such as Concanavalin A (Burger and Noonan, 1970; Shoham *et al.*, 1970). Lymphocytes are stimulated to divide in the presence of certain lectins (see review, Ling, 1968). The cell surface receptor sites for several of these lectins have been characterized as glycoproteins (Kornfeld *et al.*, 1971). Presumably these mechanisms involve carbohydrates at the cell surface, but exactly how such phenomena can take place has yet to be elucidated. The data reported here further emphasize the possible role of surface carbohydrates in cellular processes.

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Amino Acid Sequences of Mouse 2.5S Nerve Growth Factor. I. Isolation and Characterization of the Soluble Tryptic and Chymotryptic Peptides†

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ABSTRACT: The determination of the amino acid sequence of nerve growth factor (NGF), prepared as the 2.5S derivative from mouse submandibular glands, has been initiated. Tryptic digestion of *S*-carboxymethyl- and *S*-aminoethyl-NGF, followed by purification and characterization of the resulting peptides, was performed. These peptides accounted for 87 residues in the NGF molecule but contained only 5 of the 6 half-cystinyl residues and were missing the amino-terminal fragment. Digestion of the *S*-carboxymethyl derivative with chymotrypsin yielded 19 peptides covering 108 unique residues. By combined Edman degradation and carboxypeptidase A and B digestion, 96 of the 108 residues, accounted for by peptides from the three separate digests were placed in sequence.

Nerve growth factor (NGF)[1] is a protein which controls the growth of sympathetic nerve cells during development and throughout adult life (Angeletti *et al.*, 1968; Levi-Montalcini *et al.*, 1972). The biological effects accompanying these processes have been examined extensively and reported in detail (Levi-Montalcini and Angeletti, 1968). However, in

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[1] Abbreviations used are: NGF, nerve growth factor; SCM-, *S*-carboxymethyl; SAE-, *S*-aminoethyl; T-, tryptic peptides; TPCK-, (1-tosylamido-2-phenyl)ethyl chloromethyl ketone; MTH-, methylthiohydantoin; dansyl-, 5-dimethylaminonaphthosulfonic acid; ST-, SAE-NGF tryptic peptides; C-, chymotryptic peptides.

order to translate these observations into molecular terms, a more exact knowledge of the structure of NGF is required. Initial studies employing 2.5S NGF from mouse submandibular glands prepared by the method of Bocchini and Angeletti (1969), indicated that the active protein was composed of two very similar or identical polypeptide chains, each with a molecular weight of about 14,500 (Angeletti *et al.*, 1971). It was also established that the subunits, which are associated by noncovalent forces, each contained 6 residues of half-cystine bound in 3 intrachain disulfide bonds.

Based on these observations, the determination of the complete amino acid sequence was initiated. Sequence data derived from peptides isolated from 5 enzymatic digests as well as information from the protein Sequencer were required to yield an unambiguous alignment of the 118 amino acid residues in the primary NGF subunit. This paper describes the tryptic and chymotryptic peptides from *S*-carboxymethyl-NGF and the tryptic peptides of *S*-aminoethyl-NGF. The ensuing report describes the thermolytic and peptic peptides, including the alignment of the disulfide bonds and the analyses in the automatic protein Sequencer. The sum of these experiments establishes the complete covalent structure of mouse 2.5S NGF (Angeletti and Bradshaw, 1971).

Experimental Procedure

Materials. Mouse submandibular gland (2.5S) nerve growth factor was purified from adult male mouse submandibular glands by the method of Bocchini and Angeletti (1969). Trypsin, treated with TPCK, and chymotrypsin were purchased from Worthington Biochemical Corporation. The Dowex ion-exchange resins utilized in peptide purification were obtained from Beckman-Spinco (AA-15) and Bio-Rad (AG 1-X2 and AGW 50-X2). Sephadex gel filtration media were obtained from Pharmacia. Polyamide thin-layer sheets were obtained from Gallard-Schlesinger. Dithiothreitol was purchased from Nutritional Biochemicals. Pyridine was redistilled after addition of solid ninhydrin (1 g/l.) to the distillation flask. Crystalline iodo[1-^{14}C]acetic acid with a specific activity of 14.9 Ci/mol was obtained from New England Nuclear Corp. Unlabeled iodoacetic acid was recrystallized from cold petroleum ether (bp 30–60°) before use. Reagents for sequence analysis, obtained from Pierce Chemical Co., were stored under nitrogen and used without further purification, with the exception of trifluoroacetic acid, which was redistilled each week. Standard MTH- and dansylamino acids were purchased from Sigma Chemical Co.

Methods

Preparation of SCM- and SAE-NGF. SCM-NGF was prepared as described previously (Angeletti *et al.*, 1971). *S*-Aminoethyl-NGF was prepared by the method of Raftery and Cole (1966). NGF was dissolved in 2 ml of 8 M urea containing 0.28 M Tris-HCl (pH 8.6) and 0.25% EDTA to which 25 μl of 2-mercaptoethanol had been added. After incubation at room temperature in a nitrogen atmosphere for 4 hr, 100 μl of ethyleneimine was added. The reaction was allowed to proceed for 30 min in a nitrogen atmosphere and was terminated by the addition of 1 ml of glacial acetic acid. *S*-Aminoethyl-NGF was recovered by lyophilization after gel filtration on Sephadex G-25 in acetic acid or extensive dialysis against distilled water.

The extent and specificity of reaction was monitored by amino acid analysis of hydrolyzed aliquots.

Tryptic Digestion. Tryptic digestion of SCM- and SAE-NGF was carried out using TPCK-treated trypsin added in a ratio of 1:100 to NGF on a weight basis according to Bradshaw *et al.* (1969a). The reaction mixture was maintained at pH 8.8, 37° by means of a Radiometer pH-Stat, Model TTT-11. The insoluble material remaining after acidification to pH 2 with 6 N HCl was removed by centrifugation.

Chymotryptic Digest. The chymotryptic digest of [^{14}C]-SCM-NGF was performed at pH 8.5, 38°. Chymotrypsin, dissolved in 10^{-3} M HCl, was added to a 1% solution of SCM-NGF in two aliquots such that the final concentration of the enzyme is 1% (w/w). The second aliquot of chymotrypsin was added after 2 hr, and the digestion was terminated after 6 hr by the addition of 6 N HCl to bring the solution to pH 2.

