# Chemical Typing of Amyloid Protein Contained in Formalin-Fixed Paraffin-Embedded Biopsy Specimens

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**Key Words:** Amyloid; Amyloidosis; Amyloid typing; Chemical characterization; Formalin fixation



## Abstract

*The human amyloidoses represent a heterogeneous group of disorders characterized by the deposition of fibrillar protein in vital organs. Given the fact that at least 20 different molecules can form fibrils, the unambiguous identification of the type of amyloid deposited is critical to the correct diagnosis and treatment of patients with these disorders. Heretofore, this information has been inferred from particular clinical features of the disease, ancillary laboratory tests, and results of immunohistochemical analyses. However, to establish unequivocally the kind of protein that is deposited as amyloid, it is necessary to determine its chemical composition through amino acid sequencing or mass spectroscopy of material extracted from fibrillar deposits. We have developed a micromethod whereby such studies can be performed readily using sections of formalin-fixed, paraffin-embedded biopsy specimens. The ability to identify precisely the nature of the tissue deposits has diagnostic, therapeutic, and prognostic implications for patients with amyloid-associated disorders.*

Typically, the diagnosis of amyloidosis is made by pathologists and is based on the presence in tissue of material having the characteristic features of amyloid; namely, after Congo red staining, green birefringence is seen by polarizing microscopy, and nonbranching fibrils approximately 8 to 10 nm in diameter and of indeterminate length are noted by electron microscopy.[1] Amyloidosis, however, is not a single disease; rather, the term represents a group of disorders characterized by the pathologic deposition throughout the body of at least 20 different precursor proteins **Table 1**.[2] Despite the differing chemical nature of these molecules, all forms of amyloid have virtually identical tinctorial and ultrastructural features and, as a result, cannot be differentiated microscopically. Furthermore, while the various disorders associated with amyloid deposition (acquired or inherited) may be distinguished from one another on the basis of particular clinical and ancillary laboratory features, there may be considerable overlap in disease manifestations, thus making a precise diagnosis impossible.[3,4] Because the cause, treatment, and prognoses of these illnesses differ, it is essential that the exact nature of the fibrillar deposits be identified.

Historically, the two most common forms of amyloid—AL and AA—were distinguished from one another by the latter's sensitivity to potassium permanganate, ie, the loss of congophilia after pretreatment of the specimen with this compound.[4,5] However, the imprecise nature of this histochemical method has been evidenced by the fact that certain types of non-AA amyloid proteins, eg, Abeta$_2$M and AApoA1, also can be rendered negative for Congo red after exposure to potassium permanganate.[6]

A more informative means to determine the nature of pathologic fibrillar deposits involves the use of immunologic techniques that rely on antisera specific for the different types

**Table 1**
**Amyloid Nomenclature: Amyloid Fibril Proteins and Their Precursors in Humans***

| Amyloid Protein | Precursor Protein | Systemic (S) or Localized (L) | Syndrome or Involved Tissues |
|---|---|---|---|
| AL | Immunoglobulin light chain | S, L | Primary, myeloma-associated |
| AH | Immunoglobulin heavy chain | S, L | Primary, myeloma-associated |
| ATTR | Transthyretin | S | Familial (prototype Portuguese, Japanese, Swedish); senile systemic |
| | | L? | Tenosynovium |
| AA | Serum amyloid A protein (SAA) | S | Secondary, reactive |
| Abeta$_2$M | beta$_2$-Microglobulin | S | Long-term hemodialysis |
| | | L? | Joints |
| AApoA1 | Apolipoprotein A1 | S | Familial |
| | | L | Aortic |
| AGel | Gelsolin | S | Familial (prototype Finnish) |
| ALys | Lysozyme | S | Familial |
| AFib | Fibrinogen alpha chain | S | Familial |
| ACys | Cystatin C | S | Familial (prototype Icelandic) |
| Abeta | Abeta protein precursor (AbetaPP) | L | Alzheimer disease, aging; familial (prototype Dutch) |
| APrP$^{SC}$ | Prion protein | L | Spongiform encephalopathies |
| ACal | (Pro)calcitonin | L | C-cell thyroid tumors |
| AIAPP | Islet amyloid polypeptide | L | Islets of Langerhans; insulinomas |
| AANF | Atrial natriuretic factor | L | Cardiac atria |
| APro | Prolactin | L | Aging pituitary; prolactinomas |
| AIns | Insulin | L | Iatrogenic |
| AKer | Kerato-epithelin | L | Cornea |
| ABri | ABri protein precursor (ABriPP) | S, L | Familial dementia (British type) |
| ADan | ADan protein precursor (ADanPP) | ? | Familial dementia (Danish type) |
| AMed | Lactadherin | L | Aging aortic media |

