THE MAJOR PROTEINS OF HUMAN AND MONKEY AMYLOID SUBSTANCE: COMMON PROPERTIES INCLUDING UNUSUAL N-TERMINAL AMINO ACID SEQUENCES

E.P. BENDITT, N. ERIKSEN, M.A. HERMODSON and L.H. ERICSSON
Departments of Pathology and Biochemistry of the University of Washington, Seattle, Washington 98195, USA

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1. Introduction

Amyloid substance is a complex proteinaceous material found in the tissues of patients with the disease amyloidosis. Recently we have presented evidence that there are two chemically distinct kinds of amyloid substance, one associated with the classical inflammation-related amyloidosis and another, frequently designated atypical or paramyloid, occurring with tumors such as multiple myeloma or without evident pre-existing disease [1]. The classical, or inflammation-associated, substance is distinguished by: a) instability to alkali of its characteristic Congo red binding capacity; b) its amino acid composition and c) the presence of a major protein constituent, amyloid protein A. The family of proteins comprising amyloid protein A has a molecular weight range of 6000–8000 and a characteristic amino acid composition [2]; in addition, human amyloid protein A has the capacity to bind Congo red and exhibits the characteristic hyperchromism and spectral changes previously described for amyloid substance [1]. In this communication we compare the chromatographic and electrophoretic properties, the amino acid composition and the 24 amino acid N-terminal sequence of the human- and monkey-derived protein A of amyloid substance.

2. Materials and methods

Amyloid substance was concentrated and purified from liver tissue as previously described [1]. The human material was obtained from a patient who had classical generalized amyloidosis associated with tuberculosis. The clinical data on this case, designated in our series as A-4, have been recorded elsewhere [1]. The liver of a monkey (*Macaca mulatta*) having amyloidosis associated with a chronic granulomatous disease presumed to be a tuberculous infection [3] was kindly provided us by Dr. Boris Ruebner of the National Regional Primate Center at Davis, California. More than 50% of the tissue mass in each of the livers was estimated by microscopy to be amyloid substance. In both instances the amyloid substance exhibited the characteristic staining with crystal violet and Congo red and had the expected abundance of microfibrils when examined in the electron microscope.

To prepare the major constituent, protein A, the purified amyloid substances were extracted with 6 M urea – 0.01 M HCOONa – 0.5 M HCOOH, pH 3.0 (urea–formate buffer). Each 100 mg of dry starting material was suspended in 4 ml of buffer. The suspensions were stirred overnight at 4° and then centrifuged at 25,000 *g* for 30 min. Extracts were fractionated on G-100 Sephadex in the urea–formate buffer (fig. 1). The slower portion (IVb) of the major fraction obtained from each sample was dialyzed against H_2O in acetylated cellulose casing [1] until the concentration of urea had been reduced to about 0.1 M, and then lyophilized. The partly purified A proteins were further purified and freed of the bulk of the remaining urea by chromatography on

