THE STRUCTURE OF THE TERMINAL REGIONS OF THE ENCEPHALITOGENIC A1 PROTEIN*

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SUMMARY

The polypeptide chain of the encephalitogenic basic protein from bovine spinal cord was cleaved at the 2 methionine residues yielding 3 peptides (CB1, CB2, CB3). The sequence of the N-terminal region was established as N-acetyl-Ala-Ser-Ala-Gln-Lys using peptide CB1 (22 residues). The amino acid sequence of the C-terminal region of the A1 protein was established as (Lys, Ala, Ileu)-Leu-Val-His-Phe-Met-Ala-Arg-Arg-OH using carboxypeptidases and peptide CB3, a tripeptide with sequence Ala-Arg-Arg. Peptide CB2 (116 residues), located between peptides CB1 and CB3, was highly encephalitogenic in guinea pigs and reveals that the disease-inducing site remains unaltered.

Basic proteins from myelin are encephalitogenic, i.e., they induce experimental allergic encephalomyelitis (EAE), an autoimmune disease involving the central nervous system (1, 2). Recently, we have reported on the isolation of the encephalitogenic protein, referred to as the A1 protein, in high yield and purity (3), thus permitting detailed chemical and immunological studies. This protein, 16,400 daltons, is highly basic and exhibits an extended, random coil conformation (4). A peptide of 16 residues, obtained from the pepsin digest of the A1 protein, contains the encephalitogenic determinant and is of special interest in characterizing the chemical structure responsible for disease induction (5, 6). This report describes the structure of the C- and N-terminal regions of the A1 molecule and their relationship to the encephalitogenic site.

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EXPERIMENTAL

Homogeneous A1 protein, isolated from the bovine spinal cord, was purified as described elsewhere (3). Cyanogen bromide was added dropwise to the A1 protein (20 mg/ml in 70% formic acid) to reach a 400 fold molar excess over methionine. After 24 hours at 25°, the reaction mixture was lyophilized and applied to a Sephadex-G10 and G25 column (100 x 3 cm) equilibrated with 0.05 M acetic acid. The elution pattern was monitored by measuring the absorption at 235 and 280 mμ. Three fractions were prepared by combining tubes 30-47, 48-57 and 58-75. The peptides obtained were further purified by preparative high voltage electrophoresis at pH 4.7 (6).

Treatment with trypsin and pronase at 37° was performed in 0.1 M triethylamine bicarbonate buffer at pH 8.0 using an enzyme/protein ratio of 1/50. The resulting peptides were eluted from the paper following separation by high voltage electrophoresis at pH 4.7. Carboxypeptidases A and B were used at 25° in 0.05M $KHCO_3$ at pH 7.6 with an enzyme/protein ratio of 1/25. Samples were removed at timed intervals, lyophilized and redissolved in sodium acetate buffer at pH 2.2 for amino acid analysis.

Paper chromatography was performed in n-butanol:acetic acid:water (4:1:5) for 18 hours. After air drying, the chromatogram was sprayed in succession with 0.05% aqueous sodium hypochlorite, 95% ethanol and 1% soluble starch containing 1% potassium iodide (7). Gas chromatography was done on a high efficiency gas chromatography instrument, Model 402 (F. and M. Scientific Corp., Avondale, Pa.) using 3% QF-1 -OH on chromasorb W. column (200 x 0.3 cm) The column temperature was set at 170°; 1.5 ml N_2/min. was used. The silyl derivative was prepared by heating the peptide in the presence of N, O-Bis-(trimethylsilyl)-acetamide for two minutes at 100°. Acetyl groups were determined as N-2, 4-dintrophenyl acetylhydrazide (8).

The amino acid composition was determined with a Beckman amino acid analyzer. Tryptophan analysis was done using procedure K of Spies and Chambers (9); polyacrylamide gel electrophoresis at pH 4.5 (10); and N-

terminal analysis by Edman and dansylation techniques (11, 12).

RESULTS AND DISCUSSION

Treatment of the A1 protein with cyanogen bromide resulted in complete oxidation of the methionine and cleavage of the C-methionyl bond. No trace of the A1 protein was seen on polyacrylamide gel electrophoresis of the reaction mixture; rather, a single faster-moving band (peptide CB2) was observed. High voltage electrophoresis confirmed this finding and further revealed two other peptides (peptide CB1 and CB3) in addition to peptide CB2. All three peptides are basically charged at pH 4.7, i.e., they move toward the cathode on high voltage electrophoresis. Peptide CB3 exhibited a mobility only slightly slower than arginine. The amino acid composition of the three peptides following purification by gel filtration and preparative high voltage electrophoresis showed 22, 116 and 3 amino acid residues per molecule for peptides CB1, CB2 and CB3, respectively (Table I). The finding of the C-terminal homoserine in both peptide CB1 and CB2 confirms the presence of two methionine residues in the A1 protein as previously reported (4).

Peptide CB1. The partial sequence of peptide CB1 was determined from the following data: The N-terminal amino acid of peptide CB1 was not detected by either the direct Edman or the dansylation techniques. This result reveals that peptide CB1 originates from the N-terminal region of the A1 molecule, the N-terminal amino acid of which is likewise blocked. It is apparent that one of the two methionine residues found in the A1 protein is located at position #22 from the N-terminal end of the A1 molecule.