* Modified from Westermark et al.[2]

of amyloid proteins.[7-13] Unfortunately, these methods, too, have several technical limitations, eg, commercially available reagents are prepared against the native form of the amyloidogenic precursor molecule and, thus, may fail to react with the amyloid owing to conformational differences resulting from fibrillogenesis or tissue fixation. In addition, in the case of AL, fibrils most often consist of light-chain fragments.[6,14] Therefore, to establish unequivocally the nature of the amyloid protein, it is necessary to extract this material and analyze it chemically. Heretofore, such studies required relatively large amounts of fresh tissue that, most often, were not readily available. To circumvent this problem, micromethods have been developed to isolate, purify, and sequence amyloid proteins extracted from biopsy samples,[15-19] even after formalin fixation.[16,20-24] Furthermore, advances in technology, including microsequencing and mass spectroscopy, have made it possible to analyze picomole quantities of protein.

We have modified small-scale extraction, purification, and analytic techniques[18,19] to characterize chemically amyloid proteins isolated from formalin-fixed, paraffin-embedded tissues obtained by fine-needle biopsies. We now report the results of our studies involving 9 different types of amyloid (AL, AH, AA, ATTR, Abeta$_2$M, AApoA1, ALys, AANF, and AIns) in which the nature of the amyloidogenic proteins contained in the pathologic fibrillar deposits was established definitively.

## Materials and Methods

### Sample Preparation

Four-micrometer-thick sections cut from formalin-fixed, paraffin-embedded tissues were stained with a freshly prepared solution of alkaline Congo red[6] and examined under polarizing microscopy at a magnification of ×100. A qualitative assessment of amyloid deposition was made based on the relative extent of green birefringence seen in the entire specimen, and a value of 1+, 2+, or 3+ was assigned accordingly. The amount of amyloid present in the tissue section also was estimated using a quantitative imaging system. Four-micrometer-thick Congo red–stained sections were viewed at ×100 magnification and digital photographs taken using a SPOT RT 2.2.0 cooled CCD camera (Diagnostic Instruments, Sterling Heights, MI). The photographs were loaded into a calibrated image-analysis software package (ImagePro Plus, Media Cybernetics, Silver Spring, MD), and the area occupied by green birefringent material was measured.

Depending on the volume of tissue available and amount of congophilia present in the specimen, approximately 1 to 30 4-µm-thick sections were cut from formalin-fixed, paraffin-embedded tissue blocks and placed on poly-L-lysine–coated glass microscopic slides. The tissue was first deparaffinized by immersion in Americlear (Baxter, Deerfield, IL) for 48 to 72 hours and then rehydrated by exposure

for 5-minute periods to the following graded series of solvents: 100% ethanol, 95% ethanol and water, 80% ethanol and water, and, finally, distilled water. Excess liquid was drained onto a paper towel, and the slides were stored in a suitable container until completely dry. The tissue sections were loosened with a single-edge razor blade and, using a No. 11 scalpel blade, scraped from the slide into a 1.8-mL capacity Eppendorf tube to which was added 250 µL of a 0.25-mol/L tris(hydroxymethyl)aminomethane-hydrochloride, pH 8.0, 1.0-mmol/L disodium-EDTA solution and 750 µL of an 8-mol/L guanidine hydrochloride solution (Pierce, Rockford, IL). The tube was incubated at 37°C for 24 to 48 hours. In cases in which material was not completely solubilized, the incubation period was extended for an additional 48 to 72 hours, and the tubes were sonicated repeatedly until the solution was clear. The protein was reduced by addition of 20 µL of 2-mercaptoethanol (Pierce), the mixture was mixed briefly, and, after incubation for 2 hours at 37°C, alkylated by addition of 30 µL of 4-vinylpyridine followed by a 30-minute incubation at 37°C.