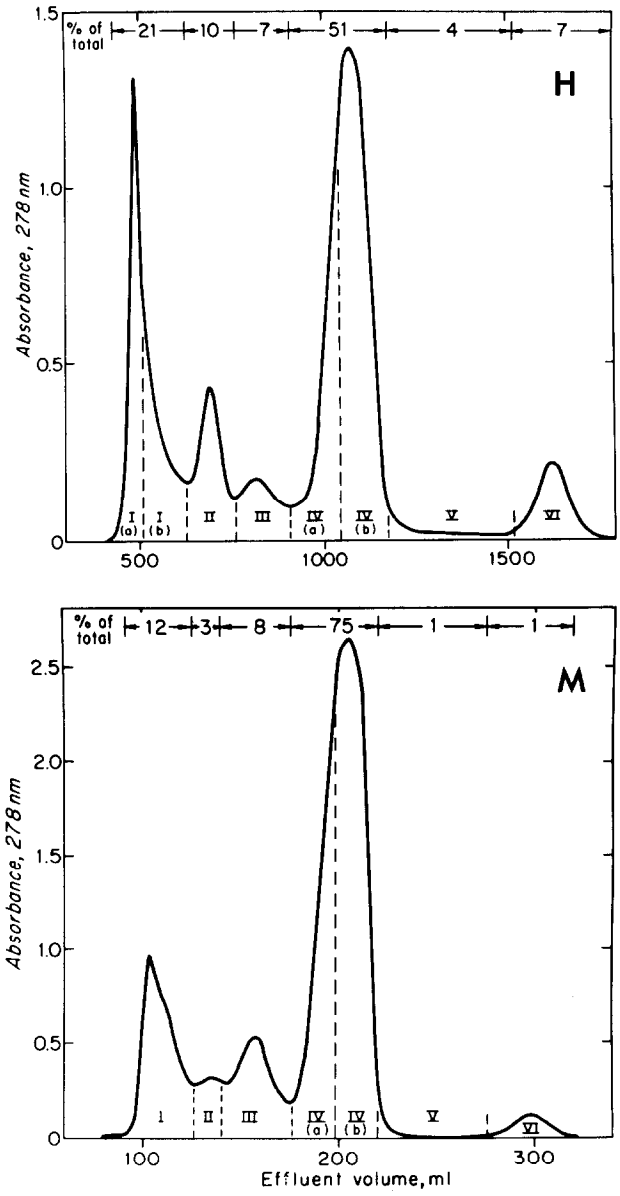

Fig. 1. Fractionation of extracts of human (H) and monkey (M) amyloid-substance concentrates on G-100 Sephadex in urea–formate buffer, pH 3. Human sample (12.4 ml derived from 400 mg purified amyloid substance) passed through a 5.0 × 88 cm column at a flow rate of 47 ml/hr; monkey sample (6.1 ml derived from 185 mg purified amyloid substance), through a 2.5 × 63 cm column at a flow rate of 12 ml/hr. Numbers at top indicate relative amounts of material contained in pooled effluent fractions (I–VI) according to absorption measurements at 289 nm (pH > 12).

Table 1
Amino acid composition* of human and monkey amyloid protein A.

Amino acid	Human (not oxidized)[a]	Human (oxidized)[b]	Monkey (oxidized)[b]
Lys	2.8	2.8	5.4
His	2.2	2.0	2.7
Arg	10.8	10.7	7.7
Asp	13.4	14.1	15.4
Thr	0.1	0.0	1.3
Ser	7.5	7.3	6.3
Glu	7.4	7.2	9.6
Pro	1.5	1.4	1.3
Gly	12.2	12.2	10.9
Ala	16.7	17.5	15.5
Cys/2	0.1	0.0[c]	0.0[c]
Val	1.4	1.3	2.0
Met	2.8	2.8[d]	2.5[d]
Ile	3.8	3.2	2.0
Leu	1.5	1.5	4.0
Tyr	5.5	5.4	6.0
Phe	8.2	7.7	3.7
Trp[e]	2.3	2.8[f]	3.7[f]
Hyp	0.0	0.0[f]	0.0[f]

* Residues per 100 found.
a Mean or extrapolated values derived from hydrolysis times of 20, 40 and 70 hr in constant boiling HCl at 108–110°; sample material isolated from washed tissue by extraction with 6 M urea, pH 3.5 [1].
b 24-hr hydrolysis of sample material oxidized with performic acid [8].
c Determined as cysteic acid; total cysteic acid < 0.05 residue per 100.
d Determined as methionine sulfone.
e Spectrophotometric estimation [9].
f Estimated in non-oxidized sample.

2.5 × 65 cm columns of G-50 Sephadex equilibrated with 0.05 M acetic acid, the developing solvent [2]. In each instance a single slightly asymmetric peak was obtained. The main portion of this rechromatographed fraction was recovered, adjusted to pH 5 to induce precipitation of the protein, dialyzed free of residual urea, and lyophilized.

The N-terminal amino acid sequence of the two proteins was determined in a Beckman Model 890 Sequencer by an adaptation [4] of the method of Edman and Begg [5].

Fig. 2. Electrophoresis of human (H) and monkey (M) amyloid protein A in polyacrylamide gel containing 5 M urea at pH 3, according to our adaptation of the method of Takayama et al. [6], as modified by Senior and MacLennan [7]. Human sample, 20 μg in 5 μl; monkey sample, 40 μg in 10 μl. Arrow indicates direction of migration (cathodic). Migration time, 4 hr at 120 V; gel length, 85 mm; stain, Coomassie blue.