Peptide Purification. The soluble tryptic and chymotryptic peptide mixtures were fractionated on 0.9 × 20 cm columns of Dowex 50-X8 equilibrated in 0.05 N pyridine–acetate (pH 2.5), and eluted with a double linear gradient of pyridine–acetate buffers as described by Bradshaw *et al.* (1969b). The separation of peptides was monitored with a Technicon AutoAnalyzer equipped for automatic alkaline hydrolysis and ninhydrin analyses (Hill and Delaney, 1967). Markers of cysteic acid (for anion-exchange resins) or arginine methyl ester (for cation-exchange resins) were added to the column, followed by one buffer wash, prior to the addition of the sample to allow for the subsequent alignment of the fraction collector and recorder tracing. The isolated peptides were recovered after removal of the solvent by rotary evaporation. High-voltage paper electrophoresis was performed on all pools on Whatman No. 1 paper in pyridine–acetate buffer (pH 3.75 at 2000 V for 1.5 hr). Peptides were detected by ninhydrin reagent, and those pools found to be heterogeneous were further purified on columns of Dowex 1-X2 (equilibrated in 3% pyridine) or Dowex 50-X2 (equilibrated in 0.2 M pyridine–acetate, pH 3.1) using the volatile pyridine–acetate buffer gradients described by Bradshaw *et al.* (1969b). Other peptides were purified on columns of Sephadex G-25 or G-50 using volatile eluents. Preparative high-voltage paper electrophoresis on Whatman No. 3MM in pyridine–acetate (pH 3.75) and paper chromatography using a solvent of pyridine–butanol–acetic acid–water (10:15:3:12) were also employed to isolate small peptides. The radioactivity present in pools derived from radioactively labeled NGF was measured in a Packard Model 3370 liquid scintillation spectrometer with a counting efficiency for ^{14}C of 84%. Each aliquot was counted in 10 ml of water-miscible scintillation liquid (Bray, 1960).

Characterization of Peptides. Aliquots from pools were hydrolyzed in the presence of 6 N HCl *in vacuo* at 110°. Amino acid analyses were performed on Spinco 120 or 120C amino acid analyzers by the method of Moore *et al.* (1958). Tryptophan was detected qualitatively by spray test, as described previously (Angeletti *et al.*, 1971) and semiquantitatively from acid hydrolysates using the automatic amino acid analyzer. The latter method was sufficiently sensitive to estimate the integral number of residues present, which was, in all cases, one.

Sequential degradation of purified peptides was carried out according to Edman (1956). The modifications of Konigsberg and Hill (1962) were adopted to facilitate the use of subtractive sequence analysis on the amino acid analyzer. Reagent specifications adopted by Waterfield and Haber (1970) were used in some instances where the residues were identified by gas–liquid chromatography of released MTH-amino acids. Aliquots were also removed at most steps to determine the N-terminal residue of the reacted peptide, using the method

TABLE 1: Amino Acid Composition of Soluble Tryptic Peptides of *S*-Carboxymethyl Nerve Growth Factor.[a]

Amino Acid	T-I-2	T-II	T-III-2	T-III-3	T-IV-2	T-IV-3	T-VI	T-VII	T-VIII	T-IX-2	T-IX-3	T-XI	T-XII	T-XIV
Lysine	1.08 (1)				1.03 (1)	1.18 (1)	0.97 (1)	1.00 (1)			1.23 (1)	1.41 (1)		1.12 (1)
Histidine												2.10 (2)		
Arginine		0.96 (1)	1.14 (1)	0.93 (1)					1.00 (1)	1.25 (1)			1.01 (1)	1.01 (1)
S-Carboxymethyl-cysteine		1.68 (2)		0.77 (1)					0.94 (1)			0.95 (1)		
Aspartic acid		1.08 (1)		1.00 (1)	1.00 (1)	1.04 (1)	0.99 (1)					1.21 (1)		
Threonine	0.96 (1)	1.00 (1)			2.86 (3)	1.87 (2)				1.00 (1)		4.05 (4)		0.92 (1)
Serine		1.00 (1)		1.79 (2)			0.98 (1)					1.11 (1)		
Glutamic acid	1.98 (2)		1.00 (1)	1.00 (1)		1.02 (1)								
Proline				1.04 (1)										
Glycine				0.92 (1)			1.07 (1)				1.00 (1)			
Alanine		1.09 (2)	2.21 (2)	0.88 (1)	1.07 (1)	0.98 (1)				0.94 (1)				1.00 (1)
Valine		1.91 (2)		0.92 (1)								1.11 (1)		
Methionine														
Isoleucine		0.96 (1)			0.88 (1)		0.98 (1)						0.94 (1)	
Leucine		0.92 (1)				0.95 (1)								
Tyrosine	1.00 (1)											1.05 (1)		
Phenylalanine	1.98 (2)											1.23 (1)	0.95 (1)	
Tryptophan			(1)[b]									(1)[b]		
Total	7	11	5	10	7	7	5	1	2	3	2	14	3	4
Per cent yield	58	74	44	52	61	83	88	53	74	29	47	43	89	34
Purification procedure[c]	DX1		DX1	DX1	DX1	DX1				DX1	DX1			
Residue no.	51–57	104–114	96–100	60–69	26–32	89–95	70–74	115	58–59	116–118	33–34	75–88	101–103	115–118

[a] Values are given in residues/mol. The assumed integral values are given in parentheses. Only the major yield peptide in each fraction is listed. Each peptide was purified until the contaminating level of any amino acid not found in the major peptide was below 0.25 residue/mol of peptide. In those instances when the identity of the contaminating peptide was known, the acceptable level was 0.25 μmol of contaminating peptide/μmol of major peptide. [b] Assumed to be one residue (see text). [c] Abbreviation used is: DX1, Dowex 1-X2.

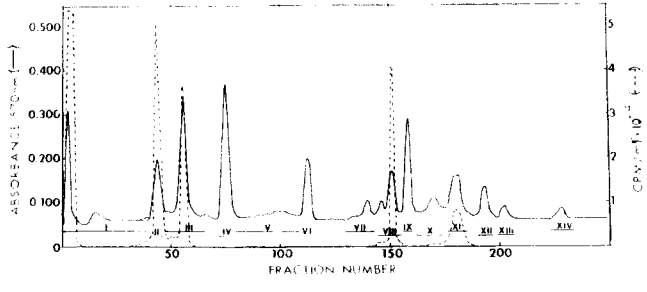

FIGURE 1: Elution profile of soluble tryptic peptides of 2.04 μmol of [^{14}C]*S*-carboxymethyl-NGF on a 0.9 × 20 cm column of Dowex 50-X8 at 55°. The column was developed at 30 ml/hr with a double linear gradient of pyridine acetate, as described in the text, and monitored automatically by ninhydrin analysis (——) after alkaline hydrolysis. Fractions of 3.0 ml were collected. Radioactivity (– – – –) was counted on 100-μl aliquots taken from alternate fractions. Fractions were pooled as indicated by the bars and corresponding Roman numerals.