### Protein/Peptide Preparation

To obtain a particulate-free solution, the reduced-alkylated protein was centrifuged at 17,000*g* for 10 minutes and the supernatant injected (ABI Model 112A injector, Applied Biosystems, Foster City, CA) onto an Aquapore 300 $C_8$ (30 × 4.0 mm) column (Brownlee Columns, Perkin Elmer, Norwalk, CT). The protein components were separated by reverse-phase high-performance liquid chromatography (HPLC) over a 45-minute period using an ABI model 140A solvent delivery system (Applied Biosystems) that produced a linear 0.1% trifluoroacetic acid water–7% acetonitrile to a 0.1% trifluoroacetic acid water–70% acetonitrile gradient at a flow rate of 1 mL/min. The absorbance was read at 220 nm using an ABI model 785A programmable absorbance detector (Applied Biosystems), and the fractions corresponding to the UV-absorbing peaks were collected manually. Each fraction was reduced to a volume of approximately 60 µL in a Speed Vac sample concentrator (Savant Instruments, Farmingdale, NY), and one fourth was placed in the glass fiber blot cartridge of an ABI model 494 Procise automated amino acid sequencer (Applied Biosystems). The N-terminal amino acid sequence was determined using the pulsed-liquid cycle according to the manufacturer's directions. The remaining material was dried in the Speed Vac and subjected, if necessary, to trypsin digestion or, in some instances, to cyanogen bromide cleavage.

In cases in which the N-terminal residue was "blocked" by the presence of pyroglutamate, it was removed by dissolving a portion of the lyophilized sample in 50 µL of a 1-mmol/L concentration of sodium phosphate buffer, pH 7.0, containing 10-mmol/L dithiothreitol and 1-mmol/L EDTA concentrations and adding 0.2 to 1 mU of *Pfu* pyroglutamate aminopeptidase (Takara Shuzo, Shiga, Japan).

After overnight incubation at 37°C, the reaction mixture was loaded onto a Prosorb cartridge (Applied Biosystems), the membrane was washed with 100 µL of 0.1% trifluoroacetic acid, and, after drying, it was removed and placed in the blot cartridge for automated sequence analysis. For digestion with trypsin, the protein fractions were reconstituted with 100 µL of a 0.1-mmol/L concentration of N-methylmorpholine–sodium acetate buffer, pH 8.0, followed by addition of a 1% solution of trypsin (Promega, Madison, WI) dissolved in the same buffer at an enzyme/protein ratio of 1:20 to 1:50 (wt/wt), as estimated from the peak height when compared with a protein standard. After incubation at 37°C for 4 hours, the sample was concentrated using the Speed Vac apparatus to a volume of approximately 25 µL. For cleavage with cyanogen bromide (Pierce), the protein fractions were dissolved in 70% formic acid, and a 100-fold molar excess of the chemical was added. The trypsin and cyanogen bromide digests were injected onto an Aquapore 300 $C_8$ microbore (150 × 0.5 mm) column and the peptides eluted by reverse-phase HPLC at a flow rate of 5 µL/min using a linear gradient of 0.1% trifluoroacetic acid water–5% acetonitrile increasing to 15% acetonitrile over the first 10 minutes with a gradual increase to 35% acetonitrile over the next 140 minutes. The absorbance was read at 215 nm and recorded on a strip chart recorder at a speed of 1 mm/min. The eluted peptides were spotted onto a polyvinylidene difluoride (PVDF) membrane (Blotter Paper/PVDF, ABI) using an ABI model 173A microblotter (Applied Biosystems) running at a synchronous rate with the chart recorder. The peptide-containing spots were cut from the PVDF membrane and placed in the blot cartridge of the Procise sequencer (Applied Biosystems).

For mass spectroscopy, the samples were applied to an Aquapore column and subjected to reverse-phase HPLC, and the eluent was directed to an ion-spray PE-Sciex type 150 EX instrument (Applied Biosystems). Mass data were analyzed using the Bio Multiview software provided by the manufacturer (Applied Biosystems).

