Beta-2 Microglobulin Is an Amyloidogenic Protein in Man

Peter D. Gorevic, Terence T. Casey, William J. Stone, Carol R. DiRaimondo, Frances C. Prelli, and Blas Frangione
Departments of Medicine and Pathology, State University of New York, Stony Brook, New York 11794; Vanderbilt University, Nashville, Tennessee 37232; and New York University School of Medicine, New York 10016

Abstract

Curvilinear fibrils with the tinctorial properties of amyloid were isolated from a patient with bone and joint involvement complicating chronic dialysis for renal disease. Subunit fractions of 24,000 and 12,000 mol wt were identified after gel filtration under dissociating conditions, the latter containing a significant amount of a dimer of the former. This was confirmed by Edman degradation of each fraction, which yielded the amino terminal sequence of normal human beta-2 microglobulin (B2M) to residues 20 and 30, respectively. The size of the subunit protein (12,000 mol wt) and the amino acid composition make it likely that intact B2M is a major constituent of the fibrils. B2M is thus another example of a low molecular weight serum protein, with a prominent beta-pleated sheet structure, that may adopt the fibrillar configuration of amyloid in certain pathologic states.

Introduction

Several low molecular weight proteins adopt the fibrillar configuration of amyloid as a concomitant of various disease states in man. These include immunoglobulin light chains in amyloidosis complicating plasma cell dyscrasias, AA protein in secondary and some heredofamilial amyloidoses, prealbumin (transthyretin) in certain kindreds affected by familial amyloidotic polyneuropathy and in senile cardiomyopathy, hormones such as calcitonin in amyloid associated with amine precursor uptake and decarboxylation neoplasms, gamma-trace protein in at least one form of hereditary hemorrhagic angiopathy in brain, and the so-called beta protein in cerebrovascular and senile plaque core amyloid in Alzheimer's Disease and Down's Syndrome with dementia (1–4). Some of these subunit proteins, notably including light chain (5), prealbumin (6), and, reportedly, beta protein (7), have a prominent beta-pleated sheet configuration, which is a characteristic feature of amyloid fibrils, as shown by x-ray diffraction and spectroscopic studies (1).

In recent years, a new form of amyloidosis of bone is being recognized with increasing frequency in patients on long-term hemodialysis (8). Amyloid deposits form tumoral masses in bone, sometimes presenting as pathologic fractures (8). They may also affect the flexor retinaculum, and contribute to the incidence of peripheral nerve entrapment syndromes often seen in dialysis patients (9). Amyloid has also been found in the skin and blood vessels on rectal biopsy, which suggests origin from the blood (10, 11). Tissue deposits show positive staining with Congo red and have characteristic apple-green birefringence by polarizing microscopy (8–11). The deposits consist of masses of fibrils with an unusual curvilinear configuration by electron microscopy that differs from the typical straight fibrils seen in most forms of amyloidosis (8, 11).

We report the isolation and partial amino acid sequence of a novel subunit protein from a case of tumoral amyloidosis of bone complicating long-term dialysis, and its definitive identification as beta-2 microglobulin (B2M).[1]

Methods

Patient (M.A.R.). A 71-yr-old black man with end-stage renal disease had been maintained on chronic center hemodialysis for 10 yr. 3 yr earlier he underwent bilateral carpal tunnel releases. During this period, he suffered a right femoral neck fracture (he had no history of antecedent trauma) that required placement of a prosthesis. A left hip fracture developed 19 mo afterwards, and subsequently a skeletal survey was found to show multiple 1–3-cm lytic lesions involving proximal humeri, right ulna, left radius, and right sixth rib. Bone aspiration showed these lesions to consist almost entirely of fibrillar material with the tinctorial properties of amyloid. Serum immunoelectrophoresis was negative for monoclonal immunoglobulin light chains, and a bone marrow biopsy was negative for tumor, granuloma, or amyloidosis.

