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Primary Structure of the Mcg λ Chain†

James W. Fett and H. F. Deutsch*

ABSTRACT: The complete primary structure of the Mcg L chain has been determined. Three new amino acid substitutions which occur in the constant region at positions 116, 118, and 167 have been detected. In addition, a substitution of threonine for glycine at position 103 in the variable region in this λ-type chain protein is noted for the first time. The primary sequence of this L chain will be of great value in relating the tertiary structure of the crystalline dimer to its ligand binding activity.

The products of a number of structural genes have been described for the C regions of immunoglobulin L chains (Hilschmann and Craig, 1965; Ein and Fahey, 1967; Milstein *et al.*, 1967; Ein, 1968; Ponstingl and Hilschmann, 1969; Hess *et al.*, 1971; Terry *et al.*, 1969; Gally and Edelman, 1972). Possible evidence for others has been indicated but not fully explored (Milstein, 1967; Milstein *et al.*, 1967). An increase in the number of variations in this region of the L chain will markedly increase the possible numbers of antibody molecules unless some linkage between these substitutions in the constant region exists or unless constant region variations will relate in some manner to variable region sequences. Different amino acid substitutions in the constant region of the L chain may also play a role in their interactions with the C_1 region of the H chain and correlate with different substitutions in this part of

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the immunoglobulin molecule. A protein which appears to be important to our understanding of these and other considerations is the Mcg molecule (Deutsch and Suzuki, 1971; Fett *et al.*, 1973), particularly so since we have found three new constant region substitutions in its L chain. Both the IgG and Bence-Jones proteins of this patient have been crystallized (Edmundson *et al.*, 1970; Schiffer *et al.*, 1970) and the three-dimensional X-ray structure of the latter at 3.5-Å resolutions has been obtained (Schiffer *et al.*, 1973). The exact interpretation of the three-dimensional X-ray model in terms of a primary sequence is obligatory. Furthermore, the L-chain Mcg dimer has been found to possess binding capacity for various dinitrophenyl derivatives and their exact location in the crystalline structure is being elucidated (Edmundson *et al.*, 1974). The complete primary sequence of the Mcg Bence-Jones protein thus will also provide data for the study of the intimate relationships of sequence to the three-dimensional structure and ligand combining properties of this protein.

Materials and Methods

The Bence-Jones protein of Mcg was prepared in crystalline form as previously described (Schiffer *et al.*, 1970). All derivatized proteins and digestion products were recovered and held in lyophilized form.

Preparation of Alkylated Protein. A 2× crystallized Mcg Bence-Jones protein preparation (172 mg) in 10 ml of pH 8.0 Tris-HCl buffer (Γ/2, 0.5) containing 0.25 M 2-mercaptoethanol (Eastman Kodak) and 8 M urea was held overnight at 37°. A 50% molar excess of iodoacetamide (0.69 g) (Pierce Chemicals) and an equal weight of Tris were then added and the reaction was allowed to proceed for 30 min at room temperature. Excess reagents in CAM-protein[1] were removed by dialysis against deionized water.

The same amount of the 2× crystallized Mcg protein preparation was reduced as described above. Aminoethylation was effected by adding 8 mmol (0.4 cm^3) of ethylenimine (Pierce Chemicals) and allowing the reaction to proceed 10 min at room temperature. The alkylation step was repeated twice (Raftery and Cole, 1966). Excess reagents were again removed from the AE-protein by dialysis against deionized water.

Cleavage with Endopeptidases. Suspensions (1%) of the reduced and alkylated Mcg proteins in pH 8.0, 0.2 M ammonium bicarbonate were subjected to digestion with trypsin, chymotrypsin, and thermolysin. Hydrolysis with trypsin (TPCK, Worthington) at a level of 4% of the amount of protein was carried out for 16 hr at 37°. Insoluble TC material was removed by centrifugation and it and the soluble supernatant peptides were freeze dried.

The reduced-alkylated protein was also subjected to digestion with 2% of its weight of α-chymotrypsin (Worthington) for 4 hr at 37° to provide a completely soluble digest. Digestion with thermolysin (Daiwa Kasei, Osaka, Japan) was carried out with 0.5% by weight of enzyme for 1 hr at 37° and this converted the protein into completely soluble material.

Fractionations of soluble peptides resulting from the endopeptidase digestions described above were carried out utilizing Dowex 50-X2 and Dowex 1-X2 ion-exchange resins. The amounts of peptide material in the effluent fractions were determined by the method of Hirs (1967). In some cases Dowex 50-X2 chromatography was employed using an automated method (Lin and Deutsch, 1973). The peptide components resolved in the various experiments were assessed for purity by electrophoresis on paper at pH 6.5 and 3.7. Larger amounts of peptides sufficient for sequence analyses were then purified by paper electrophoresis using the pH found to effect maximum separation. The mobility of peptides at pH 6.5 was of aid in determining the number of amide residues in some cases. A pK of 6.5 was assumed for imidazole groups when histidine was present.

The lyophilized TC material derived from CAM-protein was dissolved in 5 M guanidine-HCl and fractionated over a column of Sephadex G-50 (fine). The peptide fractions resolved were individually desalted over columns of Sephadex G-10 equilibrated with 5% acetic acid. One component of relatively high molecular weight, TC-I, was subjected to further cleavage with chymotrypsin and with thermolysin and the resulting digests were ii individually fractionated over columns of Dowex 50-X2.

The soluble tryptic peptide fraction derived from CAM-protein was subjected to filtration on Sephadex G-50 (Fine) in 0.2 M ammonium bicarbonate to effect separation of the larger peptides. One such component, T-S1, was subjected to further digestion with chymotrypsin followed by fractionation of the products on Dowex 50-X2.

Amino Acid Analyses. Hydrolysis of CAM-protein was carried out in evacuated, sealed tubes containing 4 N methylsulfonic acid and 0.2% 3-(2-aminoethyl)indole (Pierce Chemicals) for 20 hr at 110°.[2] Hydrolyses of TC peptides and of the relatively high molecular weight soluble tryptic peptide T-S1 were carried out in evacuated, sealed tubes containing constant boiling HCl (5.7 N) for 20 hr at 110°. All other peptides were hydrolyzed at 110° for 20 hr in an evacuated desiccator containing a reservoir of 6 N HCl (Dreyer and Bynum, 1967). The amino acid compositions of the hydrolysates were quantitated on either a Technicon TSM-1 autoanalyzer or on a Durrum D-500 autoanalyzer. The lithium buffer reagent system of Atkin and Ferdinand (1970) was used in some cases to determine the amino acids liberated in exopeptidase digests of purified peptides to permit identification of glutamine and asparagine residues.

Cleavage with Exopeptidases. Hydrolyses of purified peptides were performed in pH 8.0, 0.2 M ammonium bicarbonate. The enzymes employed were leucine aminopeptidase and carboxypeptidases A and B (all Worthington products). At various time intervals, dependent upon the particular peptide under investigation, aliquots were removed from the digestion mixture and free amino acids determined as described above.

Edman Degradations. The major portion of these were performed with an accelerated manual method (Lin and Deutsch, 1973). In several cases, lysine-rich peptides were treated with an excess of 4-sulfophenyl isothiocyanate prior to the first coupling step to decrease the hydrophobic properties of the peptide (Braunitzer *et al.*, 1970). The thiazolinone derivatives were converted into the free amino acids by treatment with 57% HI and/or with 0.2 M NaOH in the presence of dithionite (Smithies *et al.*, 1971). The yields usually ranged from about

[1] Abbreviations used are: reduced and carboxamidomethylated protein, CAM; reduced and aminoethylated protein, AE; pyrrolidonecarboxylic acid, PCA; phenylthiohydantoins, Pth; leucine aminopeptidase, LAP; carboxypeptidases A and B, CPA and CPB, respectively; tryptic "core" peptides, TC; soluble tryptic peptides derived from CAM-protein, T; soluble tryptic peptides derived from AE-protein, T-X-AE, where X will be the number of a specific peptide; chymotryptic peptides, C; thermolytic peptides, Th. When a peptide is prepared from a large tryptic peptide by digestion with chymotrypsin or thermolysin it will bear a second lettered term of C or Th, respectively. A terminal letter of N, A, or B on a peptide will indicate its being neutral, acidic, or basic, respectively, at pH 6.5. Peptides derived from Dowex 1-X2 fractionation will be designated with a prime following the enzyme designation, *i.e.* Th′, a thermolysin-derived peptide.