### Immunohistochemical Analysis

Four-micrometer-thick paraffin-embedded tissue sections were cut on a microtome, mounted on poly-L-lysine-coated slides, dried overnight at room temperature, and deparaffinized. Immunostaining was performed using the avidin-biotin complex technique (Vector Laboratories, Burlingame, CA), as described previously.[25] The primary reagents included monoclonal mouse antihuman AA (DAKO, Carpinteria, CA) and rat antihuman SAA (Biosource, Camarillo, CA) antibodies and polyclonal rabbit antihuman total and free kappa and lambda light chain

(DAKO), ATTR (DAKO), and antihuman beta$_2$M (DAKO) antibodies. A biotinylated sheep antirabbit globulin antiserum (Vector) was used as the secondary antibody.

## Results

### Biopsy Specimens

The number of sections required for chemical analyses was dependent on the amount of amyloid present in the specimen and the volume of tissue available. The amyloid content was estimated on a scale of 1+ to 3+ based on the quantity of green birefringent Congo red staining material seen by polarizing microscopy. This classification corresponded to the amyloid burden determined from computer-based quantitative imaging. Generally, for surgically derived biopsy specimens (approximately 0.5 to 1.5 cm$^3$) classified as 1+, 2+, or 3+, as few as 10, 7, or 1 4-µm-thick sections, respectively, were required. In the case of fine-needle biopsy specimens that contained congophilic material of comparable magnitude, 30, 20, or 6 sections, respectively, were used.

### Analyses of Amyloid Extracts

Amyloid-containing tissue sections were homogenized and the fibrils extracted with guanidine hydrochloride and isolated by reverse-phase HPLC. Although there were differences in the elution profiles of the amyloid extracts, typically, this material eluted from the column at an acetonitrile concentration between 35% and 45% and appeared as a relatively broad zone that contained multiple UV-absorbing peaks **Figure 1**. In contrast, guanidine extracts of normal (nonamyloid) formalin-fixed, paraffin-embedded tissue sections contained no detectable material in this area of the chromatogram. Based on the HPLC behavior of reference amyloid protein standards,[18,19] representative peaks were selected for chemical analysis. In cases in which the amyloid protein had a free N-terminal amino acid, eg, kappa and certain lambda-type AL proteins (as well as AH, AA, ALys, AApoA1, Abeta$_2$M, AANF, and AIns), it was possible to sequence this material directly and identify at least the first 10 to 15 residues of the protein. This was sufficient to establish the type of amyloid present in the specimen.

Alternatively, if no sequence was obtained when at least 4 peaks from the HPLC eluate were examined, it was presumed that the N-terminal residue was blocked, ie, that it contained pyrrolidone carboxylic acid, as occurs in certain lambda-type AL components formed from proteins of the $V_{lambda}1$ and $V_{lambda}2$ subgroups.[26] In these and other cases in which the sequence was ambiguous owing to N-terminal proteolysis (as found for ATTR), it was necessary to generate peptide fragments by trypsin or cyanogen bromide digestion of the protein contained in the selected peak. The enzymatic or chemical digests were subjected to reverse-phase HPLC using a microbore column, the peptides were spotted onto a PVDF membrane, and the amino acid sequence of selected fractions was established.

The nature of the fibrillar components also could be deduced by ion spray mass spectroscopic analysis of HPLC-separated tryptic peptides, as shown in the case of an ATTR