Several months later, the patient died of pneumonia and heart failure. At postmortem, in addition to the lytic lesions in bone, amyloid was also found in the synovium of the knee and shoulder, and a small focus was identified in an intramuscular branch of the left coronary artery. Approximately 1 g of gelatinous material was taken from the left humeral head, frozen, and subsequently used for biochemical analysis.

Isolation of subunit protein. A portion of the material was thawed and washed twice with distilled water, with the pellet collected following centrifugation for 1 h at 80,000 rpm at 4°C in a Beckman airfuge (Beckman Instruments Inc., Fullerton, CA). The pellet was suspended in 3 ml of 6 M guanidine, 0.1 M Tris, pH 10.2, containing 0.17 M dithiothreitol (DTT), and homogenized for 5 min on ice using a Virtis homogenizer. It was allowed to stand 2 d with gentle stirring at room temperature, after which 1 ml of 2 M guanidine-4 M acetic acid were added and the suspension centrifuged 2 h at 50,000 cpm in an L5-65 ultracen-

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Address correspondence to Dr. Gorevic, Division of Allergy, Rheumatology and Clinical Immunology, Department of Medicine, State University of New York, Stony Brook, New York 11794.

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1. *Abbreviations used in this paper:* B2M, beta-2 microglobulin; DTT, dithiothreitol; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

trifuge (Beckman Instruments Inc.). 19% (dry weight) of the initial material was insoluble, and the remaining supernatant was applied to a Sephadex G-75 column (6 × 80 cm) equilibrated with 5 M guanidine-1 M acetic acid, run downward at 30 ml/h. Fractions were pooled, dialyzed exhaustively against water, and lyophilized. Pooled fractions were run reduced and unreduced on 17% sodium dodecyl sulfate (SDS)-polyacrylamide gels with molecular weight standards using the Laemmli buffer system (12).

Amino acid sequence analysis. Automated sequence analyses were performed by Edman degradation using an 890C sequenator (Beckman Instruments Inc.) and a 0.1-M Quadrol program. Thiazolinone amino acids were converted to phenylthiohydantoin amino acids in a Sequemat

Figure 1. Electron micrographic appearance of bone amyloid from patient M.A.R. Fibrils are atypical, in that they are arranged in short bundles and have a curvilinear configuration (magnification × 75,000).

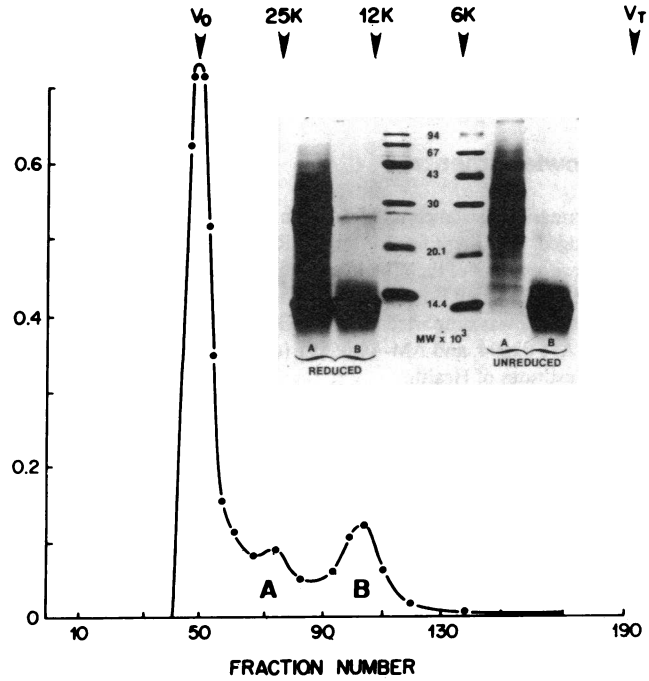

Figure 2. Chromatographic profile of tumoral amyloid solubilized in guanidine/DTT and fractionated on Sephadex G-75. The column was calibrated with ovalbumin, chymotrypsinogen, cytochrome *c*, and insulin. (*Top right*) 17% SDS-PAGE showing fractions A and B run under reducing (*left*) and nonreducing (*right*) conditions. Molecular weight markers are indicated in the center of the gel.