[2] Dr. T.-Y. Liu, personal communication.

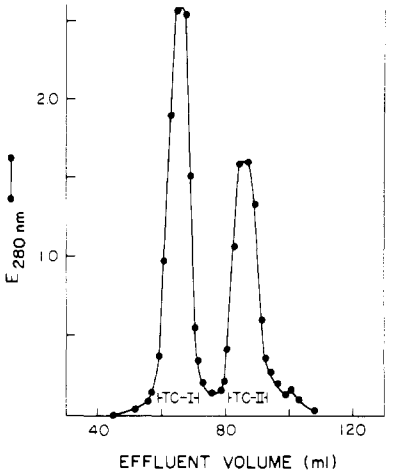


FIGURE 1: Chromatogram illustrating the separation of 20 mg of TC material on a 2 × 50 cm column of Sephadex G-50 (Fine) equilibrated with pH 5.0, 5 M guanidine-HCl; 2-ml fractions were collected.

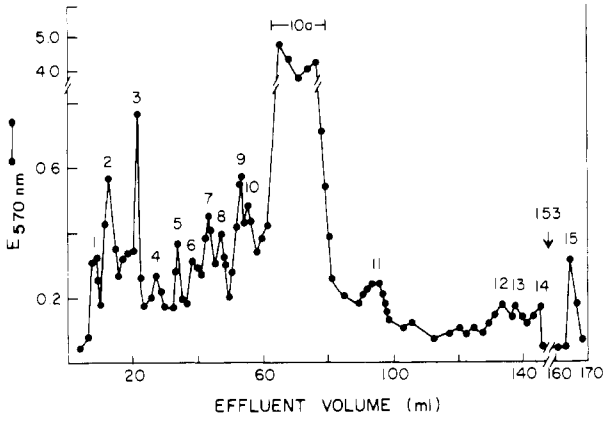


FIGURE 2: Chromatogram illustrating the separation of a chymotryptic digest of 40 mg of TC-I on a 0.9 × 20 cm column of Dowex 50-X2 equilibrated with pH 2.3, 0.2 M pyridine-formate buffer. The peptides were eluted at 50° by means of a linear gradient to pH 5.0 with 2 M pyridine-acetate followed by addition of pH 5.6, 8.5 M pyridine-acetate at tube number 153 (see arrow); 1-ml fractions were collected.

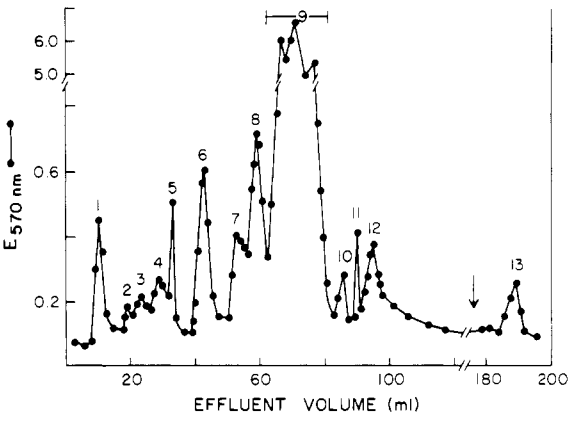


FIGURE 3: Chromatogram illustrating the separation of a thermolytic digest of 40 mg of TC-I on a 0.9 × 20 cm column of Dowex 50-X2 equilibrated with pH 2.3, 0.2 M pyridine-formate buffer. Conditions for elution of the peptides were as described in Figure 2.

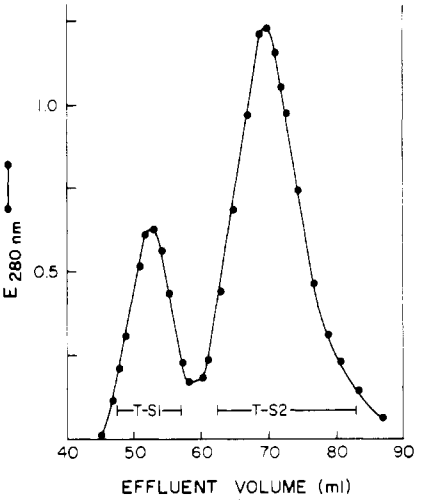


FIGURE 4: Chromatogram illustrating the separation of a tryptic digest of 50 mg of CAM-protein on a 1.5 × 39 cm column of Sephadex G-50 (fine) equilibrated with pH 8.0, 0.2 M ammonium bicarbonate; 2-ml fractions were collected.

80% at the beginning of a degradation to about 5% when it was discontinued. These results are similar to those experienced in other studies (Lin and Deutsch, 1973). Several relatively large peptides were subjected to automated, sequential Edman degradation utilizing a Beckman Sequencer 890C. In these cases the thiazolinone derivatives were converted to their Pth derivatives which were then identified directly on silica gel thin-layer sheets containing a fluorescent indicator (Eastman Kodak).

Results

The separation of the TC peptides in 5 M guanidine-HCl by gel filtration is shown in Figure 1. Two components, TC-I and TC-II, with the compositions shown in Table I were resolved. The composition of peptide TC-II was identical with that expected for the constant region 134–153 of λ chains. It was not studied in detail except for the elucidation of the tryptophan residue at position 152. This was effected by the use of a mixture of CPA and CPB which sequentially released lysine and tryptophan.

Edman degradation of peptide TC-I released no N-terminal derivative. Furthermore, its composition resembled the N-terminal sequence of other λ chains and it was assumed that this peptide was derived from this portion of the molecule. It was subjected to further enzymatic hydrolysis with chymotrypsin and with thermolysin to substantiate this consideration. The fractionations of these digests on Dowex 50-X2 columns are shown in Figures 2 and 3, respectively. Component 9 of the thermolytic digest and 10a of the chymotryptic digest contained considerable ninhydrin-negative material due apparently to undigested TC-I. Peptide components of both of these digests not retarded on Dowex 50-X2 were also found to contain ninhydrin-negative material. The detection of such peptides on the pH 6.5 paper electropherograms was made by removing guide strips, dividing these into 1-cm serial sections, and eluting each with 5% acetic acid. The determination of the presence of peptide material in these eluents was made by the method of Hirs (1967). The ninhydrin-negative chymotryptic peptide TC-I-C-1A contained Glx, Ser, Ala, and Leu (see Table II), and the thermolytic peptide TC-I-Th-1A contained Glx, Ser, and Ala (see Table III). Their homology with other λ_I and λ_{IV} subgroup N-terminal sequences suggested that these peptides were derived from this region in the Mcg protein with the terminal residue being PCA.

The soluble tryptic peptides of Mcg CAM-protein were resolved by gel filtration to give the result shown in Figure 4. Fraction T-S2 was electrophoretically heterogeneous and was not studied further. The amino acid composition of component T-S1 (see Table I) suggested that it was a single tryptic peptide

TABLE I: Amino Acid Compositions[a] of Tryptic Peptides of Reduced and Alkylated Mcg Protein Used in Sequence Experiments.