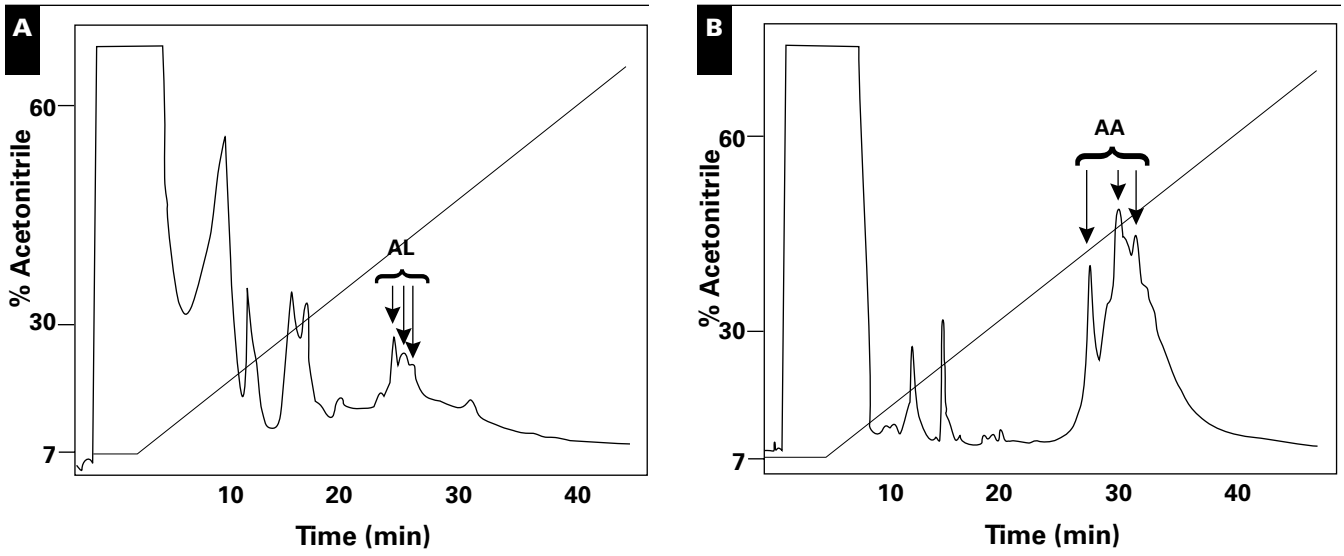


**Figure 1** Reverse-phase high-performance liquid chromatography profiles of amyloid extracted from formalin-fixed, paraffin-embedded tissue biopsy sections. **A**, Subcutaneous mass, 18 sections (6 × 2 mm). **B**, Liver, 3 sections (25 × 10 mm). Amino acid sequence analyses of protein contained in peaks designated by arrows indicated the presence of AL- and AA-related components in **A** and **B**, respectively.

protein that contained the "Appalachian" T60A mutation.[27] The primary structure of the peptide, as predicted by mass spectroscopy, was confirmed by amino acid sequence analysis **Figure 2** **Table 2**.

As summarized in **Table 3**, formalin-fixed, paraffin-embedded tissue extracts from 49 patients with amyloidosis were analyzed. In each instance, the type of amyloid was identified as AL, AH, AA, ATTR, Abeta$_2$M, AApoA1, ALys, AANF, or AIns. In AH and some cases of AL and ATTR amyloidosis, chemical analyses of the amyloidogenic precursor proteins also were performed, and these results were concordant with studies on the amyloid samples. For example, among the 24 patients with AL amyloidosis from whom urine specimens were available, 15 had kappa or lambda Bence Jones proteinuria. Of these, 9 were sequenced, and these data were in complete agreement with those obtained from analyses of the amyloid extracts.

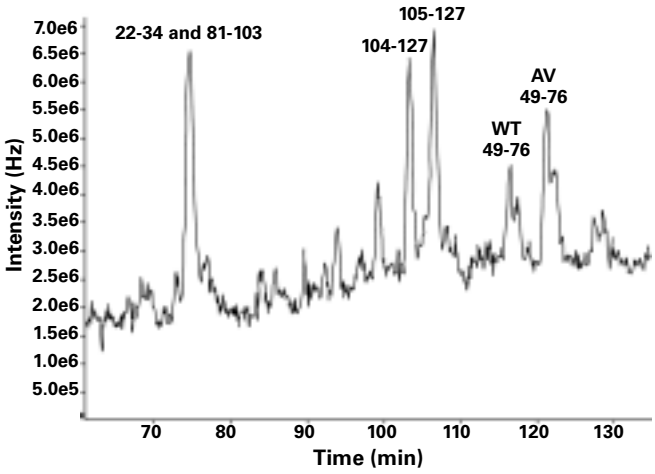


**Figure 2** Capillary liquid chromatography–ion spray mass spectroscopic analyses of peptides derived from tryptic digestion of wild-type (WT) and Appalachian variant (AV) ATTR proteins. Differences in the elution profiles of the peptides spanning residues 49-76 obtained from the WT and AV proteins are evident.

