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Amyloid plaque core protein in Alzheimer disease and Down syndrome

(protein sequence/HPLC/congophilic angiopathy/unconventional virus infection/scrapie)

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ABSTRACT **We have purified and characterized the cerebral amyloid protein that forms the plaque core in Alzheimer disease and in aged individuals with Down syndrome. The protein consists of multimeric aggregates of a polypeptide of about 40 residues (4 kDa). The amino acid composition, molecular mass, and NH_2-terminal sequence of this amyloid protein are almost identical to those described for the amyloid deposited in the congophilic angiopathy of Alzheimer disease and Down syndrome, but the plaque core proteins have ragged NH_2 termini. The shared 4-kDa subunit indicates a common origin for the amyloids of the plaque core and of the congophilic angiopathy. There are superficial resemblances between the solubility characteristics of the plaque core and some of the properties of scrapie infectivity, but there are no similarities in amino acid sequences between the plaque core and scrapie polypeptides.**

There are several closely associated morphologic changes in the brains of individuals with Alzheimer disease (AD): neurofibrillary tangles (NFTs) within neurons; plaques consisting of various proportions of amyloid cores (APCs)§ surrounded by neuritic degeneration; a variable degree of congophilic angiopathy (ACA); and widespread neuronal loss and gliosis in areas affected by NFTs and plaque formation. The composition of the NFT is still in doubt due in part to the difficulty in achieving complete solubilization (2).

Little is known about the pathogenesis of the APC, particularly whether the amyloid core precedes or proceeds from the surrounding neuritic degeneration. Since the amyloid plaque also occurs in some of the unconventional virus infections (3), and because recent data have drawn attention to the similarities between amyloid fibrils and the infectious agent of scrapie (4), speculation has naturally increased over the possible infectious nature of amyloid in AD. As a direct approach to unraveling the pathogenesis of the AD plaque, we have isolated and purified the amyloid from the cores of the plaques. For comparison, we have also studied the APC from the brains of an aged individual with Down syndrome (DS), in which the typical changes of AD also occur.

MATERIALS AND METHODS

Selection of Samples. For AD and DS cases, at the time of autopsy, one half of the brain was frozen at −70°C and the remaining half was fixed in Formalin for subsequent histological confirmation. Control brain samples were taken from individuals in whom there was no histological evidence of APC or NFT.

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Isolation and Purification of APCs. The frozen hemisphere is partially thawed and sliced at 1-cm intervals. The cortical grey matter is collected for homogenization (Sorvall Omnimixer) in buffer 1 (10 mM Tris·HCl/150 mM NaCl/5 mM EDTA/1% Triton-X 100/0.4 mM phenylmethylsulfonyl fluoride, pH 7.2) at a sample-to-buffer ratio of 1:9 (wt/vol). This homogenate is stirred on ice for 10 min, then centrifuged at 10,000 × *g* for 20 min (Sorvall RC2-B, GSA rotor, 4°C). The supernatant is discarded and the pellet is extracted with stirring for 20 min on ice in buffer 2 (as for buffer 1, with NaCl replaced by 0.6 M KI, and the concentration of Triton reduced to 0.5%) at a ratio of 1:9 (vol/vol) and centrifuged as before. The pellet is extracted with stirring for 20 min on ice in buffer 3 (as for buffer 2, with KI replaced with 1.5 M KCl), also at a ratio of 1:9 (vol/vol), and centrifuged as before. The pellet is washed twice in Tris-buffered saline (Tris/NaCl; 50 mM Tris·HCl/150 mM NaCl, pH 7.6) and once in sodium acetate buffer (0.2 M, pH 4.5). The pellet is resuspended in 100 ml of 5% (wt/vol) pepsin (Sigma) in acetate buffer and incubated with shaking at 37°C overnight.