AA	T-1N-AE	T-10A-AE	T-14B2-AE	T-S1	T′-1B	T-2A1	T-3A	T-3N	T-4N	T-5B	T-8B1	T-9A	T-13N	T-13B	TC-I	TC-II
Asp		2.09 (2)	2.18 (2)	4.64 (5)			2.12 (2)	2.10 (2)	1.15 (1)					1.02 (1)	2.30 (2)	1.30 (1)
Thr	1.85 (2)	1.97 (2)	2.00 (2)	3.60 (4)		1.55 (2)	1.80 (2)			0.94 (1)	1.92 (2)	1.82 (2)	1.25 (1)		3.75 (4)	2.30 (2)
Ser	5.62 (6)	2.75 (3)	3.08 (3)	5.40 (6)	1.10 (1)	0.90 (1)	1.91 (1)	1.20 (1)	1.25 (1)		0.97 (1)	2.60 (3)	3.45 (3)	0.93 (1)	7.50 (9)	1.40 (1)
Glu	3.25 (3)	1.05 (1)	2.10 (2)	4.13 (4)		0.92 (1)	2.90 (3)	1.27 (1)		1.15 (1)	1.08 (1)	2.96 (3)	2.30 (2)		4.85 (5)	0.30 (0)
Pro	1.66 (2)					1.00 (1)	2.60 (3)		1.17 (1)	1.05 (1)	0.87 (1)		1.40 (1)	1.83 (2)	2.20 (2)	0.98 (1)
Gly	2.00 (2)	3.10 (3)	4.25 (4)	4.45 (5)					1.10 (1)	1.05 (1)	1.07 (1)	1.07 (1)		1.07 (1)	5.40 (6)	0.97 (1)
Ala	2.00 (2)		1.20 (1)	2.75 (3)		0.98 (1)	1.85 (2)		1.18 (1)		1.00 (1)		2.30 (2)		3.00 (3)	3.00 (3)
Half-Cys	0.90 (1)[b]			0.77 (1)[d]		1.00 (1)[d]						0.94 (1)[d]			0.79 (1)[d]	0.90 (1)[d]
Val	1.07 (1)	1.15 (1)	2.22 (2)	1.92 (2)		0.98 (1)	0.91 (1)		1.10 (1)	1.75 (2)	1.02 (1)	1.89 (2)		1.07 (1)	2.86 (3)	3.10 (3)
Ile	0.83 (1)														0.54 (1)	0.91 (1)
Leu	1.92 (2)			2.02 (2)			1.80 (2)			1.13 (1)			2.20 (2)		1.75 (2)	2.04 (2)
Tyr		0.89 (1)	2.20 (3)	2.80 (3)								0.73 (1)	1.86 (2)		2.20 (3)	1.40 (1)
Phe		1.80 (2)		1.90 (2)			0.82 (1)									0.89 (1)
His			0.71 (1)		0.92 (1)							0.95 (1)			0.70 (1)	
Lys		1.00 (1)	1.00 (1)	1.00 (1)			1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	2.18 (2)	1.00 (1)	1.00 (1)		1.20 (1)	1.32 (1)
Arg					1.00 (1)									2.00 (2)		
No. of residues (yield)[e]	22 (75)	16 (9)	22[c] (7)	38	3 (36)	8 (39)	19 (37)	5 (34)	7 (55)	8 (47)	10 (40)	15 (34)	15[c] (4)	8 (11)	44[c]	20[c]
Sequence position	1–22	91–106	23–44	69–106	191–193	209–216	115–133	171–175	154–160	107–114	161–170	194–208	176–190	56–63	1–44	134–153
Charge (pH 6.5)	0	−1	+0.5		+1.5	−1	−1	0	0	+1	+1	−0.5	0	+1		

[a] The amino acid compositions except for tryptophan were determined on hydrolysates prepared at 110° for 20 hr in an evacuated desiccator containing a reservoir of 6 N HCl for all peptides except for T-S1, TC-I, and TC-II. In the latter three cases hydrolysates were prepared at 110° for 20 hr in sealed, evacuated tubes containing constant boiling HCl (5.7 N). Destruction of serine, threonine, and tyrosine was not corrected for. The numbers of residues per mole of peptide found are given along with the integral values (in parentheses) found either in eventual sequencing experiments or suggested by homologies with other λ-chain sequences. [b] Determined as *S*-aminoethylcysteine. [c] The number of residues indicated is one more than the analytical results in the columns shown. This is due to the presence of tryptophan found by eventual sequence determinations. [d] Determined as *S*-carboxymethylcysteine. [e] The open values indicate the numbers of residues in the peptide, those in parentheses their yield. Yield data are not available for peptides TS-1, TC-I, and TC-II.

TABLE II: Amino Acid Compositions[a] of Chymotryptic Peptides of Mcg CAM-Protein Used in Sequence Experiments.

AA	TC-I-C-1A	TC-I-C-2N	TC-I-C-9A	TC-I-C-12N	TC-I-C-13B	T-S1-C-9B	C′-1B	C′-2B	C-6N
Asp			1.00 (1)	1.00 (1)			1.80 (2)		0.94 (1)
Thr		1.61 (2)				1.87 (2)			1.04 (1)
Ser	0.87 (1)	3.82 (4)	1.87 (2)	1.25 (1)			0.87 (1)	1.95 (2)	
Glu	1.00 (1)	1.95 (2)			2.12 (2)		1.00 (1)		1.00 (1)
Pro		1.79 (2)					1.70 (2)		
Gly		2.10 (2)	2.20 (2)		1.00 (1)	2.12 (2)	1.19 (1)		
Ala	1.00 (1)	1.07 (1)			0.88 (1)				1.60 (2)
Half-Cys									
Val		1.02 (1)	0.87 (1)	0.88 (1)			1.99 (2)		
Ile									
Leu	0.96 (1)	1.00 (1)							1.13 (1)
Tyr			0.92 (1)	0.91 (1)				0.83 (1)	
Phe							0.83 (1)		
His					0.74 (1)			1.00 (1)	
Lys					0.83 (1)	1.00 (1)	1.15 (1)	1.40 (1)	0.78 (1)
Arg							1.75 (2)	0.92 (1)	
No. of residues (yield)[d]	4 (15)	15 (3)	7 (8)	5[b] (6)	6 (4)	5 (12)	13 (58)	6 (19)	7 (26)
Sequence position	1–4	5–19	26–32	33–37	39–44	102–106	52–64	190–195	130–136
Charge (pH 6.5)	−1	0	−1	0	+1.5	+1	+1	+2.5	0

[a–d] See footnotes *a*, *c*, *d*, and *e* of Table I.

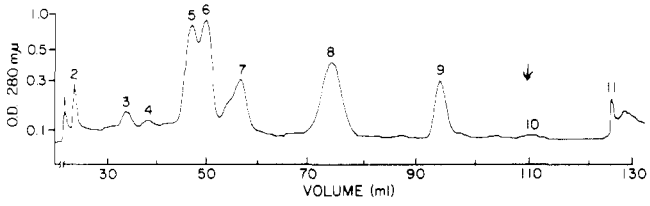


FIGURE 5: Result of automated separation of the chymotryptic digest of 40 mg of T-S1 on a 0.9 × 20 cm column of Dowex 50-X2 equilibrated with pH 3.1, 0.2 M pyridine–acetate buffer. The peptides were eluted at 50° by means of a linear gradient to pH 5.0 with 1 M pyridine–acetate followed by addition of pH 5.6, 8.5 M pyridine–acetate. The arrow represents the point of addition of the latter buffer; 1-ml fractions were collected.

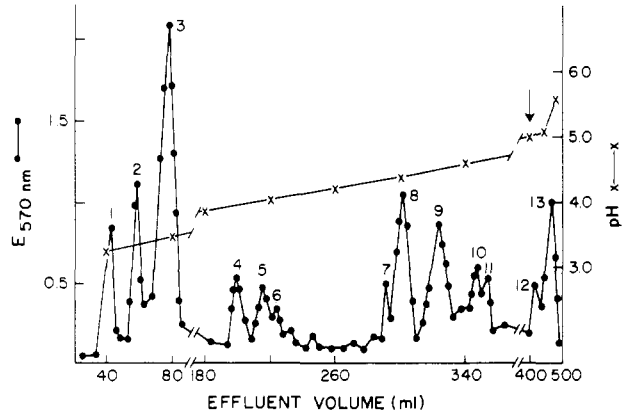


FIGURE 6: Chromatogram illustrating the separation of a tryptic digest of 70 mg of CAM-protein on a 0.9 × 60 cm column of Dowex 50-X2 equilibrated with pH 3.1, 0.2 M pyridine–acetate buffer. The peptides were eluted at 50° by means of a linear gradient to pH 5.0 with 1 M pyridine–acetate followed by the addition of pH 5.6, 4 M pyridine–acetate. The arrow represents the point of addition of the latter buffer; 2-ml fractions were collected.