### Correlation of Immunohistochemical and Chemical Classification of Amyloid

When appropriate antisera were available, immunohistochemical studies were performed on amyloid-containing tissue sections, and the results were compared with those derived by sequence and/or mass spectroscopic analyses. Most often, there was concordance; however, notable exceptions occurred. In some specimens (particularly AL), the immunohistochemical results were ambiguous, eg, the deposits reacted with the antikappa and antilambda reagents, as well as the anti-AA antiserum, as shown in **Image 1**. In these 2 cases, the amyloid proteins extracted from tissue specimens 1 and 2 were identified chemically as ALlambda and ALkappa, respectively, and no AA protein was detected. In addition, when the protein isolated from a specimen reported on the basis of immunohistochemical analysis to contain AA[28] amyloid was subjected to amino acid sequencing, it was shown to be AL. In 2 patients who received chemotherapy for AL amyloidosis that was diagnosed on the basis of a monoclonal gammopathy, 1 was found, through chemical analysis, to have AA and the other, ATTR.

## Discussion

We applied microextraction and microsequencing technology to determine the type of amyloid contained in sections cut from formalin-fixed, paraffin-embedded tissue specimens. Because the diagnosis of amyloidosis is made most often from evaluation of fine needle–derived biopsy specimens, the novelty and usefulness of this method is evidenced by the fact that it is not dependent on the availability of fresh tissue and requires only a small quantity of material. Depending on the volume of available tissue and

**Table 2**
**Expected and Observed Masses and Amino Acid Sequence Data on Five Peptides Derived From Trypsin Digestion of Wild-Type and Appalachian Variant ATTR Proteins***

| Expected Mass* | | | | |
|---|---|---|---|---|
| Wild-Type TTR | Appalachian Variant TTR | Observed Mass | Tryptic Peptide | Amino Acid Sequence |
| 1366.59 | 1366.59 | 1366.03 | 22-34 (T1) | GSPAINVAVHVFR |
| 2451.68 | 2451.68 | 2452.58 | 81-103 (T3) | ALGISPFHEHAEVVFTANDSGPR |
| 2645.99 | 2645.99 | 2645.84 | 104-127 (T4) | RYTIAALLSPYSYSTTAVVTNPKE |
| 2489.81 | 2489.81 | 2489.22 | 105-127 (T5) | YTIAALLSPYSYSTTAVVTNPKE |
| 3141.39 | | 3141.19 | 49-76 (T2) | TSESGELHGLTTEEEFVEGIYKVEIDTK |
| | 3110.99 | 3111.44 | 49-76 (T2) | TSESGELHGLTAEEEFVEGIYKVEIDTK |

* The difference in the T2 (49-76) peptide masses results from the presence at position 60 of threonine or alanine in the wild-type and Appalachian variant proteins, respectively.

**Table 3**
**Chemical Identification of Amyloid Proteins Extracted From Formalin-Fixed Paraffin-Embedded Tissue Specimens**

| Amyloid Protein | Amyloid Designation | Tissue Source* |
|---|---|---|
| Immunoglobulin light chain | AL | Kidney (2), bone (3), eye (1), colon (1), gallbladder (1), skin (3), liver (1), bladder (2), larynx (1), lung (3), salivary gland (1), tongue (1), synovium (1), gut (1), fat (2), heart (1), LN (1), nasopharynx (1), amyloidoma (1) |
| Immunoglobulin heavy chain | AH | Liver (1) |
| Serum amyloid A protein | AA | Liver (2), kidney (1), spleen (1), skin (1) |
| Transthyretin | ATTR | Heart (8) |
| beta$_2$-Microglobulin | Abeta$_2$M | Heart (2) |
| Apolipoprotein A-I | AApoA1 | Spleen (1) |
| Lysozyme | ALys | Kidney (1), cervical mass (1) |
| Atrial natriuretic factor | AANF | Heart (1) |
| Insulin | AIns | Thigh (1) |

* The number of cases is given in parentheses.

the amount of green birefringent congophilic substance present in the specimen (as judged by a qualitative microscopic grading system), it is possible to obtain from 1 to 30 4-µm-thick tissue sections a sufficient quantity of protein for amino acid sequencing and/or mass spectroscopy and, thus, to establish the nature of the fibrillar deposits. In studies involving 49 cases, 9 different types of amyloid were identified, including the most common AL, AA, and ATTR varieties. Although tissue containing other forms of amyloid (Table 1) was not available for assay, we presume that our method could be used to characterize these species as well.