The pepsin suspension is centrifuged as before and the pellet is washed in Tris/NaCl. The postpepsin pellet is resuspended in Tris/NaCl and filtered through a 52-μm nylon mesh. The filtrate is centrifuged for 10 min at 1500 × *g* (Beckman TJ-6) and the pellet is resuspended in 5–10 ml of sucrose solution A [20% (wt/vol) sucrose/1% Ficoll 400 (Pharmacia) in 10 mM sodium phosphate buffer, pH 7.4]. Two milliliters of this suspension is layered over a discontinuous gradient of 2 ml of sucrose solution B (as for solution A, but 30% sucrose) and 1 ml of sucrose solution C (as for solution A, but 45% sucrose). This gradient is centrifuged at 1500 × *g* for 30 min. Two fractions are collected: (*i*) the solution B/C interface and (*ii*) the pellet. These fractions are diluted to 10 ml with Tris/NaCl and centrifuged at 1500 × *g* for 20 min. The pellets are resuspended in 2.0 ml of Tris/NaCl and layered over a 5.0-ml Percoll (Pharmacia) solution (60 ml of 90% isosmotic Percoll with 40 ml of Tris/NaCl). This is centrifuged for 10 min at 1500 × *g* and the Tris/NaCl/Percoll interface containing the purified amyloid cores is collected and washed in Tris/NaCl.

Solubility Studies of APC. Samples of APC (1×10^4 cores in 0.1 ml of Tris/NaCl) were incubated in 1.0 ml of solvent.

Abbreviations: ACA, amyloid cerebral/congophilic angiopathy; AD, Alzheimer disease; APC, amyloid plaque core; DS, Down syndrome; NFT, neurofibrillary tangle.

§Note on nomenclature. Following published recommendations (1), we designate the amyloid in the center of the plaque as APC (amyloid plaque core) and its constituent proteins as A_4, A_8, A_{16}, etc., in which the subscript refers to the molecular mass in kDa. "APC" also serves as an abbreviation for the plaque core itself. The amyloid found in the extracellular space surrounding blood vessels we designate ACA (amyloid cerebral/congophilic angiopathy) and similarly its constituent proteins as A_4, etc.

Solubility was assessed initially on the recovery of APC from the solvent by centrifugation in a Microfuge (Beckman). If a greater than 90% reduction in the number of cores was found, the supernatant was subjected to ultracentrifugation (100,000 × *g*). The protein concentration of the supernatant before and after ultracentrifugation was determined.

PAGE. Purified APCs were dissolved in 90% (vol/vol) formic acid in water, lyophilized in a Speed Vac (Savant), redissolved in PAGE sample buffer containing 6 M urea. PAGE was performed with 15% (wt/vol) polyacrylamide slab gels.

HPLC. APCs (10–25 μg) were dissolved in 90% formic acid, lyophilized, and redissolved in 10 μl of HPLC buffer in the presence or absence of 1% (vol/vol) 2-mercaptoethanol. Two analytical I-125 protein columns (Waters) (30 cm × 7.8 mm) were connected in tandem and attached to a guard column (3 cm × 2 mm) filled with I-125 bulk packing phase. The columns were equilibrated with buffers A, B, or C. Buffer A is 0.1% $NaDodSO_4$/150 mM sodium phosphate pH 6.8; buffer B is 0.1% $NaDodSO_4$/150 mM ammonium bicarbonate, pH 8.0; buffer C is 6 M guanidine·HCl/1 M acetic acid, pH 2.5. After sample loading, the columns were developed at a flow rate of 0.2 ml/min and the protein peaks were detected by absorbance at 214 nm (buffers A and B) or 274 nm (buffer C). Proteins in peak fractions were pooled and lyophilized (buffer B) or precipitated with methanol (buffer A).

Amino Acid Analysis. APC proteins (1–4 μg) were hydrolyzed and then analyzed on an automated amino acid analyzer (Beckman 121 M).

Protein Sequence Analysis. Purified APC from AD (10 μg by amino acid analysis) and DS (4 μg) was used for NH_2-terminal sequence analysis on an Applied Biosystems model 470A gas/liquid-phase protein sequencer (Foster City, CA).