One of the peptides obtained from the tryptic digest of peptide CB1, following high voltage electrophoresis, was peptide T1. It contains one mole each of lysine, glutamine, serine, and two moles of alanine per mole of peptide (Table I). Peptide T1 also has an N-terminal block, thus originating from the N-terminal region of peptide CB1. Pronase treatment of peptide T1 liberated 0.9 moles of lysine, glutamine, and alanine and 0.3 moles of serine

TABLE I

AMINO ACID COMPOSITION

Residues/Molecules*

Amino Acid	A1 Protein	Peptide CB1	Peptide CB2	Peptide CB3	Peptide T1
Lysine	13	2	10	0	1
Histidine	9	1	9	0	0
Arginine	16	2	12	2	0
Aspartic Acid	9	0	8	0	0
Threonine	6	1	5	0	0
Serine	13	5	9	0	1
Glutamic Acid	9	2	7	0	1
Proline	9	1	8	0	0
Glycine	20	1	18	0	0
Alanine	11	4	7	1	2
Valine	4	0	4	0	0
Methionine#	2	1(0.8)	1(0.9)	0	0
Isoleucine	3	0	2	0	0
Leucine	8	1	7	0	0
Tyrosine	3	1	2	0	0
Phenylalaine	6	0	6	0	0
Tryptophan	1	0	1	0	0
Total	142	22	116	3	5

* The number of amino acid residues per molecule are computed assuming one leucine, tyrosine, glycine or proline residue for peptide CB1; two tyrosine or 3 isoleucine residues for peptide CB2; one alanine residue for peptide CB3 and one glutamic acid residue for peptide T1.

\# Methionine content of peptides CB1 and CB2 was determined as homoserine.

TABLE II

THE MOLES AMINO ACID/MOLE PROTEIN RELEASED BY CARBOXYPEPTIDASE

	A1 Protein			Peptide CB2		
Minutes	5	30	90	15	60	120
Arginine	1.9	1.9	1.9			
Alanine	0.2	0.8	1.0			
Methionine #	0.2	0.3	0.5	0.7	0.9	0.9
Phenylalanine	0.0	0.0	0.3	0.6	0.8	0.9
Histidine	0.0	0.0	0.2	0.5	0.8	0.9
Valine				0.2	0.4	0.7
Leucine				0.1	0.3	0.6
Isoleucine				0.0	0.1	0.3
Lysine				0.0	0.0	0.2
Alanine				0.0	0.1	0.2

* Carboxypeptidase B was added at zero time, followed by carboxypeptidase A after 5 minutes.

\# Carboxypeptidases A and B were added together. Samples were removed at indicated times and analyzed. Homoserine, rather than methionine is present in peptide CB 2.

per mole of peptide in addition to a peptide, referred to as T1-1, which did not stain with ninhydrin. Peptide T1-1 was isolated by eluting the anodal region of the electrophoretogram following high voltage electrophoresis of the pronase digest of peptide T1. Amino acid analysis of peptide T1-1 showed alanine and serine in a 2:1 molar ratio. Two possibilities were suggested: one peptide containing two alanine and one serine residues, or a mixture containing X-Ala and X-Ala-Ser, where X represents the blocking group. The presence of two peptides was confirmed by chromatography of peptide T1-1; two peptides were found, one having an R_f value of 0.7 identical to that of N-acetylalanine. The N-terminal block was shown to be an acetyl group by gas chromatography of the silyl derivative of T1-1; two peaks were seen, one of which corresponded to N-acetylalanine. The presence of acetate was confirmed by dinitrophenylation of the acetylhydrazide obtained by hydrazinolysis of peptides T1 and T1-1.

Treatment of peptide T1 with carboxypeptidases A and B liberated one mole of lysine/mole peptide in the first 5 minutes. This was followed by one mole of glutamine and 0.5 moles of alanine, thus positioning the other alanine residue.

These results may be summarized in the following structure for peptide T1 which constitutes the first 5 amino acid residues of peptide CB1 and the A1 protein: N-Acetyl-Ala-Ser-Ala-Gln-Lys.

Peptide CB3. The absence of homoserine suggests that peptide CB3 originates from the C-terminal end of the A1 molecule. An N-terminal alanine was found by dansylation; thus the sequence of this tripeptide is Ala-Arg-Arg.

C-Terminal Region. The sequence and origin of peptide CB3 were confirmed by treating the A1 protein with carboxypeptidases A and B(Table II). It can be seen that two moles of arginine/mole peptide were released during the first 5 minutes, followed by alanine, methionine, phenylalanine, and histidine.

Peptide CB2. The largest of the three peptides isolated from the

cyanogen bromide treated A1 protein, contains one tryptophan i.e., residue number 87 from the N-terminal of the A1 protein (13). An N-terminal alanine was found for peptide CB2 by means of the dansylation procedure. From the carboxypeptidase data of Table II, it is evident that the C-terminal region has the following sequence: (Lys, Ala, Ileu)-Leu-Val-His-Phe-Hser. It should be noted that the C-terminal region of peptide CB2, taken together with peptide CB3, confirmed the C-terminal sequence established for the A1 protein by direct treatment with carboxypeptidase (Table II). These data testify further to the purity of the A1 protein preparation.

Our results are at variance with those of Chao and Einstein (14) who reported a Gly-Ala-Arg sequence at the C-terminal end of a basic protein from bovine spinal cord. This difference may possibly arise from the presence of contaminating peptides.

Of the peptides derived from cyanogen bromide treatment, only peptide CB2 was encephalitogenic. On a molar basis, it induced EAE (3) in guinea pigs at the same doses as the A1 protein, i.e., at 10 μg/animal or greater peptide CB2 induced clinical disease in 80% of the 30 animals tested; histologic lesions are found in the central nervous system of all animals tested at 1 μg or more. These data are compatible with previous findings of an encephalitogenic determinant found in a peptide of 16 amino acids. This region, containing the single tryptophan residue of the A1 protein, is located at residues 61-46 from the C-terminal arginine and thus remains intact in peptide CB2. The complete amino acid sequence of the A1 molecule is currently under investigation.

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