TABLE II: Characterization of Peptides from Tryptic Fractions I, II, and III.

Fraction I:
Peptide T-I-2:
Sequence: Gln(Tyr,Phe,Phe,Glu,Thr)Lys
Carboxypeptidase B: 5 min: Lys, 1.00
Carboxypeptidase A: 4 hr: Thr, 0.88; Glu, 0.91; Tyr, 0.99; Phe, 2.00
High-voltage electrophoresis, pH 6.5: acidic
Fraction II:
Peptide T-II:
Sequence: Ile-Asx-Thr-Ala-Cys-Val-Cys-Val-Leu-Ser-Arg
Carboxypeptidase B: 5 min: Arg, 0.85
Carboxypeptidase A: 1 hr: Ser, 0.79; Leu, 0.70; Val, 0.45; CM-Cys, 0.19
Fraction III:
Peptide T-III-2:
Sequence: Gln-(Ala-Ala-Trp)-Arg
Carboxypeptidase B: 5 min: Arg, 1.00
Carboxypeptidase A: 30 min; Trp, 0.88; Ala, 2.00
High-voltage electrophoresis, pH 6.5: neutral
Peptide T-III-3:
Sequence: Ala-Ser-Asx-Pro-Val-Glx-(Ser,Gly,Cys)-Arg
Carboxypeptidase B: 5 min: Arg, 0.94
High-voltage electrophoresis, pH 6.5: acidic

TABLE III: Characterization of Peptides from Tryptic Fractions IV, VI, VII, and VIII.

Fraction IV:
Peptide T-IV-2:
Sequence: Thr-Thr-Ala-Thr-Asn-Ile-Lys
Carboxypeptidase B: 5 min: Lys, 1.00
Carboxypeptidase A: 30 min: Ile, 0.88
High-voltage electrophoresis, pH 6.5: basic
Peptide T-IV-3:
Sequence: Ala-Leu-Thr-Thr-(Asp,Glu)-Lys
Carboxypeptidase B: 5 min: Lys, 0.93
High-voltage electrophoresis, pH 6.5: acidic
Fraction VI:
Peptide T-VI:
Sequence: Gly-Ile-Asp-Ser-Lys
Carboxypeptidase B: 5 min: Lys, 0.96
High-voltage electrophoresis, pH 6.5: neutral
Fraction VII:
Peptide T-VII:
Sequence: Lys
Fraction VIII:
Peptide T-VIII:
Sequence: Cys-Arg

of Hartley (1970) and Gray and Smith (1970). Dansylated amino acid derivatives were identified by chromatography on polyamide sheets by a modification of the procedure described by Woods and Wang (1967). Polyamide sheets were cut into 3.5 × 7.0 cm rectangles and developed in 1.5% formic acid (width) followed by benzene–glacial acetic acid (88:10) in the second dimension.

Carboxyl-terminal sequences of several peptides were determined using carboxypeptidases A and B.

Results

Tryptic Digest of S-Carboxymethyl Nerve Growth Factor. Previous studies (Angeletti *et al.*, 1971) indicated that the protein contained 9 lysine and 7 arginine residues per 14,500 molecular weight. Thus, on a theoretical basis, a maximum of 17 peptides should be recovered from a tryptic digest of [^{14}C]SCM-NGF, no more than 6 of which should contain residues of radioactive *S*-carboxymethylcysteine. In fact, after tryptic digestion of 2.04 μmol of [^{14}C]SCM-NGF for 6 hr, 63% of the radioactivity was solubilized, leaving a significant acid-insoluble core. The soluble portion of the digest was applied to a column of Dowex 50-X8 and eluted with a double linear gradient of pyridine–acetate buffers. The elution profile of the ninhydrin-positive material and the radioactivity in the fractions are shown in Figure 1. As indicated by the bars and Roman numerals, 14 fractions were made, 5 of which contained radioactivity. A summary of the 14 pure peptides derived from these fractions is given in Table I. Each peptide is designated by its position of elution from the initial Dowex column. The composition of each peptide as well as the total number of residues, per cent yield, secondary purification procedures, if any, and the residue numbers comprised in the final sequence (Angeletti *et al.*, 1973) is shown. It is of interest to note that only 77 unique residues were recovered in soluble peptides and, if one examines the difference in the total compositions, it is easily noted that the core peptides must

TABLE IV: Characterization of Peptides from Tryptic Fractions IX, XI, XII, and XIV.

Fraction I:
Peptide ST-I-3:
Sequence: Asp-Ser-Val-Ser-(Val,Trp,Val,Gly,Asp)-Lys
Carboxypeptidase B: 5 min: Lys, 0.93
High-voltage electrophoresis, pH 6.5: acidic
Fraction III:
Peptide ST-III-2:
Sequence: Ile-Asp-Thr-Ala-Cys
High-voltage electrophoresis, pH 6.5: neutral
Fraction IX:
Peptide ST-IX:
Sequence: Thr-Thr-Thr-His-Thr-Phe-Val-Lys
Carboxypeptidase B: 5 min: Lys, 0.92
Fraction X:
Peptide ST-X:
Sequence: Val-Cys
Fraction XII:
Peptide ST-XII:
Sequence: Arg
Fraction XVII:
Peptide ST-XVII:
Sequence: His-Trp-Asn-Ser-Tyr-Cys
High-voltage electrophoresis, pH 6.5: basic

TABLE V: Amino Acid Composition of the Soluble Tryptic Peptides of *S*-Aminoethyl Nerve Growth Factor.[a]

Amino Acid	ST-I-3	ST-II-2	ST-II-3	ST-III-2	ST-III-3	ST-IV-2	ST-V-2	ST-VII-2
Lysine	1.09 (1)	0.69 (1)	0.84 (1)			1.00 (1)		
SAE-Cysteine				1.00 (1)			1.00 (1)	
Histidine								
Arginine					0.96 (1)		1.00 (1)	1.00 (1)
Aspartic acid	2.22 (2)	1.00 (1)	0.96 (1)	1.10 (1)		0.98 (1)	1.04 (1)	
Threonine		2.80 (3)	1.82 (2)	1.00 (1)				
Serine	1.76 (2)					0.84 (1)	1.76 (2)	0.97 (1)
Glutamic acid			1.03 (1)		1.04 (1)		1.05 (1)	
Proline							1.10 (1)	
Glycine	1.28 (1)					0.95 (1)	1.02 (1)	
Alanine		1.05 (1)	1.00 (1)	1.00 (1)	1.78 (2)		0.88 (1)	
Valine	2.96 (3)						0.87 (1)	1.10 (1)
Methionine								
Isoleucine		0.89 (1)		1.00 (1)		0.95 (1)		
Leucine			1.03 (1)					1.08 (1)
Tyrosine								
Phenylalanine								
Tryptophan	(1)[b]				(1)[b]			
Total	10	7	7	5	5	5	10	4
Per cent yield	8	11	14	22	38	43	30	19
Purification procedure[c]	DX1	DX1	DX1	DX1	DX1	DX1	DX1	DX1
Residue no.	16–25	26–32	89–95	104–108	96–100	70–74	60–69	111–114

[a] Values are given in residues/mol. The assumed integral values are given in parentheses. See Table I for further details. [b] As-

have an elevated content of both acidic and hydrophobic residues, including the sixth, and last, unique *S*-carboxymethylcysteine. The recovery of this residue after thermolytic digestion of the tryptic insoluble core has been described previously (Angeletti *et al.*, 1971) and will be documented in detail (Angeletti *et al.*, 1973).