RESULTS

Isolation and Purification of APCs. There are two types of APC seen in the isolated state: the predominant form is the dense spherical core with a Maltese cross in polarized light (Fig. 1*a*), and a smaller population is composed of amorphous forms (Fig. 1*b*). By electron microscopy, the preparations of APC have a purity greater than 90% (Fig. 1*c*), and both the dense spherical cores and the amorphous cores are composed of interlacing bundles of amyloid fibrils (Fig. 1*d*). From optimal material, more than 5×10^5 APCs per g of starting grey matter may be recovered.

Solubility Characteristics of APC. The solubility profile of APC is summarized in Table 1. The major classes of detergents were ineffective, although repeated extraction with 10% $NaDodSO_4$/10% 2-mercaptoethanol, when applied with sonication and heating (60°C for 10 min), did achieve partial solubilization.

While investigating the effect of performic acid oxidation, we found that formic acid alone (at concentrations ≥70%) was very effective in achieving complete and rapid solubilization. At high pH, solubilization of the APC with NaOH (0.1–1.0 M) was found to be dependent on time and temperature, but there was considerable variability from case to case.

Most inorganic ions had no effect on the APC, and only the strongest chaotrope, guanidinium thiocyanate showed effective solubilization. Guanidine·HCl and urea had no effect on the APC prior to solubilization in formic acid (see below). Of the organic solvents examined, only phenol showed complete and rapid solubilization.

Molecular Weights of Proteins Associated with APC. APC-associated proteins of two individuals with AD and one with DS were examined by HPLC. Two major AD-derived protein species of apparent molecular mass 16 kDa (A_{16}) and 8 kDa (A_8) were eluted from the gel permeation column with buffer A (Fig. 2*a*). About 15% of the protein eluted in the void volume. Samples pretreated with 2-mercaptoethanol and chromatographed in buffer A resulted in a similar elution profile (Fig. 2*b*). The same AD preparation dissolved in buffer B also eluted at two major peak positions (Fig. 2*c*), the first peak eluting in the void volume (≥60 kDa) and the second peak at apparent molecular mass of 16–22 kDa. However, the majority of the solubilized AD-derived protein dissolved in buffer C eluted from the protein column at a position corresponding to 4.3–4.6 kDa (data not shown) as reported (5) for the major ACA proteins.

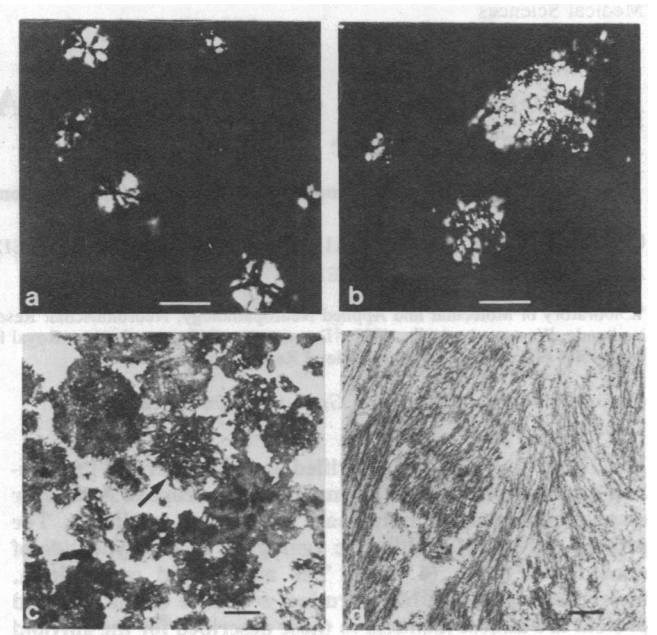

FIG. 1. Morphology of amyloid deposits: isolated and purified spherical plaque cores (*a*) and the amorphous variety (*b*) seen by polarization microscopy with Congo red; plastic-embedded sections (*c*) showing a mixture of spherical cores and an amorphous deposit (arrow), which is composed of interwoven masses of amyloid filaments (*d*). (Scale bars: *a* and *b*, 20 μm; *c*, 10 μm; *d*, 300 nm.)