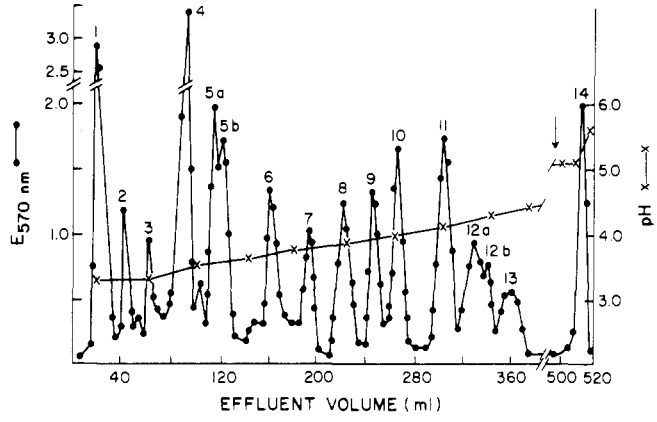


FIGURE 7: Chromatogram illustrating the separation of a tryptic digest of 80 mg of AE-protein on a 0.9 × 60 cm colmn of Dowex 50-X2 equilibrated with pH 3.1, 0.2 M pyridine–acetate buffer. The elution conditions were as described in Figure 5; 2-ml fractions were collected.

derived from the variable region. Eventual sequence experiments localized it to residues 69–106. Chymotryptic degradation products of it were fractionated on Dowex 50-X2 to give the result presented in Figure 5.

Fractionations of the soluble tryptic peptides of CAM- and AE-protein on Dowex 50-X2 gave the results presented in Figures 6 and 7, respectively. Similar results for the chymotryptic and for the thermolytic digests of CAM-protein are shown in Figures 8 and 9, respectively. The amino acid compositions of all of the soluble tryptic, chymotryptic, and thermolytic peptides used in the determination of the primary sequence are shown in Tables I, II, and III, respectively. The data of these tables include the number of residues for each peptide, their change at pH 6.5, and their residue positions as eventually established in the sequence experiments.

Highly basic tryptic, chymotryptic, and thermolytic peptides

C-7A1	C-7A2	C-9A1	C-11N1	C-11N2	C-12A2	C-13B	C-18N	C-19B	C-20B	C-21B
		0.95 (1)						1.70 (2)	1.20 (1)	
	2.60 (3)	1.00 (1)			0.98 (1)	0.89 (1)			1.75 (2)	
1.85 (2)	1.78 (2)	1.08 (1)		2.13 (2)	1.09 (1)	0.98 (1)		0.95 (1)	2.02 (2)	
2.00 (2)	2.72 (3)				2.00 (2)	1.00 (1)			2.10 (2)	2.20 (2)
1.86 (2)	0.96 (1)	1.30 (1)			0.97 (1)				1.85 (2)	0.83 (1)
	1.04 (1)	1.04 (1)							2.00 (2)	1.20 (1)
	1.00 (1)	1.00 (1)		2.00 (2)			0.98 (1)		1.97 (2)	2.02 (2)
	0.87 (1)[c]		0.93 (1)[c]			0.87 (1)[c]				
	1.65 (2)	0.91 (1)	1.00 (1)			0.80 (1)	1.00 (1)		2.10(2)	0.78 (1)
		0.84 (1)								1.55 (2)
2.00 (2)			1.12 (1)		1.84 (2)					
		0.65 (1)		1.31 (1)				0.87 (1)		0.91 (1)
1.25 (1)		0.80 (1)								
						0.90 (1)				1.40 (1)
	0.93 (1)							1.00 1)	3.30 (4)	2.00 (2)
9 (27)	15 (11)	10 (6)	3 (39)	5 (47)	8[b] (29)	6 (22)	3[b] (33)	5 (53)	19 (16)	13 (17)
121–129	202–216	140–149	137–139	177–181	182–189	196–201	150–152	172–176	153–171	39–51
−2	−2	−1	0	0	−1	+0.5	0	+1	+2	+1.5

derived from reduced and alkylated proteins which could not be eluted from Dowex 50-X2 were purified by individually fractionating these digestion mixtures on columns of Dowex 1-X2 and eluting with a pH 9.4, 0.12 M *N*-ethylmorpholine–0.20 M α-picoline–0.12 M pyridine–acetic acid buffer. The compositions of the peptides purified by this procedure are included in the data of Tables I, II, and III.

The methods and results employed in the determination of the complete primary sequence of Mcg protein are presented in Table IV. Peptides whose compositions suggested that they were derived from the variable region of the protein were subjected to sequence analyses. Peptides whose compositions exactly matched those of λ-type constant region peptides were not sequenced, although in some instances exopeptidase digestions were employed to verify the locations of tryptophan residues and some of the amide containing residues. CPA digestion of peptide C-7A2 was also employed to demonstrate the presence of the C-terminal serine characteristic of λ-type chains (Milstein, 1965).

The discussion of the sequencing experiments will be initiated at the N terminus of the molecule even though in the actual experiments the locations of peptides which had been sequenced were first effected by arranging them according to compositional homologies with known λ-chain sequences. Eventual determinations of overlaps with other petides provided confirmation of these positionings. Although chymotryptic peptides were isolated which entailed almost all of the residues of the variable region, the sequences of tryptic and thermolytic peptides provided for most of the primary sequence of the variable region.

The following discussion will relate to the methods and results of the sequence experiments presented in Table IV.

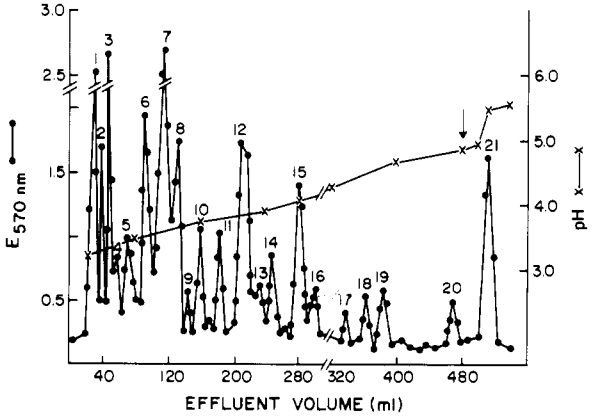


FIGURE 8: Chromatogram illustrating the separation of a chymotryptic digest of 70 mg of CAM-protein on a column of Dowex 50-X2 equilibrated with pH 3.1, 0.2 M pyridine–acetate buffer. The elution conditions were as described in Figure 5. 2-ml fractions were collected.

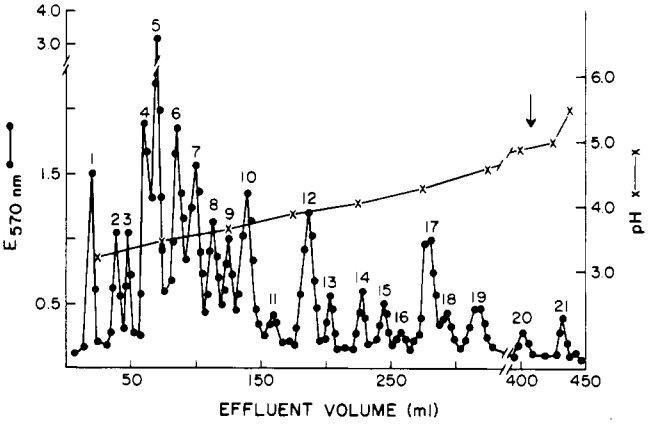


FIGURE 9: Chromatogram illustrating the separation of a thermolytic digest of 70 mg of CAM-protein on a column of Dowex 50-X2 equilibrated with pH 3.1, 0.2 M pyridine–acetate buffer. The elution conditions were as described in Figure 5: 2-ml fractions were collected.

TABLE III: Amino Acid Compositions[a] of Thermolytic Peptides of Mcg CAM-Protein Used in Sequence Experiments.