FRACTION I. This fraction contained considerable radioactivity, but when subfractionated on Dowex 1-X2, all of the radioactivity was found to be present in a subfraction which contained no peptide material as judged by amino acid analysis, and was thus assumed to be SCM-mercaptoethanol remaining from the original reaction mixture. A single, unlabeled pure peptide T-I-2, was recovered from this fraction. Carboxypeptidase B digestion readily confirmed that the carboxyl-terminal residue of this peptide is lysine, but attempts to determine the sequence from the N terminus by the Edman degradation procedure were not successful (see Table II). Since this peptide was known to contain 2 glutamic acid residues, it was concluded that the N-terminal residue was probably pyrrolidonecarboxylic acid formed from the cyclization of an amino-terminal glutamine. Consecutive digestions by carboxypeptidase B and carboxypeptidase A yielded stoichiometric amounts of all residues except glutamic acid which was one residue short. This is the expected result if the amino-terminal residue is pyrrolidonecarboxylic acid. Electrophoretic analysis revealed that the remaining glutamyl residue must be present in the acid form.

FRACTION II. As judged by both high-voltage paper electrophoresis and amino acid analysis, this fraction consisted of a single, pure peptide, T-II. As described in Table II, a combination of the Edman degradation procedure and carboxypeptidase A and B digestion, allowed the elucidation of the sequence of the entire peptide. This peptide contains 2 residues of *S*-carboxymethylcysteine, thus accounting for the larger radioactive peak on the fractionation (Figure 1).

FRACTION III. When analyzed by high-voltage paper electrophoresis at pH 3.75, this fraction showed one major ninhydrin-positive spot, and one clearly separate, ninhydrin-negative spot containing tryptophan. Chromatography on Dowex 1-X2 separated these two peptides. In view of the fact that peptide T-III-2 was both ninhydrin negative and resistant to Edman degradation (Table II), it was concluded that it possessed a block α-amino group probably in the form of pyrrolidonecarboxylic acid. Electrophoretic analysis at pH 6.5 was consistent with this hypothesis. Further proof was obtained from digestion with carboxypeptidases A and B, in which all the amino acids of the peptide were released except glutamic acid.

The second peptide isolated from this fraction, T-III-3, contains the third *S*-carboxymethylcysteinyl residue, as indicated by radioactive analysis. Six successful rounds of Edman degradation were performed, and the carboxyl-terminal residue was confirmed to be arginine by carboxypeptidase B hydrolysis. The peptide migrates to the positive pole at pH 6.5 and therefore, at least one of the two dicarboxylic amino acids, in addition to SCM-Cys, is in the free acid form.

FRACTION IV. This fraction was impure, and was found to contain two peptides, which were separated on a column of Dowex 1-X2. The first, T-IV-2, was a heptapeptide, whose structure was established unequivocally by 4 successful rounds of Edman degradation plus consecutive carboxypeptidase A and B hydrolyses (see Table III). Electrophoretic analysis at pH 6.5 showed that this peptide was basic, and therefore, that the aspartyl residue is present as asparagine.

Peptide T-IV-3, the second peptide eluted, was also composed of 7 residues. The positions of the first four amino acids

ST-VIII	ST-IX	ST-X	ST-XI	ST-XII	ST-XIII	ST-XIV	ST-XVI	ST-XVII
1.00 (1)	0.79 (1)				0.82 (1)		0.95 (1)	
		1.06 (1)						1.07 (1)
	1.06 (1)							0.91 (1)
			1.15 (1)	1.00 (1)		1.29 (1)	1.00 (1)	
								1.05 (1)
	4.00 (4)		1.05 (1)				0.98 (1)	
								1.00 (1)
					1.00 (1)			
			1.00 (1)				0.75 (1)	
	0.77 (1)	1.00 (1)						
						0.90 (1)		
								1.08 (1)
	1.00 (1)					1.00 (1)		
								(1)[b]
1	8	2	3	1	2	3	4	6
8	25	13	30	18	17	19	5	8
								G-25
115	81–88	109–110	116–118	NA[d]	33–34	101–103	115–118	75–80

sumed to be 1 residue (see text). [c] Abbreviations used are: DX1, Dowex 1-X2; G-25, Sephadex G-25. [d] No assignment.

were ascertained by Edman degradation, while by carboxypeptidase B digestion the C-terminal residue was confirmed to be lysine. According to electrophoretic analysis at pH 6.5 both acidic residues are present in the acid form.

FRACTION V. This fraction contained no peptidic material.

FRACTION VI. A single, pure peptide T-VI was obtained from this fraction without further subfractionation. Three successful turns of Edman degradation and carboxypeptidase B digestion yielded the complete sequence of this pentapeptide (Table III). Electrophoretic analysis indicated that the aspartyl residue was in the acidic form.

FRACTION VII. This fraction contained one major component, peptide T-VII, which was free lysine, indicating the probable presence of an Arg-Lys or Lys-Lys sequence in the NGF molecule.

FRACTION VIII. A radioactive dipeptide, T-VIII, was found in homogeneous form in this fraction. A single turn of subtractive Edman degradation confirmed the sequence to be SCM-Cys-Arg (Table III).

FRACTION IX. Electrophoretic analysis at pH 3.75 indicated the presence of two components in this fraction, which were separated by subfractionation on Dowex 1-X2. The structure of the first, the tripeptide T-IX-2, was elucidated by two turns of Edman degradation. The second peptide isolated from this fraction, T-IX-3, is a dipeptide whose sequence was confirmed by one turn of Edman degradation (Table IV).

FRACTION X. No appreciable peptide material was found in this fraction as judged by amino acid analysis.