The APC protein fraction of the DS individual was separated on the same columns employed for APC of AD, using buffer A and buffer C (data not shown). Under the latter buffer conditions, the same 4.3- to 4.6-kDa major component was detected. When buffer A was employed, most of the DS-derived protein eluted at a volume corresponding to an apparent mass of about 8.7 kDa. A peak corresponding to the AD-derived material A_{16} was observed as a leading shoulder of the A_8 peak but amounted to less than 10% of the material collected in peak 2. In addition, proteins eluting in the void volume and at a volume corresponding to an apparent mass of 40 kDa were also observed as minor fractions. HPLC of a control brain sample did not show any peaks (buffer A or B).

APC proteins were also analyzed by $NaDodSO_4$/PAGE. In this system, a broad smear was obtained around the 60- to 16-kDa region for APC-AD (data not shown). The AD material gave discrete but still somewhat broadened bands of apparent mass 16, 12, and 8 kDa when 6 M urea was included in the sample buffer in the absence of 2-mercaptoethanol (Fig. 3*a*). After reduction with 2-mercaptoethanol, the majority of the protein migrated on $NaDodSO_4$/urea/PAGE at positions corresponding to 8 kDa (A_8) and 4 kDa (A_4) (Fig. 3*b*). The aggregational properties of the APC proteins in $NaDodSO_4$ buffers in the absence of urea observed by gel permeation chromatography or $NaDodSO_4$/PAGE are clearly pH dependent. At alkaline pH there are dominant forms of A_{16} and higher aggregates [in HPLC buffer B (Fig. 2*c*) and

Table 1. Comparison of the APC solubility profile with the inactivation of scrapie

Conditions	APC solubility	Inactivation of scrapie
Detergents*		
Triton X-100 (1%)	−	−
CTAB (1%)	−	−
Sarkosyl (10%)	−	−
DOC (10%)	−	−
$NaDodSO_4$	±†	+‡
Acid		
Acetic acid (100%)	−	?§
Formic acid (≥70%)	+	?§
Alkali		
NaOH (0.2–1.0 M)	+	+
Inorganic ions		
Na^+	−	−
K^+	−	−
Cl^-	−	−
Chaotropic ions		
Guanidine·HCl	− (6.0 M)	+ (0.2 M)
Guanidinium thiocyanate	+ (6.0 M)	+ (0.2 M)
Potassium thiocyanate	− (6.0 M)	+ (0.2 M)
Trichloroacetate	− (1.0 M)	+ (0.2 M)
Denaturing agent		
Urea (8.0 M)	−	−
Organic solvents		
Ethanol	−	−
Methanol	−	−
Phenol	+ (≥80%)	+ (5–90%)

*All percents are wt/vol. CTAB, cetyltrimethylammonium bromide; Sarkosyl, sodium dodecyl sarcosinate; DOC, sodium deoxycholate.
†APCs are only partially dissolved when 10% (wt/vol) $NaDodSO_4$, 10% (wt/vol) 2-mercaptoethanol, sonication, and heat (60°C) are used for repeated extractions.
‡Inactivation occurs at a ratio of ≥1.5 g of $NaDodSO_4$ per g of protein.
§Scrapie infectivity is precipitable at pH 5 and is stable down to pH 2.1, but there is complete destruction of infectivity at more extreme acidity.

Laemmli sample buffer]. The A_{16} and A_8 forms become predominant at neutral pH (Fig. 2 *a* and *b*) and A_8 as well as A_4 were detected when HPLC separation on the I-125 protein columns was done in 0.1% $NaDodSO_4$/1 M acetic acid (data not shown). The pH-dependent aggregation is reduced by the addition of urea and 2-mercaptoethanol (Fig. 3) or guanidine·HCl. In the presence of guanidine·HCl and acid, the predominant form is A_4. However, at neutral pH the APC structure remains unchanged in urea and guanidine·HCl (Table 1), and no significant solubilization of proteins is obtained without preextraction with 90% formic acid. The same holds true for solubilization employing $NaDodSO_4$ alone.