AA	TC-I-Th-1A	TC-I-Th-6N	Th'-1B	Th'-2B	Th-3N	Th-4A1	Th-4A2	Th-5A1
Asp			2.03 (2)					2.14 (2)
Thr		0.72 (1)		0.90 (1)	0.75 (1)	0.72 (1)	0.87 (1)	2.73 (3)
Ser	0.88 (1)	0.98 (1)	0.83 (1)	1.92 (2)	2.63 (3)	0.89 (1)	1.80 (2)	2.68 (3)
Glu	1.00 (1)	0.89 (1)		2.04 (2)	1.03 (1)	1.00 (1)	1.99 (2)	
Pro			1.64 (2)	0.83 (1)	1.42 (2)	0.85 (1)	1.52 (2)	
Gly		1.00 (1)	1.10 (1)		0.95 (1)			2.94 (3)
Ala	1.03 (1)				1.13 (1)	1.01 (1)		
Half-Cys						0.95 (1)[c]		1.10 (1)[c]
Val		1.03 (1)	1.83 (2)			0.99 (1)	0.73 (1)	2.10 (2)
Ile								1.00 (1)
Leu		0.77 (1)		0.92 (1)	1.00 (1)		0.80 (1)	
Tyr								1.75 (2)
Phe							1.00 (1)	
His				0.94 (1)				
Lys			1.00 (1)	1.00 (1)				
Arg			1.62 (2)	0.87 (1)				
No. of residues (yield)[d]	3 (9)	6 (9)	11 (33)	11[b] (39)	10 (41)	7 (69)	10 (21)	17 (52)
Sequence position	1–3	14–19	53–63	184–194	4–13	210–216	119–128	18–34
Charge (pH 6.5)	−1	0	+2	+1.5	0	−1	−2	−1

AA	Th-5A2	Th-6N	Th-7N1	Th-9N	Th-10A	Th-10N1	Th-10N2	Th-11A
Asp	3.92 (4)				1.00 (1)			
Thr		0.87 (1)			0.93 (1)			
Ser	2.48 (3)	0.98 (1)	1.13 (1)		1.07 (1)	1.67 (2)	1.22 (1)	
Glu	4.20 (4)		1.00 (1)				1.00 (1)	1.00 (1)
Pro					0.69 (1)			
Gly	1.00 (1)	0.97 (1)	0.92 (1)		1.19 (1)			
Ala	1.88 (2)				1.12 (1)	2.00 (2)		
Half-Cys	1.07 (1)[c]			1.00 (1)[c]			1.09 (1)[c]	
Val		1.08 (1)		0.87 (1)	0.85 (1)			
Ile					1.00 (1)			1.43 (2)
Leu	1.12 (1)	1.00 (1)	0.98 (1)	0.96 (1)	0.84 (1)			
Tyr	2.73 (3)				0.91 (1)	0.75 (1)	1.05 (1)	0.86 (1)
Phe	0.69 (1)				1.06 (1)			
His								
Lys								
Arg								
No. of residues (yield)[d]	20 (13)	5 (22)	4 (35)	3 (31)	11 (20)	5 (38)	4 (41)	4 (20)
Sequence position	80–99	75–79	14–17	136–138	139–149	176–180	195–198	49–52
Charge (pH 6.5)	−6	0	0	0	−1	0	0	−1

AA	Th-12B	Th-15B	Th-17A	Th-17B	Th-18B	Th-19N	Th-20B2
Asp	1.05 (1)	1.00 (1)				1.00 (1)	
Thr	0.76 (1)	0.87 (1)	2.63 (3)	2.80 (3)			
Ser		3.63 (4)	0.86 (1)			0.87 (1)	
Glu	1.04 (1)		1.87 (2)		1.87 (2)		
Pro	1.81 (2)					1.20 (1)	0.70 (1)
Gly	1.00 (1)	2.10 (2)	1.00 (1)	2.00 (2)		2.12 (2)	1.00 (1)
Ala	1.18 (1)	1.15 (1)				2.03 (2)	1.95 (2)
Half-Cys							
Val	0.99 (1)		2.02 (2)	2.12 (2)		1.01 (1)	0.92 (1)
Ile							
Leu	0.98 (1)						
Tyr					0.92 (1)		
Phe		1.19 (1)		1.01 (1)			
His			0.80 (1)		1.00 (1)		
Lys	0.79 (1)	1.00 (1)	0.80 (1)	0.98 (1)		1.12 (1)	1.72 (2)
Arg							
No. of residues (yield)[d]	10 (58)	11 (30)	11 (48)	9 (57)	4 (32)	9 (9)	7 (42)
Sequence position	109–118	64–74	199–209	100–108	38–41	154–162	42–48
Charge (pH 6.5)	+1	+1	−0.5	+1	+0.5	0	+2

[a–d] See footnotes *a*, *c*, *d*, and *e* of Table I.

As previously discussed, the thermolytic and chymotryptic cleavages of TC peptide TC-I yielded two ninhydrin-negative peptides, TC-I-Th-1A and TC-I-C-1A, respectively. Both were shown to contain N-terminal PCA and to derive from the N terminus of the molecule. CPA sequentially released leucine, alanine, and serine from TC-I-C-1A which indicated that the terminal sequence was PCA-Ser-Ala-Leu.

A thermolytic decapeptide, Th-3N, was shown to overlap TC-I-C-1A and to extend from residues 4 to 13.[3] Both acid and alkaline hydrolyses of the thiazolinone derivatives were carried

[3] All residue positions refer to those of the intact Mcg protein and not to the positions of residues of the particular peptide under discussion.

out in order to detect the threonine and serine residues. The charge properties of this peptide indicated that the glutamic acid residue was in the amide form.

Pentadecapeptide TC-I-C-2N was found to overlap residues 13–14 and to extend from positions 5 to 19. No amino acid was detected after alkaline hydrolysis of the thiazolinones derived from Edman cycles 5, 7, and 9, but amino acid composition data indicated that serine residues were present at these positions. The absence of charge at pH 6.5 indicated that both glutamic acid residues of the acid hydrolysate existed in their amide form. The products of CPA digestion of peptide Th-7N1 established the Gln-Ser sequence at positions 16–17. Peptide TC-I-Th-6N, which overlapped TC-I-C-2N and Th-7N1, established positions 18–19 as being Val-Thr.

The above data and the composition of the large docosapeptide T-1N-AE indicated that the latter extended from the N-terminal PCA residue to the invariant half-cystine at position 22. The C-terminal *S*-aminoethylcysteine residue of this peptide was released by treatment with CPB. Further digestion with CPA released an additional four residues.

The peptide Th-5A1 overlapped the half-cystine at position 22 and was found to comprise residues 18–34. The complete sequence of this peptide was obtained by performing 15 cycles of Edman degradation and by digestion with CPA. The assignment of serine residues to positions 26 and 27 was based on amino acid composition data. CPA released tyrosine and asparagine in its initial action. Since peptide Th-5A1 possessed one negative charge at pH 6.5, the CPA result indicated that the residue at position 28 was aspartic acid. The sequence data obtained for peptides T-14B2-AE and TC-I-C-12N firmly established the position at 33 as being asparagine. The presence of aspartic acid at position 28 was also confirmed by LAP digestion of peptide TC-I-C-9A.

Peptide T-14B2-AE was found to overlap peptides Th-5A1, TC-I-C-9A, and TC-I-C-12N and to extend from positions 23 to 44. The location of tryptophan at position 37 was established by digestion of peptide TC-I-C-12N with CPA. The peptide Th-18B overlapped positions 38–39. The results of CPA and LAP digestions of it and its slightly basic properties at pH 6.5 indicated that glutamine residues were present at positions 39 and 40.

Peptide TC-I-C-13B overlapped Th-18B. It was inert to Edman degradation suggesting that cyclization of the glutamine residue at position 39 had occurred. This must have occurred during elution and subsequent manipulation of the peptide after its separation by paper electrophoresis, since its mobility at pH 6.5 indicated a charge of +1.5.

Peptide Th-20B2, which was eventually found to extend from positions 42 to 48, was difficult to sequence by the manual Edman technique because of marked losses due to the solubility of its phenylthiocarbamyl derivative during the diethyl ether extraction of the thiazolinones. When it was converted to the 4-sulfophenyl isothiocyanate form the five N-terminal amino acids could be readily identified by the Edman procedure.

The thermolytic tetrapeptide Th-11A was found to extend from residues 49 to 52 and by virtue of its anodic charge at pH 6.5 to contain glutamic acid at position 52. The chymotryptic tridecapeptide C-21B was found to overlap peptides Th-18B, TC-I-C-13B, Th-20B2, and Th-11A and to extend to position 51. C-21B appeared to initiate at the same position as TC-I-C-13B since it was also inert to Edman degradation due to the cyclization of the glutamine residue at position 39. In this case, however, cyclization must have occurred prior to the purification by paper electrophoresis since at pH 6.5 it possessed a charge of +1.5.