FRACTION XI. This fraction contained a pure, radioactive, 14 residue peptide, T-XI. In addition to [^{14}C]SCM-Cys, this peptide also contained both of the 2 histidyl residues and one of the 2 tryptophanyl residues found among the soluble tryptic peptides. As shown in Table IV, only 4 successful turns of Edman degradation were accomplished. Lysine was confirmed to be the C-terminal amino acid by carboxypeptidase B digestion.

FRACTION XII. This fraction was a pure tripeptide, T-XII, whose full structure was easily elucidated by combined Edman

TABLE VI: Characterization of the Unique Peptides Isolated from *S*-Aminoethyl Tryptic Fractions I, III, IX, X, XII, and XVIII.

Fraction IX:
  Peptide T-IX-2:
    Sequence: Ala-Thr-Arg
  Peptide T-IX-3:
    Sequence: Gly-Lys
Fraction XI:
  Peptide T-XI:
    Sequence: His-Trp-Asx-Ser-(Tyr,Cys,Thr,Thr,Thr,His,-Thr,Phe,Val)-Lys
    Carboxypeptidase B: 5 min: Lys, 0.95
Fraction XII:
  Peptide T-XII:
    Sequence: Phe-Ile-Arg
    Carboxypeptidase B: 5 min: Arg, 0.94
Fraction XIV:
  Peptide T-XIV:
    Sequence: Lys-Ala-Thr-Arg
    Carboxypeptidase B: 5 min: Arg, 0.97

TABLE VII: Amino Acid Composition of the Chymotryptic Peptides of *S*-Carboxymethyl Nerve Growth Factor.[a]

Amino Acid	C-II-1	C-III-1	C-III-2	C-IV-1	C-IV-2	C-IV-3	C-V-1	C-VI-1	C-VII-1
Lysine								0.96 (1)	3.21 (3)
Histidine					0.84 (1)	1.10 (1)	0.93 (1)		
Arginine			1.00 (1)						
S-Carboxymethyl-cysteine	0.94 (1)		1.82 (2)		1.00 (1)	0.90 (1)	0.78 (1)	1.00 (1)	2.01 (2)
Aspartic acid	1.28 (1)	1.30 (1)	1.36 (1)		0.84 (1)	1.00 (1)		1.71 (2)	3.77 (4)
Threonine			1.18 (1)	1.17 (1)	3.00 (3)	3.90 (4)	3.60 (4)		
Serine	2.79 (3)	1.20 (1)			0.85 (1)	1.38 (1)			
Glutamic acid								1.90 (2)	1.02 (1)
Proline									
Glycine									2.00 (2)
Alanine			1.12 (1)					1.82 (2)	1.05 (1)
Valine	3.10 (3)		1.94 (2)						3.00 (3)[d]
Methionine									
Isoleucine			1.94 (2)						0.94 (1)
Leucine			1.12 (1)						1.00 (1)
Tyrosine		1.00 (1)			0.75 (1)	0.68 (1)			
Phenylalanine				1.00 (1)		0.85 (1)	0.70 (1)		
Tryptophan	(1)[b]							(1)[b]	
Total	9	3	11	2	8	10	7	9	18
Per cent yield	39	11	20	20	20	10	31	68	25
Purification procedure	DX1[c]	DX1	DX1	DX1	DX1	DX1	DX1	DX1	DX1
Residue no.	13–21	77–79	102–112	85–86	77–84	77–86	80–86	91–99	22–39

[a] Values are given in residues/mol. The assumed integral values are given in parentheses. See Table I for further details. [b] As-

degradation and carboxypeptidase B digestion (see Table IV).

FRACTION XIII. This pool did not provide any peptides in sufficient yield to be identified.

FRACTION XIV. Although this pool consisted of a single, pure peptide, T-XIV, its composition suggested that it was not unique as both lysine and arginine were present. By the methods outlined in Table IV, it was shown that lysine is the N-terminal residue, while arginine occupies the C-terminal position. The sequence of this tetrapeptide suggests that it is an incompletely digested fragment consisting of peptides T-VII (Lys) and T-IX-2 (Ala-Thr-Arg).

Tryptic Digest of S-Aminoethyl Nerve Growth Factor. Since the digestion of the SCM derivative of NGF with trypsin produced peptides accounting for only about 69% of the polypeptide chain, various methods were considered to recover the remaining portions of the molecule. One procedure was suggested by the presence of one half-cystinyl residue in the insoluble fraction. Conversion of cysteinyl residues to SAE-cysteine is known to make them susceptible to tryptic attack, and mixtures of such peptides are often more soluble than those prepared from SCM derivatives.

Accordingly, 3.75 μmol of SAE-NGF was digested with TPCK-trypsin as described above. A small acid-insoluble core was still present after 6-hr digestion. Following its removal by centrifugation, the soluble peptide mixture was applied to a column of Dowex 50-X-8 and eluted with a double linear gradient of pyridine–acetate buffers. The elution profile of the separation is shown in Figure 2. The 17 fractions are indicated by Roman numerals and bars. A summary of the subsequent treatment of these fractions and the peptides derived from them is shown in Table V, where the amino acid composition, total number of residues, per cent yield, secondary fractionation procedures, and the residues encompassed in the total sequence are listed for the purified peptides, designated ST. A more detailed analysis of those peptides, which are unique to this digest or add useful information to the tryptic peptides already described, is given below.

FRACTION I. After subfractionation on Dowex 1-X2 to remove the cysteic acid marker (see Methods), one pure peptide was isolated from this fraction. The peptide, ST-I-3, was derived from a previously insoluble tryptic peptide and contained a new lysine residue. Four turns of Edman degradation were accomplished, and the C-terminal residue was confirmed to be lysine by carboxypeptidase B digestion (Table VI). Electrophoretic analysis showed the peptide to be acidic, indicating both aspartyl residues were present in the acid form. This peptide is also unique in that it contains the last of the 3 tryptophanyl residues present in the NGF polypeptide chain.

FRACTION II. Two pure peptides, ST-II-2 and ST-II-3, were recovered from this fraction after further fractionation on a column of Dowex 1-X2. The first was identical in composition with T-IV-2 and the second with T-IV-3 and consequently, they were not analyzed further.