Amino Acid Composition of APC and Its Constituent Proteins. The amino acid compositions of APCs from AD and DS individuals are almost indistinguishable (Table 2) and are in good agreement with the compositions reported for ACA (5, 6) and other APC preparations (7). However, the composition differs from that of the scrapie protein (8, 9). Purified APC protein obtained by gel permeation chromatography does not have N-linked sugars due to the absence of glucosamine (Table 2). This is true for both AD- and DS-derived proteins, and may be taken as an indication of the purity of the samples employed for sequencing. The compositions of A_{16} and A_8 of APC-AD and of A_8 of APC-DS are very similar to those of the corresponding crude APC, which also indicates that only one protein species is involved. AD and DS proteins eluting in the void volume also show amino acid compositions similar to the composition of purified A_{16} (peak 2 of Fig. 2*a*) or A_8 (peak 3 of Fig. 2*a*). This suggests that A_{16} and A_8 are aggregates of A_4.

The composition of the material eluting at the salt fronts (Fig. 2) differs clearly from that of A_{16} and A_8 (Table 2). It could be derived from APC by proteolysis of A_4, but the material eluting at the salt front constitutes only about 10% of A_{16} or A_8 as determined by amino acid analysis of whole peaks of single HPLC separations. This analytic approach clearly reveals the presence of a single major protein species in the APC of AD and DS individuals that is A_4 by several criteria. The amino acid composition of an A_4 preparation from guanidine·HCl buffers could, however, not be determined, due to proteinaceous contaminants in guanidine·HCl. Therefore, reliable values for A_4 monomers could not be directly obtained.

Sequence Analyses. Crude APC from AD and DS individuals as well as purified A_{16} and A_8 were analyzed by gas/liquid-phase sequencing. The results (Fig. 4) were compared to the sequence of ACA A_4 subunits, which had been purified by reversed-phase HPLC (5, 6). APC from AD individuals has the same sequence as reported for ACA-AD. But the APC-AD sequence differs from that of ACA-AD in two respects. First, APC-AD has glutamic acid at position 11, where ACA-AD is reported to have a glutamine residue (Fig. 4). Second, APC-AD has NH_2-terminal ragged ends, but ACA-AD does not. Only 12% of the sequenced protein is NH_2-terminally of the same length as ACA-AD, whereas the majority (64%) of the APC begins with a phenylalanine residue corresponding to position 4 of the full-length sequence. Two other APC minor species were also detected. All these shorter chains are clearly produced by proteolysis of the chain, starting with an aspartic acid residue. A_{16} and A_8 isolates from the HPLC separations of APC from AD have the same ragged NH_2-terminal ends as found for total APC-AD.

Total APC from the DS individual has ragged ends differing quantitatively from the ends found for the APC-AD material (Fig. 4). Only three different species in respect to NH_2-