Three peptides, C′-1B, Th′-1B, and T-13B, were found to overlap the region of 52–64. The undecapeptide, Th′-1B, possessed a charge of +2 at pH 6.5 and contained two arginine, a lysine, and two aspartic acid residues. This indicated that one of the aspartic acid residues was present in its amide form. LAP digestion of it demonstrated a Val-Asn N-terminal sequence which indicated that the peptide originated at position 53 with the asparagine residue occupying position 54. Failure to detect an amino acid in the sixth Edman cycle following alkaline hydrolysis along with the composition data for peptide Th′-1B indicated that a serine residue occupied position 58.

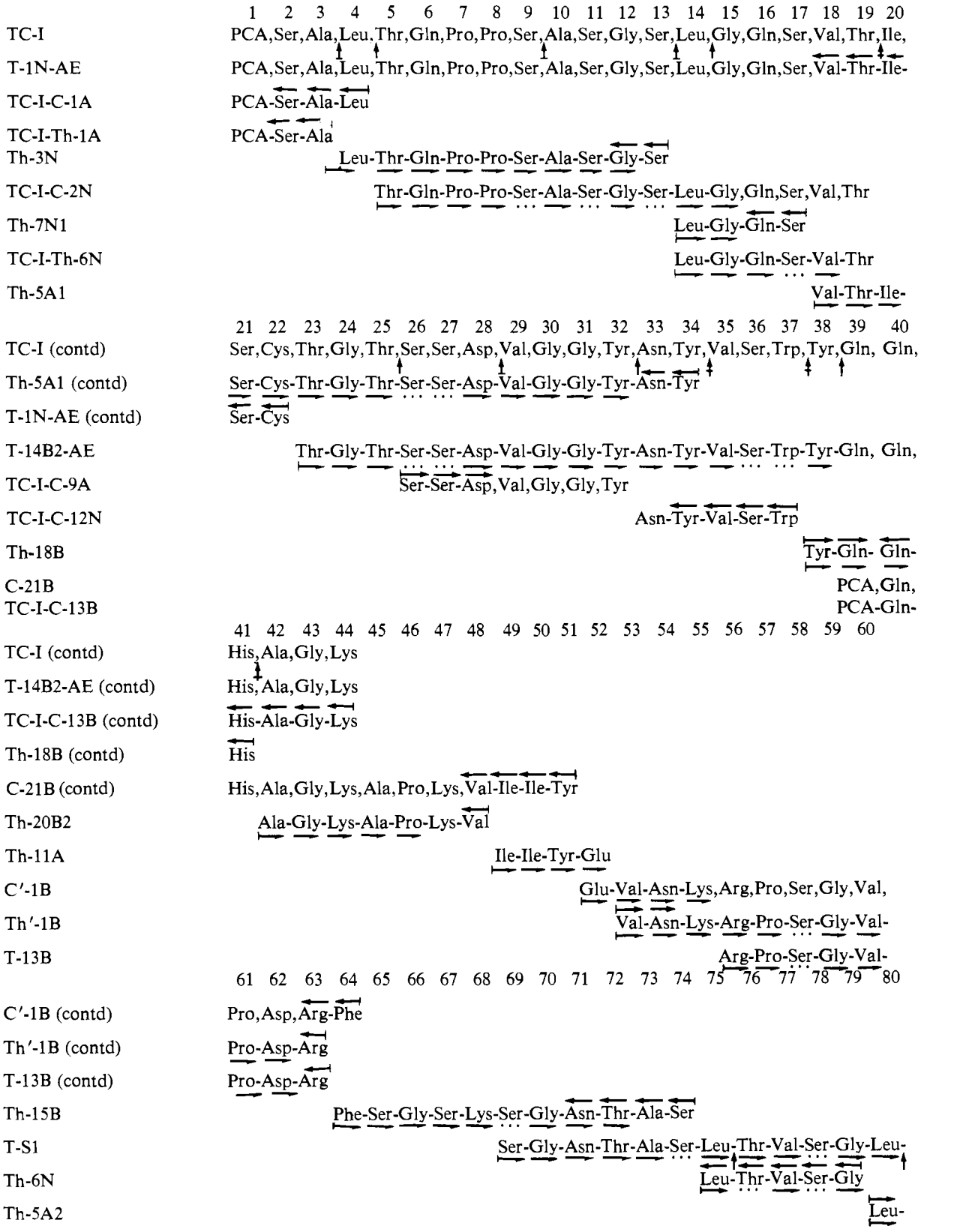
The thermolytic undecapeptide Th-15B was found to extend from positions 64 to 74. It was subjected to nine cycles of Edman degradation and was digested with CPA. No amino acid was detected for Edman cycle six after either acid or alkaline hydrolysis of the thiazolinone derivatives, but compositional data indicated that position 69 was occupied by a serine residue. The peptide charge at pH 6.5 indicated that residue 71 was present as asparagine. This was confirmed by digestion with CPA.

The large tryptic peptide T-S1 was found to extend from positions 69 to 106. Thirteen cycles of Edman degradation were performed. The sequences obtained for peptides overlapped by T-S1 were useful in establishing the overall sequence of it. The pentapeptide Th-6N was found to extend from positions 75 to 79. The thermolytic peptide Th-5A2 was subjected to 19 cycles of Edman degradation and was found to contain residues 80–99. No detectable amino acids were found for this peptide for Edman cycles 12, 13, and 17 following alkaline hydrolysis of the thiazolinone derivatives. Its amino acid composition indicated that these positions were occupied by serine. In order to locate amide residues peptide Th-5A2 was also subjected to ten cycles of automated Edman degradation utilizing a Beckman Sequencer 890C. The resulting thiazolinones were converted to their corresponding Pth derivatives and identified by silica gel thin-layer chromatography utilizing a heptane–*n*-butyl alcohol–formic acid solvent system (Jeppsson and Sjoquist, 1967). This resulted in establishing glutamine at position 81, glutamic acid at positions 83 and 85, and aspartic acid at positions 84 and 87. The sequence of the first four amino acids in this peptide was also confirmed by digestion with LAP. The determination of whether amide forms of the acids were present at positions 94, 97, or 98 was eventually elucidated by the sequence studies on the hexadecapeptide T-10A-AE. Its complete sequence was determined by means of digestion employing LAP, CPA, and CPB and by manual and automated Edman degradations. The thiazolinones resulting from automated sequencing were again converted to their Pth derivatives followed by direct chromatographic identification. Serine residues were found at positions 91, 92, and 96 and glutamic acid, aspartic acid, and asparagine residues at positions 94, 97, and 98, respectively. These studies established the sequence up to position 107.

The nonapeptide Th-17B, which was completely sequenced, was found to span positions 100–108 and thus extended two residues beyond peptide T-10A-AE. Assignment of the threonine residue at position 105 was based on amino acid composition. The presence of the threonine at position 105 was also confirmed from the result of sequencing studies on peptide T-S1-C-9B. The sequences of peptides Th-17B and T-S1-C-9B are of interest since all other λ-type proteins have been found to possess a -Gly-Gly-Gly- sequence at positions homologous to the 102–104 -Gly-Thr-Gly- sequence of Mcg.

