N-Terminal Sequence of an Encephalitogenic Protein from Human Myelin

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Experimental allergic encephalomyelitis is produced by injection of a basic protein from central-nervous tissue mixed with Freund's complete adjuvant into guinea pigs (Kies, 1965; Carnegie, Bencina & Lamoureux, 1967). The encephalitogenic basic protein is a component of myelin (Laatsch, Kies, Gordon & Alvord, 1962; Roboz-Einstein, Csejtey & Davis, 1965; Eng, Chao, Gerstl, Pratt & Tavaststjerna, 1968).

Human brain was defatted with chloroform-methanol (2:1, v/v) and the encephalitogenic protein extracted at pH 2.5–3.0. The basic proteins were collected on CM-Sephadex C-25 and eluted with 0.1N-HCl. Gel filtration on Sephadex G-100, G-75 and G-50 was used to purify the encephalito-

genic protein. In calculating the amino acid composition shown in Table 1 the protein was assumed to contain six histidine residues, since six histidyl peptides were observed in a peptide 'map' of a tryptic digest. The values for serine, threonine and tyrosine are extrapolated to zero time of hydrolysis with constant-boiling HCl. Tryptophan was estimated spectrophotometrically and from peptide 'maps' of a tryptic digest. No N-terminal amino acid could be detected with leucine aminopeptidase, by the 'dansyl' method, or by the Edman method. The protein had the same amino acid composition and electrophoretic mobility as the encephalitogenic basic protein isolated by Eng *et al.* (1968) from human myelin. The protein

Table 1. Amino acid composition of peptides from the N-terminal region of an encephalitogenic protein from human myelin

The values for serine, threonine and tyrosine are extrapolated to zero time. Tryptophan was estimated spectrophotometrically. Mobilities are relative to aspartic acid at pH 6.5. G, Gel filtration; E, electrophoresis at pH 6.5, except where another pH is stated; C, paper chromatography.

Amino acid composition (residues/mol.)											
Amino acid	Protein	CNBr poly-peptide	Tryptic peptides				Chymotryptic peptides				Subtilisin peptide
			Ta	Tb	Tc	Td	Ca	Cb	Cc	Cd	
Thr	5.6	2.1	2.3	—	—	—	2.1	0.2	—	—	—
Ser	12.1	4.0	1.4	1.0	0.9	1.0	1.3	1.7	1.0	1.0	1.0
Glu	6.1	2.2	—	1.0	—	1.0	—	—	1.0	1.0	1.1
Pro	8.9	1.0	—	—	—	1.1	—	—	0.8	—	0.9
Gly	16.2	1.1	0.3	—	1.2	—	—	1.4	0.1	—	0.2
Ala	8.2	3.1	2.4	1.0	—	—	2.0	0.3	—	1.0	0.1
Met or homoserine	1.0	0.7	0.8	—	—	—	0.8	—	—	—	—
Leu	4.9	1.0	1.0	—	—	—	0.9	0.1	—	—	—
Tyr	2.7	1.0	0.9	—	—	—	—	1.0	—	—	—
Lys	7.6	1.9	—	0.9	1.0	—	—	1.0	0.9	—	0.3
His	6.0	1.1	—	—	1.0	—	—	1.0	—	—	1.0
Arg	9.1	1.9	—	—	—	2.0	—	—	1.9	—	2.0
Asp	7.1	—	—	—	—	—	—	—	—	—	—
Val	2.6	—	—	—	—	—	—	—	—	—	—
Ile	2.3	—	—	—	—	—	—	—	—	—	—
Phe	5.5	—	—	—	—	—	—	—	—	—	—
Trp	1.0	—	—	—	—	—	—	—	—	—	—
Yield (%)		50	33	26	44	55	64	68	54	61	17
Mobility		+0.6	0.0	0.0	+0.5	+0.7	0.0	+0.4	+0.7	−0.6	+0.7
Purification		G,E	G,C	G,C	G,E,C	G,E,C	E	E	E	E	E,E, 3.5

(200mg.) was digested with cyanogen bromide (800mg.) at room temperature in 70% (w/v) formic acid. The products were separated by gel filtration on Sephadex G-50 with 0.1% acetic acid as solvent. A polypeptide was eluted at 1.9 times the void volume, and further purified by paper electrophoresis at pH 6.5. The amino acid composition of this polypeptide is given in Table 1. No *N*-terminal residue was detected. Homoserine was confirmed to be *C*-terminal by digestion with carboxypeptidase A.

In general the techniques described by Ambler (1963) were used for the enzymic digestion and purification of peptides. Enzymes were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Amino acid compositions were determined on a Technicon analyser.

Sequence determination was by the 'dansyl'-Edman method (Gray, 1967) with chromatography of the 'dansyl' amino acids on polyamide sheets (Woods & Wang, 1967). The subtractive Edman technique (Dopheide, Moore & Stein, 1967) was used where necessary.