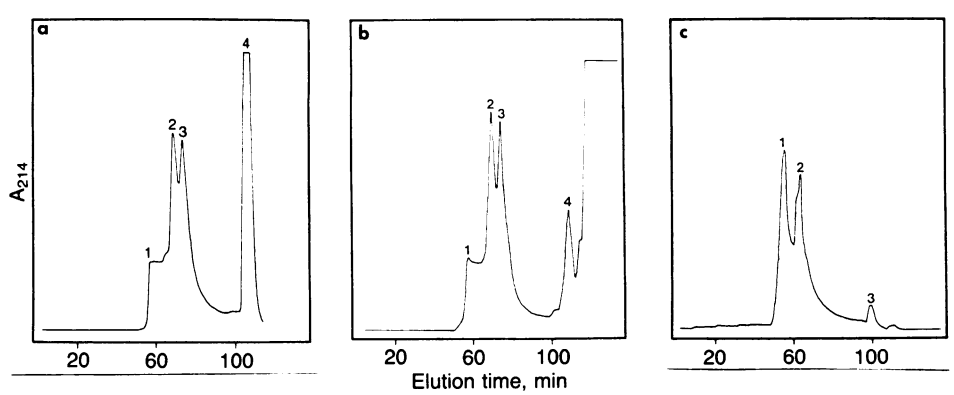

FIG. 2. HPLC separation of APC proteins. (*a*) APC-AD chromatographed in buffer A. Peak 1, with the void volume; peak 2, A_{16}; peak 3, A_8; and peak 4, with the salt front. (*b*) Same as *a* but with the sample treated with 10% 2-mercaptoethanol. Peak assignment as in *a*. The peak beyond 110 min is due to the 2-mercaptoethanol in the sample buffer. (*c*) APC-AD chromatographed in buffer B. The protein in peak 1 is included in the void volume and is greater than 60 kDa, peak 2 includes protein of 16–18 kDa, and peak 3 is the salt front.

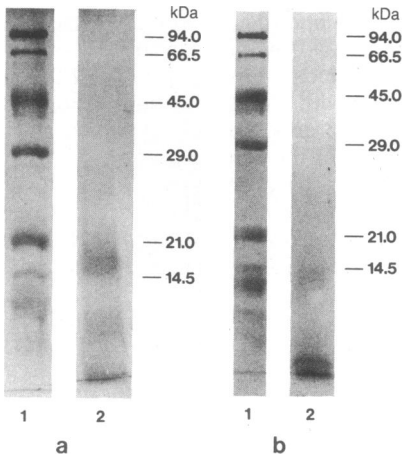

FIG. 3. $NaDodSO_4$/PAGE of APC proteins from an AD individual (Coomassie brilliant blue stain). Lanes 1, marker proteins. (*a*) APC proteins (lane 2) were electrophoresed in buffer containing 6 M urea and 0.1% $NaDodSO_4$. The broad bands correspond to molecular masses of about 16, 12, and 8 kDa. (*b*) APC proteins as in *a* but in the presence of 5% 2-mercaptoethanol and treated for 30 min at 37°C prior to loading of the gel. The band moving above the front is A_8 in lane 2. A_4 moves in this system with the front.

terminal heterogeneity are observed. Full-length chains account for 22% of the protein.

DISCUSSION

Analysis of the composition of the APCs has been hindered by their unusual solubility profiles, but our finding them soluble in formic acid has enabled characterization by HPLC and $NaDodSO_4$/urea/PAGE. APC from AD and DS individuals are composed of a single major protein component of about 4–5 kDa (A_4). The chromatographic behavior of A_{16} and A_8 in HPLC buffer A and the partial sequences of these proteins are in agreement with the assumption that A_{16} and A_8 are tetramers and dimers of A_4. The monomeric form A_4 is found only in strong denaturants such as 6 M guanidine·HCl at low pH. In urea/$NaDodSO_4$ buffers at alkaline pH, A_8 and A_4 coexist. A_4 of APC-AD and APC-DS has a strong tendency to form dimers (A_8), which are the predominant forms for APC-DS in 0.1% $NaDodSO_4$-containing buffers at pH 6.8. At this pH, the proteins from APC-AD are dimers and tetramers, but they form tetramers and higher oligomers at pH 8.0. Therefore, aggregation of A_4 is pH dependent. Preliminary experiments indicate that this dependence has a titration behavior typical of histidine residues.

The sequences determined for AD and DS-derived APC proteins clearly establish their common origin. Both APC proteins have ragged NH_2 termini, which differ between the AD and DS preparations. The APC protein sequences derived from AD and DS individuals are completely homologous with the corresponding ACA protein sequences (5, 6) with one difference: we found glutamic acid at position 11 of the full-length chain, whereas glutamine was reported at this position for the ACA-AD protein. The ragged ends found for the APC proteins were not reported for the ACA proteins (5, 6). We found more extensive degradation of the proteins isolated from APC of AD individuals and slightly less for DS proteins. Since the NH_2-terminal heterogeneity encountered does not show major proteolytic cuts on the COOH-terminal side of hydrophobic residues, as would be expected for pepsin, the protease employed during purification, we take this as a possible indication for processing occurring *in situ*. Further work is necessary to clarify this point.