P-6 autoconverter at 65°C using methanol/HCl (7:1 vol/vol). Identification of the phenylhydantoin amino acids was achieved by high pressure liquid chromatography using an IBM 5-μm octadecyl column developed with a methanol/water gradient. Cysteine was detected by measuring the radioactivity of phenylthiohydantoin ^{14}C-carboxymethylcysteine (13).

Electron microscopy. Tissue was placed in 2% glutaraldehyde treated with Millonigs OsO_4 and 1% uranyl acetate, and embedded via propylene oxide into araldite plastic. 500 A sections were stained with 3.8% uranyl acetate and viewed on a Hitachi H-600 electron microscope.

Results

Fibrils. Material obtained at postmortem was found to stain positively with Congo red and have characteristic birefringence by polarizing microscopy. It was composed almost entirely of atypical curvilinear fibrils (Fig. 1) ultrastructurally.

Fractionation of subunit proteins. After washing to remove contaminating proteins, it was established on gels that the remaining fibrillar material was composed of low molecular weight proteins that could be visualized in reducing and dissociating buffers. Consequently, a portion of the amyloid was homogenized in 6 M guanidine, pH 10.2, with 0.17 M DTT, resulting in the solubilization of 81% (dry weight) of the starting material. Fractionation of the solubilized amyloid on Sephadex G-75 in guanidine yielded a void and two retarded peaks (Fig. 2), the latter designated A and B, respectively, comprising 53, 35, and 12% of the applied sample (dry weight). The molecular weights of the two retarded peaks were 24,000 and 12,000 mol wt, determined by calibration of the gel filtration column. These molecular weights were confirmed by SDS-polyacrylamide gel electrophoresis (PAGE), which showed B to be a single band of 12,000 mol wt. Under nonreducing conditions, the A peak consisted of three bands, the bottom of which had a molecular weight of 24,000. In reducing buffer, this band largely disappeared with the appearance of material with identical molecular weight as the B peak (Fig. 2, inset). It seemed likely, therefore, that A contained a dimer of B.

Amino acid sequence. The amino terminal sequence of A and B was established by Edman degradation after reduction and alkylation. B had an unambiguous amino terminal residue (isoleucine) and sequenced to position 30 (Fig. 3). A gave an identical sequence to position 20, though with considerably lower yield and significant contamination with other amino acids only at the amino terminal position. Residue 13 was indeterminate in both A and B, and residue 17 could also not be determined in peak A. These findings show the identity of B and a significant portion of the A peak material.

The sequence obtained was identical to the amino-terminal sequence of human beta-2 microglobulin (B2M), with no amino acid substitutions among the residues identified (Fig. 3). The size of the monomer (12,000 mol wt) and the amino acid composition (not shown) make it likely that the intact B2M molecule is present in the fibrils.

Discussion

Amyloidosis is a generic term that refers to a group of diseases in which fibrillar material forms as a consequence of several different pathogenic mechanisms (14). These include structural amyloidogenicity of certain proteins, polymerization, and proteolysis. Structural amyloidogenicity may relate to the beta-pleated sheet configuration or the presence of polymorphic or abnormal molecules in the blood.

For some years, it has been recognized that B2M has a prominent beta structure (15), which has been suggested to be important in the stabilization of the tertiary conformation of class I HLA heavy chains and other differentiation antigens with which it is associated on cell surfaces (16). A recent detailed x-