FRACTION III. Electrophoretic analysis also showed this fraction to contain two major peptides, which were readily separated on a column of Dowex 1-X2. The first, ST-III-2, was shown by amino acid analysis to correspond to the N-terminal 5 residues of the cysteinyl-containing tryptic peptide, T-II. As shown in Table VI, four successful turns of Edman degradation confirmed the sequence already derived for T-II. The peptide was shown to be neutral by electrophoresis at pH 6.5, indicating that the aspartyl residue was present in the

C-VII-2	C-VIII-2	C-X-1	C-X-2	C-X-3	C-XI-1	C-XIII	C-XIV	C-XV	C-XVI
		1.10 (1)	2.00 (2)	2.10 (2)	2.35 (2)		1.00 (1)		1.00 (1)
0.95 (1)	1.92 (2)		1.24 (1)	1.05 (1)	0.87 (1)				
			2.20 (2)	2.00 (2)	2.32 (2)	1.15 (1)	1.68 (2)	1.08 (1)	1.68 (2)
			1.67 (2)	1.60 (2)	1.79 (2)				
	1.00 (1)		2.00 (2)	2.20 (2)	2.21 (2)				
1.25 (1)	1.82 (2)		1.30 (1)	1.20 (1)			1.00 (1)		0.90 (1)
1.67 (2)	0.96 (1)		2.70 (3)	3.00 (3)	2.78 (3)		1.02 (1)		
			2.00 (2)	2.20 (2)	1.37 (1)	1.00 (1)			
0.85 (1)	0.75 (1)		1.10 (1)	0.92 (1)	0.88 (1)				
	1.12 (1)		1.86 (2)	2.20 (2)	2.12 (2)				
		0.88 (1)	1.04 (1)	1.22 (1)	1.31 (1)		0.93 (1)		0.95 (1)
1.00 (1)	1.25 (1)	0.95 (1)	1.36 (1)	0.98 (1)	1.19 (1)				
	0.50 (1)								
			1.16 (1)	0.80 (1)	1.00 (1)				
		1.00 (1)							
						0.93 (1)			
0.96 (1)	1.92 (2)		1.06 (1)	1.92 (2)				0.95 (1)	
			(1)[b]	(1)	(1)				
7	12	4	23	24	20	3	6	2	5
24	23	36	45	13	11	81	8	26	14
DX1	DX1	DX1	DX1	DX1	G-25				
1–7	1–12	87–90	54–76	53–76	57 76	50–52	113–118	100–101	114–118

sumed to be 1 residue (see text). [c] Abbreviations are as in Table V. [d] 72-hr hydrolysis.

acid form. The second peptide isolated in major quantity from this fraction, ST-III-3, was identical in composition with T-III-2 and thus was not analyzed further.

FRACTION IV. This fraction was purified by fractionation on a Dowex 1-X2 column, but only one major peptide, ST-IV-2, was isolated, identical in composition with T-VI.

FRACTION V. After subfractionation on a column of Dowex 1-X2, one high yield peptide, ST-V-2, was isolated. This peptide had a composition identical with T-III-3, and was not further studied.

FRACTION VI. This fraction was purified on a Dowex 1-X2 column, from which one major peptide, ST-VI-2 was isolated. This peptide is identical in composition with ST-V-2. The basis of the partial separation on the Dowex 50-X8 column of fractions V and VI is not apparent.

FRACTION VII. Only one major yield tetrapeptide, ST-VII-2, was isolated from this fraction following subfractionation on a Dowex 1-X2 column. From its amino acid composition, it appears to correspond to the C-terminal portion of the double cysteinyl-containing tryptic peptide T-II, thus adding confirmatory evidence to the sequence of this 11 residue peptide.

FRACTION VIII. This fraction containing one peptide, ST-VIII, consisted of free lysine, as did the tryptic peptide T-VII.

FRACTION IX. No further purification was required for this fraction, in which peptide ST-IX was found. Its composition corresponds to the carboxy-terminal portion of the cysteinyl-containing tryptic peptide, T-IX, which had not been successfully characterized in its entirety. By a combination of Edman degradation and carboxypeptidase B digestion, the entire sequence of ST-IX was derived as shown in Table VI.

FRACTION X. The dipeptide ST-X was found to be pure. By one round of Edman degradation its sequence was confirmed to be Val-Cys (Table VI). This peptide plus peptides ST-III-2 and ST-VII-2 comprise all of the complete cleavage products which could be isolated from the portion of the NGF molecule corresponding to tryptic peptide T-II.

FRACTION XI. This fraction contained only the tripeptide, ST-XI, which is identical in composition with T-IX-2 and thus was not further analyzed.

FRACTION XII. Peptide ST-XII which was shown to be free arginine, was present in this fraction. It was not possible to assign the exact position of this residue although it probably arises from peptide T-III-3.

FRACTION XIII. This fraction contained a dipeptide, ST-XIII, identical in composition with T-IX-3, and was thus not further analyzed.

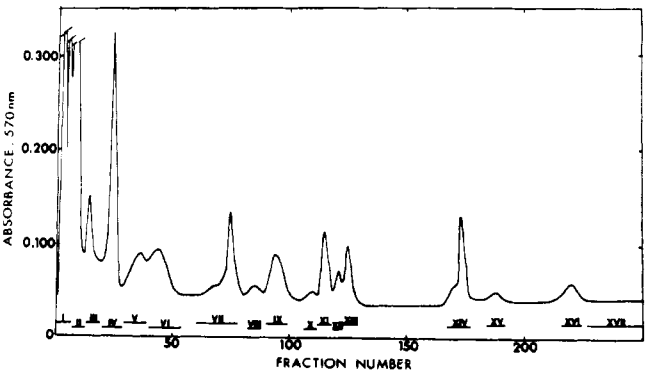

FIGURE 2: Elution profile of soluble tryptic peptides of 3.75 μmol of *S*-aminoethyl-NGF on a 0.9 × 20 cm column of Dowex 50-X8 at 55°. Conditions were the same as those used in Figure 1.

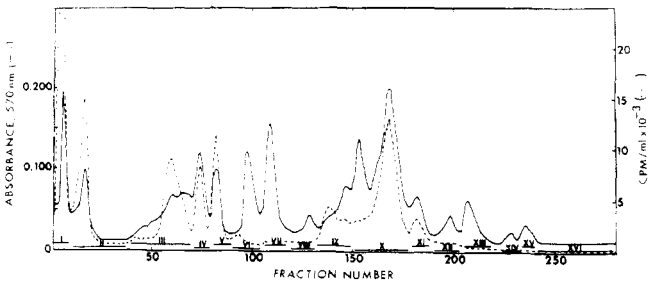

FIGURE 3: Elution profile of the soluble chymotryptic peptides of 3.75 mol of [^{14}C]*S*-carboxymethyl-NGF on a 0.9 × 20 cm column of Dowex 50-X8 at 55°. The column was developed at 30 ml/hr with a double linear gradient of pyridine acetate and monitored automatically by ninhydrin analysis (——) after alkaline hydrolysis. Fractions of 3.0 ml were collected. Radioactivity was counted on 100-μl aliquots taken from alternate fractions (- - - -). Fractions were pooled as indicated by the bars and corresponding Roman numerals.