Amino acid analyses of purified A_{16} and A_8 from AD cores and of A_8 from DS cores reveal the complete absence of N-linked carbohydrate, since no glucosamine residues were detected. Total APCs do show the presence of glucosamine,

Table 2. Amino acid compositions of APC proteins from AD and DS individuals

	AD							DS				
	mol %					No. of residues per monomer		mol %			No. of residues per monomer of A_8	No. of residues in AD/DS A_4†
Amino acid*	Total APC	HPLC‡ Peak 1	HPLC‡ A_{16}	HPLC‡ A_8	HPLC‡ Peak 4	A_{16}	A_8	Total APC	HPLC Peak 1	HPLC A_8		
Asp	8.73	11.05	8.89	9.36	8.72	3.5	3.6	9.66	9.67	9.40	3.7	4
Thr	1.21	2.06	1.30	0.74	1.42	0.5	0.3	1.30	1.30	0.90	0.3	(1)
Ser	6.24	6.40	6.49	7.53	7.55	2.6	2.9	6.50	8.37	7.16	2.9	3
Glu	11.20	10.74	11.19	11.43	10.38	4.4	4.5	11.93	11.97	11.93	4.8	5
Pro	0.65	1.55	0.96	0.74	2.12	0.4	0.3	1.12	2.25	1.12	0.4	(1)
Cys	0.68	+	+	+	+	(1)	(1)	+	+	+	(1)	(1)
Gly	17.43	16.84	16.13	15.20	23.11	6.2	5.9	15.68	18.02	15.68	6.2	6
Ala	9.38	9.71	9.54	9.71	5.90	3.7	3.8	8.05	7.77	8.05	3.2	4
Val	9.34	8.99	10.05	9.24	7.55	3.9	3.6	9.38	4.95	9.38	3.7	4
Met	2.50	+	2.47	2.48	+	0.9	0.9	1.75	1.32	1.75	0.7	1
Ile	4.40	4.24	4.15	4.34	4.01	1.6	1.7	4.06	3.28	4.06	1.6	2
Leu	6.91	6.51	6.38	7.00	6.84	2.5	2.7	7.01	7.14	7.01	2.8	3
Tyr	2.24	3.51	2.26	2.80	2.59	0.9	1.1	2.67	3.40	2.67	1.1	1
Phe	6.26	5.06	6.04	5.99	6.37	2.4	2.4	5.56	3.63	5.56	2.2	3
His	5.69	4.55	5.80	5.20	4.01	2.3	2.1	5.01	2.30	5.01	2.0	3
Lys	5.68	5.68	5.70	5.52	4.95	2.3	2.2	5.90	5.01	5.90	2.3	2
Arg	3.79	3.10	2.64	2.66	4.48	1.0	1.0	3.44	4.61	2.60	1.1	1
GlcN	0.36							+	+			
nmol protein		0.30	0.77	0.90	0.11				0.20	1.20		
Total						40	40				40	45

*Tryptophan was not determined.
†The calculated composition of the A_4 subunit of APC from AD and DS individuals.
‡Proteins from experiment shown in Fig. 2*a* (whole peaks analyzed).