3. Results

Of the purified amyloid substance of the human case 75–80% was found to be soluble in the urea–formate solvent when used as described; as shown in fig. 1, 51% of this soluble portion was recovered in pool IV (a + b), according to optical absorption at 289 nm of aliquots adjusted to pH > 12. Of the monkey material 65–70% was found to be soluble in the urea–formate buffer, and 75% appeared in pool IV (fig. 1). According to electrophoretic analysis, the material in pool IV from both species is almost entirely protein A; electrophoretograms of the final protein preparations, i.e., after passage through G-50 Sephadex, are shown in fig. 2. The proteins derived from the human and from the monkey amyloid substances have nearly identical electrophoretic mobilities. The protein of human origin shows a major band with a faint but distinct trailing shadow; that from the monkey shows two distinct components, the leading one being the more intensely stained. In addition, the monkey protein shows a faint slower band.

Amino acid compositions of human and monkey amyloid protein A are clearly similar, as shown in table 1. They share the characteristic features of low or absent threonine, low proline and lack of cysteine, cystine, and hydroxyproline. Both proteins contain substantial amounts of alanine, glycine, and amino acids with acidic, basic, and aromatic side chains.

The amino acid sequences of the N-terminal 24 amino acids of the human and the monkey proteins are shown in fig. 3. The sequences are identical with the exception of conservative replacements at positions 3 and 11, which seem entirely compatible with other known evolutionary changes and with the preservation of structural–functional characteristics of proteins. In the human case, 13% of the purified protein A lacks amino acids 1, 2 and 3. This appears to be compatible with the presence of a trailing shadow in the electrophoretic pattern of the human protein. In the instance of the monkey amyloid protein A approximately 22% of the sample lacks the N-terminal arginine, and the electrophoretic pattern at pH 3 exhibits a discrete second band of a lesser staining than the leading band. A third, slower moving band is visible in the monkey material and this may represent another larger and/or less positively charged but related peptide. Inspection of the experimental sequence data indicates that the level of contamination by extraneous proteins with a free N-terminal probably does not exceed 2% in either of the two protein A preparations.

	1	2	3 [a]	4	5	6	7	8	9	10	11	12
Human:	Arg	Ser	Phe	Phe	Ser	Phe	Leu	Gly	Glu	Ala	Phe	Asp
Monkey:	Arg [b]	Ser	Trp	Phe	Ser	Phe	Leu	Gly	Glu	Ala	Tyr	Asp

	13	14	15	16	17	18	19	20	21	22 [c]	23	24
Human:	Gly	Ala	Arg	Asp	Met	Trp	Arg	Ala	Tyr	Ser	Asp	Met
Monkey:	Gly	Ala	Arg	Asp	Met	Trp	Arg	Ala	Tyr	Ser	Asp	Met

[a] Approximately 13% of the human amyloid protein A lacks the N-terminal Arg-Ser-Phe.

[b] Approximately 22% of the monkey amyloid protein A lacks the N-terminal Arg.

[c] Identification of Ser at position 22 in both chains should be considered tentative.

Fig. 3. N-terminal amino acid sequences of human and monkey amyloid protein A.

4. Conclusion

The data indicate that in cases of the typical or classical variety (i.e., the variety associated with chronic inflammation) amyloid substance has a major component which is homologous in man and monkey. Thus far we have had the opportunity to examine amyloid substances from eight cases of amyloidosis of this type in human beings and have found them to be essentially the same according to amino acid composition, alkali instability of the Congo red binding, and the presence of protein A [1, 2]. Four cases of monkey amyloidosis of this variety have shown essentially identical amyloid substance by the same criteria [2].

Amyloidoses of the "atypical" variety, that is those associated with tumors and multiple myeloma, and the so-called primary kind, can be distinguished chemically from the typical inflammation-related variety [1]. It has recently been suggested that in some cases amyloid proteins represent N-terminal fragments of light chains of immunoglobulins or of light chains with internal deletions [10]. While this may be true for certain types of paramyloidosis, it does not seem to be true for the classical variety, because protein A of "typical" amyloid substance accounts for 50% or more of the recoverable material of purified amyloid substance in both man and monkey and, as we have shown elsewhere [1], carries the Congo red binding marker of amyloid substance. Amyloid protein A appears to be a new protein since, to our knowledge, no peptide having the amino acid sequence reported here has been described in the literature. Further study of this protein, or family of proteins, will provide us with some new biological insights as we determine its source, structure and function.

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