FRACTION XIV. This fraction contained a tripeptide, ST-XIV, corresponding to the tryptic peptide, T-XIII. No further characterization was performed.

FRACTION XV. No appreciable peptide material could be found in this fraction as judged by amino acid analysis.

FRACTION XVI. Only one peptide, ST-XVI, was found in this fraction. Its composition is identical to T-XIV and was not further analyzed.

FRACTION XVII. The last fractions to be eluted from the Dowex 50-X8 column were pooled together, and passed through a Sephadex G-25 column (0.9 × 125 cm), in 0.1 M acetic acid. A single peptide, ST-XVII, which contained histidine, tryptophan, and AE-cysteine, was thus separated from minor contaminants. From its amino acid composition, this peptide corresponded to the amino-terminal portion of the cysteine-containing tryptic peptide, T-XI. Five rounds of Edman degradation were performed, confirming this hypothesis and extended the sequence information available for this region (Table VI). Electrophoretic analysis indicated that the aspartyl residue was present in the amide form.

Chymotryptic Digest of S-Carboxymethyl Nerve Growth Factor. The digestion of [^{14}C]SCM-NGF with chymotrypsin and acidification in preparation for chromatography, resulted in the formation of a considerable insoluble fraction. After the digest was centrifuged at 5000*g*, the supernatant was applied to a Dowex 50-X8 column, and eluted with pyridine–acetate buffers (Figure 3). The radioactively labeled peptides are indicated by the dashed line. The fractions were pooled as indicated by the bars and Roman numerals and examined for purity in the same manner as tryptic peptides. Table VII summarizes the amino acid composition, method of purification, and yield of each of the pure peptides obtained from this digest. The chymotryptic peptides, designated C, are numbered in the order of elution from the Dowex 50-X8 column. The corresponding residue numbers are given in the last line.

FRACTION I. This fraction contained the cysteic acid marker plus non-peptide radioactive material assumed to be [^{14}C]-SCM-mercaptoethanol incompletely removed from SCM-NGF by dialysis.

FRACTION II. After purification on Dowex 1-X2, one pure, radioactive peptide was recovered from this fraction. This nonapeptide, C-II-1, contains both cysteine and tryptophan, and represents the sixth cysteinyl residue not found in any of the soluble tryptic peptides. Three successful turns of Edman degradation positioned the cysteinyl residue (Table VIII).

TABLE VIII: Characterization of the Chymotryptic Peptides from Fractions II, III, V, and VI.

Fraction II:	
Peptide C-II-1:	
Sequence:	Ser-Val-Cys-(Asx,Ser,Val,Ser,Val,Trp)
Fraction III:	
Peptide C-III-1:	
Sequence:	Asn-Ser-Tyr
	High-voltage electrophoresis, pH 6.5: Neutral
Peptide C-III-2:	
Sequence:	Ile-Arg-Ile-Asx-Thr-Ala-Cys-Val-(Cys,Val,Leu)
Fraction V:	
Peptide C-V-1:	
Sequence:	Cys-Thr-Thr-Thr-His-Thr-Phe
Fraction VI:	
Peptide C-VI-1:	
Sequence:	Thr-Thr-Asx-Glx-Lys-(Glx,Ala,Ala,Trp)

FRACTION III. When subfractionated on Dowex 1-X2, this radioactive fraction yielded two peptides, only one of which, contained radioactivity. Peptide C-III-1 was characterized completely by two rounds of Edman degradation (Table VIII). Electrophoretic analysis showed that the aspartic acid residue is present in the amide form. The second peptide, C-III-2, which contained two residues of *S*-carboxymethylcysteine was subjected to 8 successful turns of Edman degradation (Table VIII).

FRACTION IV. This radioactive fraction was subfractionated on Dowex 1-X2, yielding three peptides, whose compositions are shown in Table VII. The first, C-IV-1, is a dipeptide which was not further analyzed. The second and third peptides, C-IV-2 and C-IV-3 containing 8 and 10 residues, respectively, both contained radioactivity. Neither of these peptides was characterized further. However, it should be noted that the two are very similar, differing only by one threonine and one phenylalanine. It was concluded that both C-IV-1 and C-IV-2 were derived from C-IV-3.

FRACTION V. When purified by chromatography on Dowex 1-X2, only one pure peptide, C-V-1, was isolated from this fraction. Six successful turns of Edman degradation completely structured this peptide which is clearly related to T-XI and ST-IX.

FRACTION VI. This fraction was purified on Dowex 1-X2, and provided only one major yield peptide. Five turns of Edman degradation were successfully performed upon this peptide (Table VIII), permitting its alignment.

FRACTION VII. Electrophoretic analysis revealed the presence of more than one peptide in this fraction, which was therefore subfractionated on Dowex 1-X2 to yield one 18-residue peptide, C-VII-1, and one 7-residue peptide, C-VII-2. The amino terminus of C-VII-1 was determined by the dansylation technique and the carboxy-terminal portion by digestion with carboxypeptidase A (Table IX). This information was sufficient to position this peptide in the final structure (Angeletti *et al.*, 1973). Peptide C-VII-2 was found to contain N-terminal serine, but no further characterization was performed.

FRACTION VIII. This fraction was chromatographed on Dowex 1-X2 from which a single 12-residue peptide, C-VIII-

2, was isolated. From amino acid composition data this peptide contains the two histidine residues and the methionine residue not previously isolated among the soluble tryptic peptides, and appears to be related to peptide C-VII-2. No further characterization was performed.

FRACTION IX. No peptides were isolated from this fraction.

FRACTION X. When purified further on Dowex 1-X2, this fraction provided several useful peptides. Peptide C-X-1, a tetrapeptide, was fully characterized using the dansyl and subtractive Edman procedures (Table IX), thus providing an important overlap peptide. C-X-2 is a 23 residue peptide apparently spanning several tryptic peptides, as judged by the lysine content. The first four residues from the amino terminus were determined by Edman degradation (Table IX). C-X-3 is identical in composition with C-X-2, except for an additional phenylalaninyl residue. No further characterization was performed.

FRACTION XI. Only one major peptide was isolated from this fraction, C-XI-1. From the amino acid composition data, this peptide is apparently related to, but 3 residues shorter than, C-X-2. Five successful turns of Edman degradation were performed after minor contaminants were removed by gel filtration on Sephadex G-25.

FRACTION XII. This fraction contained less than 0.3 μmol of material, which upon further purification on Dowex 1-X2, yielded only trace amounts of the same peptides found in fractions C-XI and C-XIII.

FRACTION XIII. This fraction consisted of a single tripeptide C-XIII in high yield which was fully characterized by Edman degradation (Table IX).