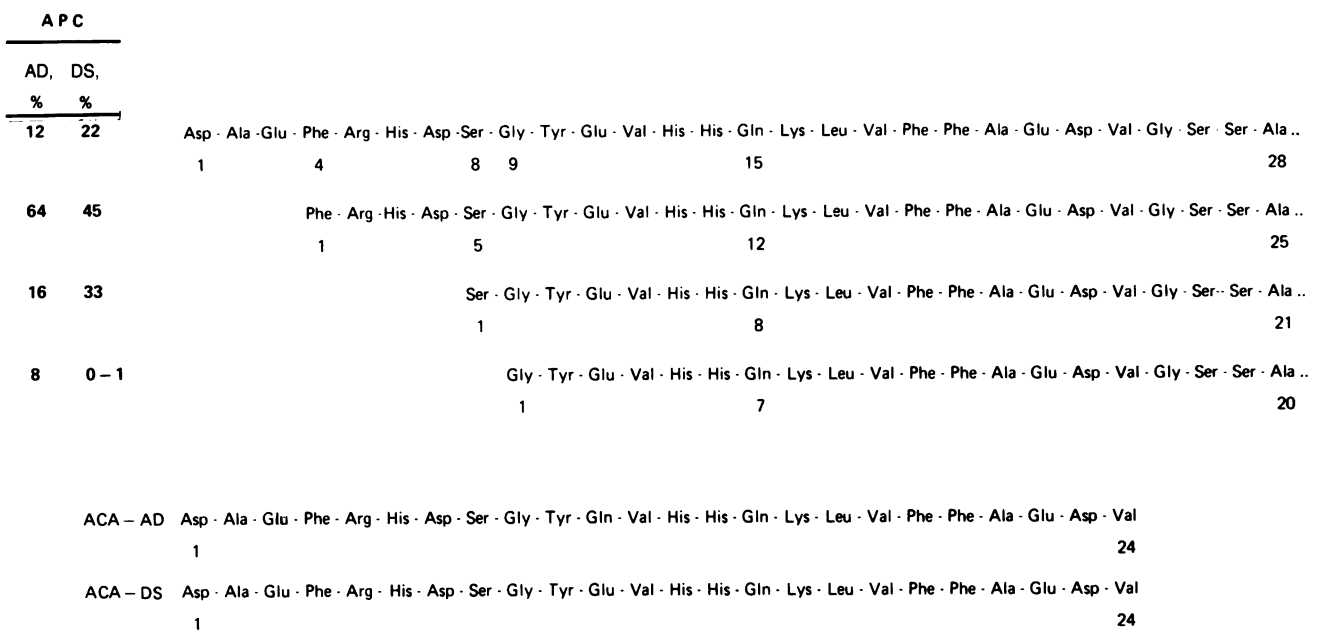

FIG. 4. NH_2-terminal amino acid sequence of APC from individuals with AD and DS. The sequences of ACA from AD (ACA-AD) and DS (ACA-DS) are taken from Glenner and Wong (5, 6). The numbers on the left are percentages of total protein loaded on the filter of the gas-phase sequencer.

possibly attached to contaminants. The compositions calculated for the A_4 forms of the AD and DS proteins are almost indistinguishable from each other and from the compositions of the ACA proteins (5, 6). The compositions also reveal the surprising fact that roughly half-mole equivalents for proline and threonine result if they are calculated for the monomer of about 4 kDa. This may reflect either COOH-terminal ragged ends arising in the same way as discussed for the NH_2 termini or the presence of two genes for A_4 or pre-A_4. If the latter is true, the "proline-containing gene" and the "threonine-containing gene" have to be expressed in roughly equimolar amounts.

Thus, only minor differences between the APC-AD and APC-DS are seen (the DS protein contains little A_{16}, and there is slightly less heterogeneity of the NH_2 termini). Morphologically, the amyloid cores from the DS individual were predominantly of the amorphous type. These biochemical and morphological differences may reflect the age of the amyloid itself, the APC-DS being of more recent origin, with less time for processing of NH_2 termini.

The APC protein sequences reported here are not homologous to known protein sequences (Protein and Nucleic Acid Sequence Databases, National Biomedical Research Foundation, April 1984), nor are they related to the sequences reported for the scrapie-associated protein (8, 9). Furthermore, the apparent masses of these proteins differ by a factor of about 4 to 5, since the scrapie-associated protein is reported to be 20 to 30 kDa (8). The similarities between the solubility profile of APC and the chemical inactivation profile of scrapie (Table 1) suggest that common physiochemical properties stabilize the macromolecular forms of both the aggregated amyloid fibrils and the putative fibrillar forms of the scrapie virus (4).

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