The specific type of amyloid present in all samples studied, as established through chemical analysis of protein extracted from formalin-fixed, paraffin-embedded biopsy specimens, was consistent with the clinical manifestations of disease and other laboratory criteria, eg, in the case of systemic AL amyloidosis, by the presence of a monoclonal gammopathy or plasma cell population, and in ATTR by DNA and mass spectroscopic analyses of serum transthyretin. In addition, the results of sequence analyses of material extracted from relatively large amounts (ie, >1 g) of fresh tissue in cases of AL, AH, AA, and ATTR amyloid

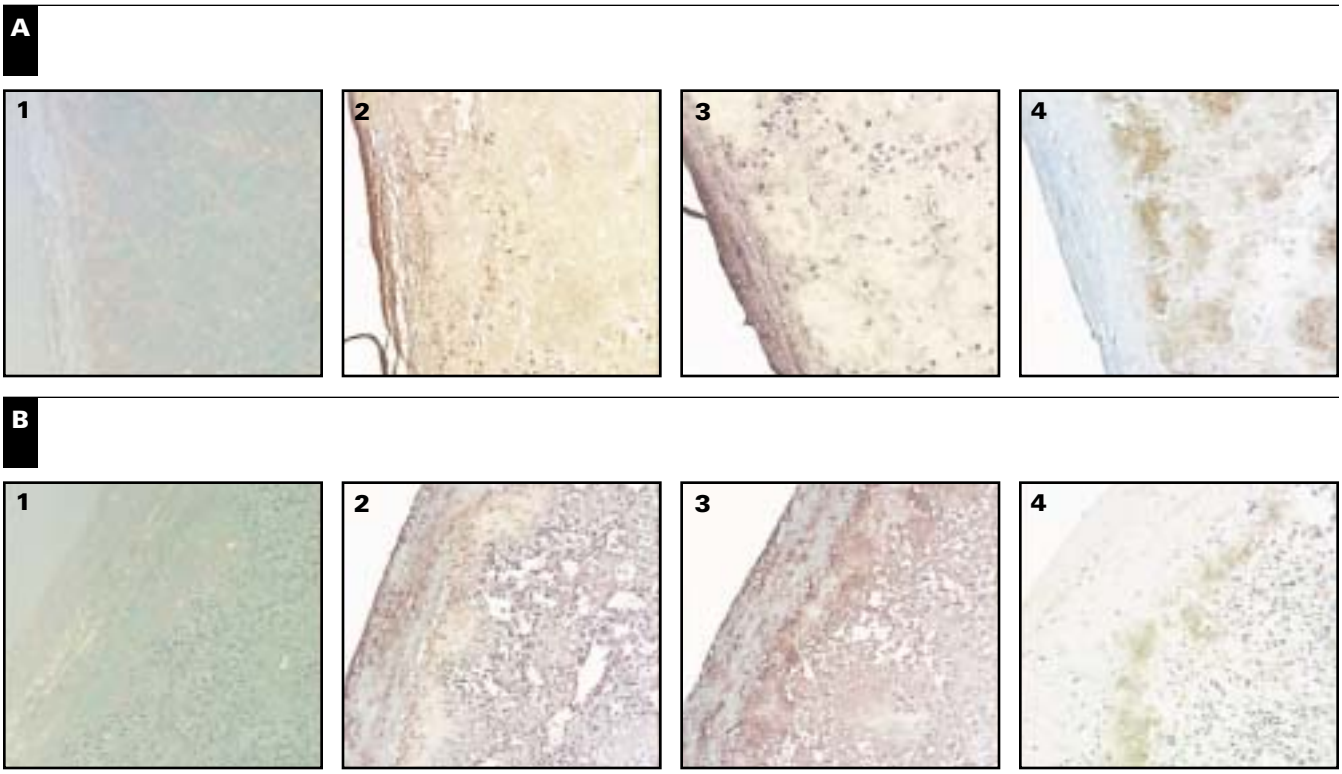


**Image 1** Immunohistochemical analyses of amyloid-laden splenic tissue. **A**, Patient 1 (AL lambda). **B**, Patient 2 (AL kappa). In both cases, the amyloid reacted with all 3 antisera (**A** and **B**: 1, Congo red; 2, anti–free kappa antisera; 3, anti–free lambda antisera; 4, anti-AA antisera; original magnification ×200).

were concordant with those obtained using material derived from formalin-fixed, paraffin-embedded sections.