Peptide T-5B was found to initiate at position 107 and thus

TABLE IV: Sequence of Tryptic, Chymotryptic, and Thermolytic Peptides Establishing the Primary Structure of Mcg λ Chain.[a]



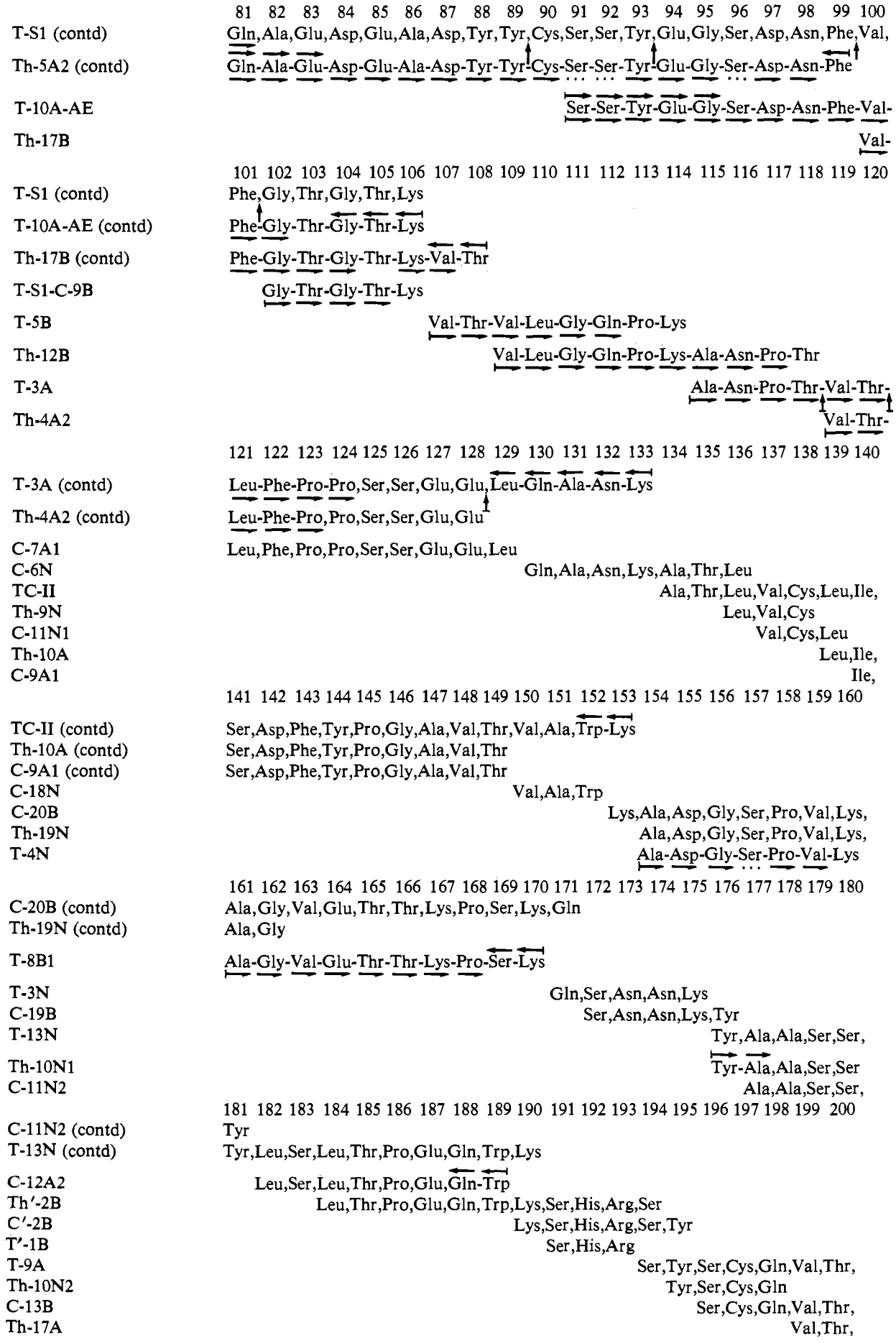

81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
T-S1 (contd) Gln,Ala,Glu,Asp,Glu,Ala,Asp,Tyr,Tyr,Cys,Ser,Ser,Tyr,Glu,Gly,Ser,Asp,Asn,Phe,Val,
Th-5A2 (contd) Gln-Ala-Glu-Asp-Glu-Ala-Asp-Tyr-Tyr-Cys-Ser-Ser-Tyr-Glu-Gly-Ser-Asp-Asn-Phe
T-10A-AE Ser-Ser-Tyr-Glu-Gly-Ser-Asp-Asn-Phe-Val-
Th-17B Val-
101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
T-S1 (contd) Phe,Gly,Thr,Gly,Thr,Lys
T-10A-AE (contd) Phe-Gly-Thr-Gly-Thr-Lys
Th-17B (contd) Phe-Gly-Thr-Gly-Thr-Lys-Val-Thr
T-S1-C-9B Gly-Thr-Gly-Thr-Lys
T-5B Val-Thr-Val-Leu-Gly-Gln-Pro-Lys
Th-12B Val-Leu-Gly-Gln-Pro-Lys-Ala-Asn-Pro-Thr
T-3A Ala-Asn-Pro-Thr-Val-Thr-
Th-4A2 Val-Thr-
121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140
T-3A (contd) Leu-Phe-Pro-Pro,Ser,Ser,Glu,Glu,Leu-Gln-Ala-Asn-Lys
Th-4A2 (contd) Leu-Phe-Pro,Pro,Ser,Ser,Glu,Glu
C-7A1 Leu,Phe,Pro,Pro,Ser,Ser,Glu,Glu,Leu
C-6N Gln,Ala,Asn,Lys,Ala,Thr,Leu
TC-II Ala,Thr,Leu,Val,Cys,Leu,Ile,
Th-9N Leu,Val,Cys
C-11N1 Val,Cys,Leu
Th-10A Leu,Ile,
C-9A1 Ile,
141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160
TC-II (contd) Ser,Asp,Phe,Tyr,Pro,Gly,Ala,Val,Thr,Val,Ala,Trp-Lys
Th-10A (contd) Ser,Asp,Phe,Tyr,Pro,Gly,Ala,Val,Thr
C-9A1 (contd) Ser,Asp,Phe,Tyr,Pro,Gly,Ala,Val,Thr
C-18N Val,Ala,Trp
C-20B Lys,Ala,Asp,Gly,Ser,Pro,Val,Lys,
Th-19N Ala,Asp,Gly,Ser,Pro,Val,Lys,
T-4N Ala-Asp-Gly-Ser-Pro-Val-Lys
161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
C-20B (contd) Ala,Gly,Val,Glu,Thr,Thr,Lys,Pro,Ser,Lys,Gln
Th-19N (contd) Ala,Gly
T-8B1 Ala-Gly-Val-Glu-Thr-Thr-Lys-Pro-Ser-Lys
T-3N Gln,Ser,Asn,Asn,Lys
C-19B Ser,Asn,Asn,Lys,Tyr
T-13N Tyr,Ala,Ala,Ser,Ser,
Th-10N1 Tyr-Ala,Ala,Ser,Ser
C-11N2 Ala,Ala,Ser,Ser,
181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200
C-11N2 (contd) Tyr
T-13N (contd) Tyr,Leu,Ser,Leu,Thr,Pro,Glu,Gln,Trp,Lys
C-12A2 Leu,Ser,Leu,Thr,Pro,Glu,Gln-Trp
Th′-2B Leu,Thr,Pro,Glu,Gln,Trp,Lys,Ser,His,Arg,Ser
C′-2B Lys,Ser,His,Arg,Ser,Tyr
T′-1B Ser,His,Arg
T-9A Ser,Tyr,Ser,Cys,Gln,Val,Thr,
Th-10N2 Tyr,Ser,Cys,Gln
C-13B Ser,Cys,Gln,Val,Thr,
Th-17A Val,Thr,

TABLE IV (*Continued*)

	201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216
Th-17A (contd)	His,Glu,Gly,Ser,Thr,Val,Glu,Lys,Thr
T-9A (contd)	His,Glu,Gly,Ser,Thr,Val,Glu-Lys
C-13B (contd)	His
C-7A2	Glu,Gly,Ser,Thr,Val,Glu,Lys,Thr,Val,Ala,Pro,Thr,Glu,Cys,Ser
C-2A1	Thr,Val,Ala,Pro,Thr,Glu,Cys,Ser
Th-4A1	Val,Ala,Pro,Thr,Glu,Cys,Ser

[a] A hyphen between any two amino acids indicates that the sequence was established; other residues are separated by commas. Horizontally placed arrows above an amino acid pointing right indicate degradation by leucine aminopeptidase; those directed left indicate degradation by carboxypeptidase. Half-arrows below the lines (all pointing to the right) show degradations by the Edman method. All arrows showing the initiation point of a degradation carry a bar at their distal end. Secondary points of cleavage are shown by vertical arrows below a given peptide as follows: a simple arrow, chymotrypsin; an arrow with a bar at its distal end, thermolysin; an arrow with a bar at its midpoint, both chymotrypsin and thermolysin.

overlap Th-17B. This peptide bridges the variable and constant regions of Mcg, the latter being initiated at position 112 in the numbering system employed. Peptide T-5B was found to extend to position 114 and to contain a glutamine residue since the peptide is a cation at pH 6.5.

