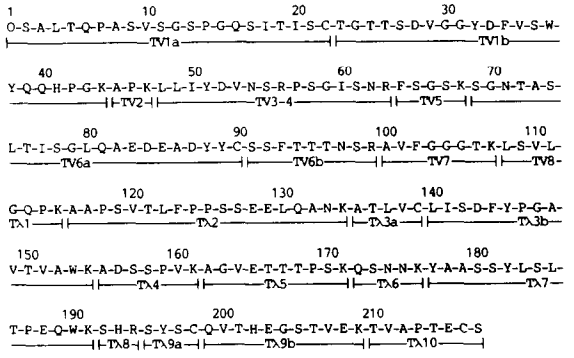

Fig.1. The proposed amino acid sequence for an amyloidogenic Bence Jones protein NIG-84. One-letter code for amino acids: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; O, Pca; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

Although the NIG-84 sequence is most homologous with that of the V λ II subgroup, it also has individual characteristics: (i) whereas all the reported V λ II specimens have a tyrosine residue at position 93, NIG-84 has phenylalanine; (ii) insertion of an extra alanine residue at position 100; (iii) a rare replacement of serine for threonine at position 109, near the V-C junction. On the basis of these data, the reported V λ II specimens together with NIG-84, can be classified into the alternate subset, provisionally designated sub-subgroups V λ IIa and V λ IIb as exemplified by us for the V λ I subgroup [10], and by others for V κ III subgroup [11]. In these cases, all the specimens of ALs and BJPs, but not in those without amyloidosis, were shown to have either insertion of an extra amino acid residue(s) and/or an uncommon sequence including very rare replacement at one or more positions. These structural characteristics probably cause local distortions in the conformation of these proteins, and these in turn might lead to their being more prone to association with the amyloid process. However, it is not yet possible to identify definitely any specific sequence or sequences which is essential for the amyloidogenicity of such proteins. Apparently further sequence data must be obtained before clarifying the problem.

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