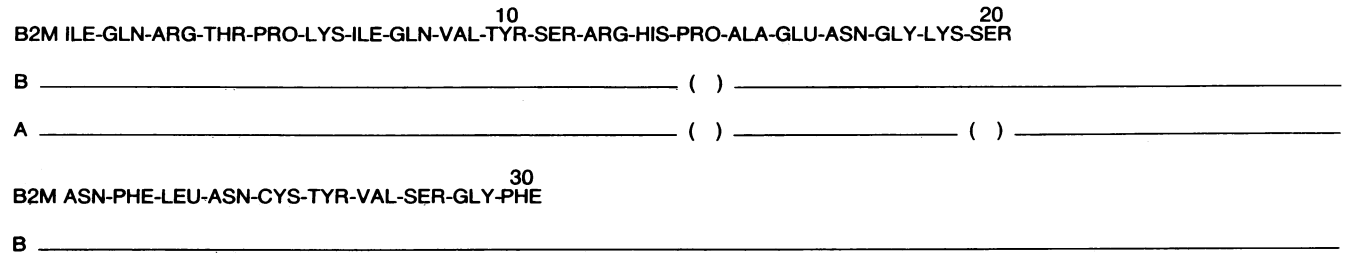

Figure 3. Comparison of the amino terminal sequence of MAR amyloid fractions A and B (Fig. 2) with the published sequence (20) of B2M, ——, homology; ( ), undetermined.

ray crystallographic study (17) of bovine B2M demonstrated 50% beta structure, with particular structural homology to the CH_3 domain of immunoglobulin G1. In addition, comparison of the amino acid sequences of B2M obtained from various species has shown striking conservation over half of the total residues, and that the conserved residues are specifically those that participate in the two antiparallel beta-pleated sheets present in the molecule. This would imply that the beta structure and its interaction with associated molecules may be central to the biological functions of B2M.

Largely on the basis of the crystallographic data (17), and the configuration of B2M on the cell surface, it has been assumed that B2M differs from immunoglobulin constant domains in not dimerizing in vivo, and that the circulating form of the molecule is exclusively the monomer. Few studies have specifically corroborated this point, however, and we are unaware of any that have sized B2M in pathologic states. Our demonstration of a dimeric form of B2M, and possibly higher molecular weight polymers, as major constituents of atypical amyloid fibrils, in our patient, will necessitate a clarification of this issue.

Allelic forms of B2M have been identified in mice (18) and in the owl monkey (19). Sequence studies of human B2M, however, have failed to establish true genetic polymorphism (20). Although posttranslational changes resulting in charge heterogeneity may explain the existence of more than one species of B2M in human urine (21), there is no evidence for multiple circulating forms in man. This may be in contradistinction to the prealbumin form of amyloidosis, where both normal and variant molecules circulate, the latter being the predominant and perhaps exclusive form that deposits in the fibrils (22). Both amino and in some cases carboxyterminal heterogeneity is frequent among amyloid proteins, and has been taken as an indication that they arise by proteolysis due to the action of endopeptidases on a precursor protein. In light chain amyloidosis, the amino terminus of fibril subunit proteins is usually unambiguous, but fragments of immunoglobulin light chain due to proteolytic cleavage in the constant region of the molecule are often seen. In some instances, copolymerization of intact light chains with proteolytic digestion products can be demonstrated when fibrils are fractionated under dissociating conditions (1, 23). Our observation that a molecule with only constant region homology is capable of adopting a fibrillar configuration raises questions as to how important is the variability of light chains in light chain amyloidosis. It seems more likely that framework region determinants, perhaps related to the beta structure, may be more significant and relevant to fibril formation. The full amino acid sequence of our subunit molecules will thus be necessary to establish whether the protein is intact B2M, whether there is carboxyterminal heterogeneity, and if a variant or mutant molecule is present in this patient.

Elevated levels of B2M have been found in association with chronic renal insufficiency, lymphoproliferative disorders, some rheumatic diseases, and the acquired immunodeficiency syndrome (24–26). In these disorders, the potential exists for the development in certain susceptible individuals of a pathologic disorder analogous to so-called light chain disease, in which immunoglobulin light chains deposit in tissue in an amorphous or fibrillar configuration (27). Although B2M has been shown to deposit in renal allografts by immunohistological techniques (28), other tissues of patients with chronic renal disease have not been examined. Such studies would clearly be of interest in the light of our observations.

Note added in proof. Gejyo et al. (29) have recently reported amino acid sequence data to show that beta-2-microglobulin was a subunit protein present in amyloid isolated from the carpal tunnel of a patient on chronic dialysis, thus providing independent corroboration of our findings.

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