Tryptic peptides. The polypeptide (2.3 μ moles) was digested with L-1-chloro-4-phenyl-3-toluene-*p*-sulphonamidobutan-2-one-treated trypsin (50 μ g.) for 2½ hr. at 30° at pH 8.5. The products were separated by gel filtration on Sephadex G-25 and purified by paper electrophoresis (Offord, 1966) and by paper chromatography.

The composition and mobilities of the four peptides obtained are given in Table 1.

Peptide Ta contained homoserine and was thus the *C*-terminal peptide. The sequence was established by the 'dansyl'-Edman method to be Tyr-Leu-Ala-Thr-Ala-Ser-Thr-homoserine.

Peptide Tb. The yield of this peptide was lower since a considerable proportion was present in the zone between the first and second peaks from the gel filtration column. It gave no 'dansyl' amino acid. A mixture of carboxypeptidase A (120 μ g.) and B (25 μ g.) was used to digest the peptide (0.14 μ mole) for 17 hr. at 30°. Lysine (1.0 mole/mole), glutamine (0.6) and serine (0.2) were released. Since carboxypeptidase A releases serine slowly and alanine rapidly (Ambler, 1967) these results suggested the sequence Ala-Ser-Gln-Lys. The carboxypeptidase digest was fractionated by paper electrophoresis at pH 6.5 with 3000 v for 1½ hr. Zones were cut from the anodic side and eluted with 0.01N-NH₃. Hydrolysis of the zone with mobility between 0.75 and 0.80 (relative to aspartic acid) showed the presence of alanine and serine in approximately equal amounts, but the zone gave no colour with ninhydrin. From the mobility of the zone a molecular weight between 190 and 210 was calculated (Offord, 1966) for the blocked peptide. Hydrazinolysis (Fraenkel-Conrat & Tsung, 1967)

of this zone for 5 hr. at 105° yielded serine and a small amount of alanine. Acetohydrazine was identified by paper electrophoresis at pH 2.1. Samples of *N*-acetylalanine and *N*-acetylglycine were used as reference compounds. Acetohydrazine had a mobility relative to lysine of 0.9. Therefore the sequence for the *N*-terminal peptide was established as: Acetyl-Ala-Ser-Gln-Lys.

Peptide Tc was shown by the 'dansyl'-Edman and subtractive Edman methods to have the sequence His-Gly-Ser-Lys.

Peptide Td. The 'dansyl'-Edman and subtractive Edman methods gave the sequence Arg-Pro-Ser-Glx-Arg. Glutamine was established from the electrophoretic mobility of the peptide at pH 6.5 and from the products of digestion with carboxypeptidase A and B.

Overlap peptides. The order of the tryptic peptides was established by the isolation and sequence of peptides produced by the digestion of 0.9 μ mole of the polypeptide with chymotrypsin (55 μ g.) for 4 hr. and 0.5 μ mole with subtilisin (50 μ g.) for 4½ hr. at 30°. Peptides were fractionated by paper electrophoresis.

Peptide Ca was found by the 'dansyl'-Edman method to have the sequence Leu-Ala-Thr-Ala-Ser-Thr-homoserine.

Peptide Cb was shown by the 'dansyl'-Edman and subtractive Edman methods to have the sequence His-Gly-Ser-Lys-Tyr.

For peptide Cc, the 'dansyl'-Edman and subtractive Edman methods established the sequence Lys-Arg-Pro-Ser-Glx-Arg. This unexpected split by chymotrypsin at arginine could be due to traces of trypsin in the enzyme preparation.

Peptide Cd was acidic and gave no colour with ninhydrin. It was obtained by eluting zones between

Fig. 1. Amino acid sequence of a polypeptide from a cyanogen bromide digest of an encephalitogenic basic protein from human myelin. T, Tryptic peptides; C, chymotryptic peptides; S, subtilisin peptides.

aspartic acid and the neutral amino acids. Hydrolysis with acid gave alanine, serine and glutamic acid.

Peptide Sa was the histidine peptide with the greatest cationic mobility in a subtilisin digest. Its composition is given in Table 1. When it was digested with carboxypeptidase A only histidine (0.9 mole/mole) was released.

From the above information the sequence shown in Fig. 1 is proposed for the polypeptide from the cyanogen bromide digest of the encephalitogenic basic protein. As the protein has only one methionine residue and has a blocked *N*-terminal group, it is suggested that the polypeptide originates from the *N*-terminal region. Preliminary examination of a chymotryptic digest of the protein yielded a ninhydrin-negative peptide with the composition of peptide Cd, but the yield was low.

It is noteworthy that the polypeptide split off from the basic protein with cyanogen bromide is still encephalitogenic in guinea pigs. Doses of 0.005–0.033 μ mole mixed with Freund's complete adjuvant induced mild clinical disease and severe histological damage in ten out of 17 guinea pigs injected. The high-molecular-weight fragment from the cyanogen bromide digest also induced typical experimental allergic encephalomyelitis (P. R. Carnegie, T. A. McPherson & G. Robson, unpublished work).

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