FRACTION XIV. This fraction yielded a pure 6-residue peptide, C-XIV, whose composition showed it to contain both lysine and arginine. Four successful turns of Edman degradation were performed (Table IX).

FRACTION XV. From this fraction a pure dipeptide, C-XV, was obtained and readily characterized by one turn of Edman degradation, as shown in Table IX.

FRACTION XVI. A 5-residue peptide, C-XVI, was found pure in this fraction. No further characterization was performed.

TABLE IX: Characterization of Selected Chymotryptic Peptides from Fractions VII, X, XI, XIII, XIV, and XV.

Fraction VII:	
Peptide C-VII-1:	
Sequence:	Val-(Gly,Asx,Lys,Thr,Thr,Ala,Thr,Asx,Ile,-Lys,Gly,Lys,Glx)-Val-Thr-Val-Leu
Carboxypeptidase A:	5 min: Thr, 0.61; Val, 0.64; Leu, 0.70
	1 hr: Thr, 0.90, Val, 1.74; Leu, 0.90
Peptide C-VII-2:	
Sequence:	Ser-(Ser,Thr,His,Pro,Val,Phe)
Fraction X:	
Peptide C-X-1:	
Sequence:	Val-Lys-Ala-Leu
Peptide C-X-2:	
Sequence:	Phe-Glx-Thr-Lys-(Cys,Arg,Ala,Ser,Asx,-Pro,-Val,Glx,Ser,Gly,Cys,Arg,Gly,Ile,Asx,Ser,Lys,His,Trp)
Fraction XI:	
Peptide C-XI-1:	
Sequence:	Lys-Cys-Arg-Ala-Ser-(Asx,Pro,Val,Glx,Ser,-Gly,Cys,Arg,Gly,Ile,Asx,Ser,Lys,His,Trp)
Fraction XIII:	
Peptide C-XIII:	
Sequence:	Arg-Glx-Tyr
Fraction XIV:	
Peptide C-XIV:	
Sequence:	Ser-Arg-Lys-Ala-(Thr,Arg)
Fraction XV:	
Peptide C-XV:	
Sequence:	Arg-Phe

Discussion

The sum of the amino acid compositions of the tryptic peptides isolated from *S*-carboxymethyl and *S*-aminoethyl nerve growth factor accounts for 87 residues after correction for overlapping segments. The remaining residues were not recovered from the acid-insoluble core. In this regard, it is interesting to note that the amino-terminal peptide was not isolated by either separation, as judged by the absence of any peptide with amino-terminal serine (Angeletti *et al.*, 1971). In addition, there was no obvious carboxyl-terminal peptide found, one missing lysine or arginine. Subsequent analyses (Angeletti *et al.*, 1973) have indicated that peptide T-IX-2 (or T-XIV), which possesses carboxyl-terminal arginine, is the carboxyl-terminal peptide of the polypeptide chain.

An examination of the terminal residues of the isolated tryptic peptides failed to reveal any evidence for cleavages other than at lysyl or arginyl residues (or SAE-cysteine). Thus, NGF appears to be an unusually specific substrate for trypsin.

Considerably more of the NGF structure was represented in the chymotryptic peptides isolated. The nineteen peptides listed in Table VII account for 183 amino acids which correspond to 108 unique residues. Based on the completed structure (Angeletti *et al.*, 1973), only 10 residues, later identified as residues 40–49, were not located in these peptides. This region was also missing from the isolated tryptic peptides as well.

As with the tryptic peptides, the cleavages affected by chymotrypsin followed very much along those conventionally ascribed to it (Hill, 1965). Only the hydrolyses at the Thr-Lys (56–57) and Ser-Arg (113–114) bonds may be considered unusual.

Despite the extensive overlapping and residue placement provided by the peptides from the three separate digestions, these data were still insufficient to deduce the amino acid sequence. The data necessary to determine the complete covalent structure, provided by the thermolytic and peptic peptides and by degradation using the automatic sequenator, are described in the ensuing communication (Angeletti *et al.*, 1973).

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Amino Acid Sequences of Mouse 2.5S Nerve Growth Factor. II. Isolation and Characterization of the Thermolytic and Peptic Peptides and the Complete Covalent Structure†

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ABSTRACT: The isolation and characterization of the thermolytic peptides of *S*-carboxymethyl nerve growth factor and the peptic peptides of native nerve growth factor are described. The peptides derived from the latter digestion were also used for the determination of the pairing of the half-cystinyl residues. In addition, the two fragments produced by cyanogen bromide cleavage were isolated and used, together with the analyses of the protein Sequencer, to establish the structure of the amino-terminal portion of the protein. It was determined that some preparations of nerve growth factor contained polypeptide chains that were eight residues shorter than the primary subunit. These chains have been designated A (long) and B (short) and apparently arise from limited proteolysis. Carboxypeptidase B digestion of *S*-carboxymethyl nerve growth factor defined the carboxyl-terminal portion of the molecule. These data are combined with those derived from the tryptic and chymotryptic peptides described in the preceding communication to construct an internally consistent primary and secondary structure of nerve growth factor. In addition, each of the side chains of Glx and Asx residues was identified as to its acidic or neutral character. Thus, mouse nerve growth factor consists, in the unaltered state, of a dimer of two polypeptide chains containing 118 amino acids, each with a molecular weight of 13,259. The disulfide pairs are formed by Cys-15 and Cys-80, Cys-58 and Cys-108, and Cys-68 and Cys-110.

The preceding communication (Angeletti *et al.*, 1973) describes the isolation and characterization of the soluble peptides from tryptic and chymotryptic digests of *S*-carboxymethyl nerve growth factor (NGF)[1] and the tryptic digest of *S*-aminoethyl-NGF. Because these data in their entirety did not provide sufficient information, especially with respect to the amino terminus, to construct a tentative sequence for NGF, additional peptides from digestion of NGF with thermolysin and pepsin were prepared and characterized. Furthermore, as it was known that the subunit of NGF is a single polypeptide chain containing three internal disulfide bridges and no free sulfhydryl groups (Bocchini, 1970; Angeletti *et al.*, 1971), the peptic digestion was performed on native NGF so as to allow the determination of the disulfide pairing. In addition, experiments were also conducted using the Beckman protein Sequencer, on the one hand, and carboxypeptidase digestion, on the other, to elucidate the nature of the amino and carboxy termini, respectively. The Sequencer

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[1] Abbreviations used are: NGF, nerve growth factor; CM-, carboxymethyl: Th-, thermolytic peptides; P-, peptic peptides; SE-, sulfoethyl; PTh-, peptides from the thermolytic digest of a peptic fragment; PC-, peptic acid insoluble peptides; CNBr-, cyanogen bromide fragments; Pth, phenylthiohydantoin.