It has long been presumed that it is difficult, if not impossible, to extract and sequence the protein contained in formalin-fixed tissue owing to the cross-linking effect and chemical modification of amino acid residues[29] that result from such treatment. Nevertheless, Layfield et al[22,23] were able to identify 2 types of amyloid (AL and ATTR) by heating homogenized formalin-preserved tissues in a standard sodium dodecyl sulfate (SDS)-urea–containing electrophoresis sample buffer, subjecting the protein extracts to SDS-polyacrylamide gel electrophoresis (PAGE), and, after transfer onto PVDF membranes, selecting for sequence analysis components of appropriate molecular mass. They postulated that the beta-pleated structure of amyloid proteins made them uniquely resistant to the effects of formaldehyde treatment; however, Ikeda et al[24] demonstrated, using comparable techniques, that many types of membrane-bound components also can be extracted from similarly fixed tissue. Castaño et al[16] used 99% formic acid to isolate amyloid from formalin-fixed tissue and, after HPLC purification and SDS/PAGE, identified by amino acid sequencing the presence of an AL protein. In contrast, we found it advantageous to use guanidine hydrochloride for extraction, since it yields amyloid components virtually free of other molecules. Furthermore, to ensure maximum exposure to the guanidine solvent and to obtain the highest yield of protein, we chose to extract the amyloid by homogenizing thinly cut sections rather than tissue blocks. We found that, with rare exception, formalin fixation did not result in structural modification of lysyl or other amino acid residues typically affected by this chemical agent,[29] even in tissue preserved in formaldehyde for 100 years.[30] The remarkable stability of amyloid fibrils in the presence of formalin also may account for their apparent resistance to in vivo degradation.

Because at least 20 different proteins can form amyloid deposits and result in diseases that, in some cases, are very similar in their clinical and pathologic manifestations,[3,4] it is necessary to know, for diagnostic, prognostic, and therapeutic purposes, the nature of the amyloidogenic constituent. This is particularly important for patients with cardiac, renal, hepatic, or neurologic involvement, as is commonly found in AL, AA, ATTR, AFib, and ALys forms of the disorder. In addition, AA amyloidosis, although occurring most commonly in patients with chronic infectious or inflammatory diseases, also may be a manifestation of an occult neoplasm.[31] While the type of amyloid often can be inferred from immunohistochemical analyses[7-12] or immunologic (enzyme-linked immunosorbent) assays[13] of tissue biopsy specimens or aspirates, the results may be inconclusive or inaccurate owing to the lack of appropriate antisera or loss of particular protein-specific epitopes. Thus, to establish unequivocally the nature of the deposited amyloid protein, it is essential that the material be extracted from the specimen and analyzed chemically. The advances in microinstrumentation and the method described herein have made it possible to obtain such information from minute amounts of formalin-fixed, paraffin-embedded tissues that, by histochemical study, have been deemed to contain amyloid. The ability to diagnose precisely the type of congophilic protein present has become increasingly important given the development of specific therapies for patients with amyloid-associated disorders. These include high-dose chemotherapy[32] and organ transplantation[33] for AL and ATTR, respectively, as well as the use of inhibitors of fibril formation,[34] amyloidolytic drugs,[35] and compounds that block interaction of molecules that codeposit with amyloid and seemingly promote fibrillogenesis or inhibit amyloid degradation.[36-38] Furthermore and more recently, active[39] or passive[40,41] immunotherapy has been used to prevent as well as to eliminate pathologic fibrillar deposits. Thus, to provide optimal patient care, we recommend that pathologists not only establish a diagnosis of amyloidosis but also, as part of the routine workup, ascertain the specific type of protein that is deposited as amyloid.

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