The sequence of the decapeptide Th-12B, which overlapped T-5B, was determined by performing nine cycles of Edman degradation and was found to represent residues 109–118. The cationic charge of Th-12B at pH 6.5 indicated that both of the residues at positions 112 and 116 were present as amides. The sequence of this peptide which reveals the presence of asparagine at position 116 and threonine at 118 demonstrates two new constant region substitutions hitherto undetected.

The composition of peptide T-3A also suggested a two amino acid substitution in this portion of the constant region. The partial sequence of the nonadecapeptide was performed and the results likewise confirmed these two substitutions. Treatment of peptide T-3A with thermolysin generates a tetrapeptide of net charge 0 at pH 6.5 containing these two new residues.

The compositions of various chymotryptic, thermolytic, and tryptic peptides covering the region from residue 119 to 153 indicated that this portion of Mcg had a sequence identical with that of other λ-type proteins. The presence of tryptophan at position 152 was confirmed by subjecting TC-II to digestion with a mixture of CPB and CPA.

In order to determine the Kern isotypic specificity of the Mcg protein, which is defined by a Gly-Ser interchange at position 156 (Mcg numbering) (Ponstingl and Hilschmann, 1969; Hess *et al.*, 1971), the tryptic heptapeptide T-4N which was found to extend from positions 154 to 160 was studied in detail. Its compositional data indicated that Mcg was a Kern(+) protein since it contained one glycine and only one serine residue. Six cycles of Edman degradation confirmed that position 156 was occupied by the glycine residue which is characteristic of the Kern(+) specificity.

Our attention was directed to the tryptic decapeptide T-8B1 for two reasons. Its amino acid composition indicated its homology with the constant region sequence 161–170, but it differed from the expected in possessing an additional lysine residue while lacking a threonine. The cationic properties of T-8B1 at pH 6.5 were also inconsistent with the usual λ-type peptide of this region which should be neutral. Since it was a tryptic peptide containing two lysine residues, it was most logical to postulate a Lys-Pro sequence at positions 167–168. The peptide was subjected to Edman degradation and to digestion with a mixture of CPA and CPB. Initial Edman degradations were accompanied by large losses apparently due to extraction of the hydrophobic phenylthiocarbamylated peptide. To circumvent this the peptide was allowed to react with an excess of 4-sulfophenyl isothiocyanate prior to the first coupling step. This derivative could be sequenced in good yields for eight cycles. The postulated Lys-Pro sequence at 167–168 was confirmed. Thus, in addition to the constant region amino acid substitutions at positions 116 and 118, the Mcg protein also shows the substitution of a threonine by a lysine residue at position 167.

Amino acid compositions of chymotryptic, thermolytic, and tryptic peptides which spanned the region 171–216 indicated that the C-terminal 46 residues of the molecule were identical with the typical λ-chain constant region sequence for this portion of the protein. The location of the tryptophan residue at position 189 was confirmed by subjecting peptide C-12A2 to digestion with CPA.

The presence of an arginine residue at position 193 which is implied from the amino acid composition of the peptide T′-1B indicates that the Mcg L chain possesses an Oz(−) isotypic specificity (Ein and Fahey, 1967; Ein, 1968).

The release of serine from peptide C-7A2 with CPA is characteristic of λ chains.

The complete primary sequence of Mcg protein is presented in Figure 10. The amino acid composition of Mcg L chain as determined by sequence studies agrees well with that determined for the methylsulfonic acid hydrolysis products. Values for serine, threonine, and isoleucine were found to be slightly low, most likely due to destructive losses and to incomplete release of β-branched amino acids during hydrolysis. Trytophan was recovered in 93% of the theoretical amount.

Discussion

The primary sequence of Mcg L chain is permitting a direct determination of the relation of its amino acid side chains to the binding of a series of small molecules in three sites of the variable region of the disulfide linked dimer (Edmundson *et al.*, 1974). The locations of these binding regions as determined by crystallographic techniques are being related to both the sequence and the three-dimensional structure.

It is surprising that the substitutions noted for Mcg L chain at positions 116, 118, and 167 in the constant region have previously escaped detection. However, with the asparagine for alanine at 116 and threonine for serine at 118 substitutions, the

20
PCA-Ser-Ala-Leu-Thr-Gln-Pro-Pro-Ser-Ala-Ser-Gly-Ser-Leu-Gly-Gln-Ser-Val-Thr-Ile-Ser-Cys-Thr-Gly-Thr-Ser-Ser-Asp-Val-Gly-Gly-Tyr-
40 60
Asn-Tyr-Val-Ser-Trp-Tyr-Gln-Gln-His-Ala-Gly-Lys-Ala-Pro-Lys-Val-Ile-Ile-Tyr-Glu-Val-Asn-Lys-Arg-Pro-Ser-Gly-Val-Pro-Asp-Arg-Phe-
80
Ser-Gly-Ser-Lys-Ser-Gly-Asn-Thr-Ala-Ser-Leu-Thr-Val-Ser-Gly-Leu-Gln-Ala-Glu-Asp-Glu-Ala-Asp-Tyr-Tyr-Cys-Ser-Ser-Tyr-Glu-Gly-
100 120
Ser-Asp-Asn-Phe-Val-Phe-Gly-Thr-Gly-Thr-Lys-Val-Thr-Val-Leu-Gly-Gln-Pro-Lys-Ala-Asn-Pro-Thr-Val-Thr-Leu-Phe-Pro-Pro-Ser-Ser-
140
Glu-Glu-Leu-Gln-Ala-Asn-Lys-Ala-Thr-Leu-Val-Cys-Leu-Ile-Ser-Asp-Phe-Tyr-Pro-Gly-Ala-Val-Thr-Val-Ala-Trp-Lys-Ala-Asp-Gly-Ser-
160 180
Pro-Val-Lys-Ala-Gly-Val-Glu-Thr-Thr-Lys-Pro-Ser-Lys-Gln-Ser-Asn-Asn-Lys-Tyr-Ala-Ala-Ser-Ser-Tyr-Leu-Ser-Leu-Thr-Pro-Glu-Gln-
200 216
Trp-Lys-Ser-His-Arg-Ser-Tyr-Ser-Cys-Gln-Val-Thr-His-Glu-Gly-Ser-Thr-Val-Glu-Lys-Thr-Val-Ala-Pro-Thr-Glu-Cys-Ser

FIGURE 10: The complete primary structure of Mcg λ chain.

peptides from this portion of the molecule will show little change in chromatographic and electrophoretic properties. The lysine for threonine substitution at 167 induces a readily noted charge difference. Investigators generally accept reported constant region sequences except for the well-known Oz and Kern substitutions and this probably relates to the lack of detailed study of the constant region of different λ-chain proteins. Substitutions in the constant region of both κ- and λ-type chains other than those responsible for the Inv (Hilschmann and Craig, 1965; Terry *et al.*, 1969), Oz (Ein and Fahey, 1967; Ein, 1968), and Kern (Ponstingl and Hilschmann, 1969; Hess *et al.*, 1971) genetic differences have been noted (Milstein, 1967; Milstein *et al.*, 1967). Steinberg *et al.* (1974) have recently also found that the substitution of alanine or valine at position 153 of κ chains relates to the well-known leucine and valine exchanges at 191 in establishing the three Inv allotypes. Thus, a larger number of constant region variations than generally assumed may actually exist. The presence of additional substitutions in this region of L chains will increase the total number of possible antibody molecules unless there is some linkage between these substitutions and the numerous different variable region sequences.

The Mcg L-chain sequence demonstrates homologies to both λ_I- and λ_{IV}-type proteins. For the first 112 residues (V region) Mcg shows that 81% of its residues are identical with both protein Vil (Ponstingl and Hilschmann, 1971), a λ_I type, and with protein Bo (Wikler and Putnam, 1970), a λ_{IV} type. Comparisions with two λ_{IV}- and seven λ_I-type proteins for the first ~20 N-terminal residues (Smith, 1973) indicate that Mcg shows greater homology with the λ_{IV} subgroup.

We have instituted investigations of other λ chains derived from Bence-Jones, meyloma IgG and IgA sources, Waldenstroms IgM proteins, and normal human IgG in order to determine the relationships of the three new Mcg substitutions to the Kern and Oz variations, to see if there is any significant tendency toward occurrence with a given class of immunoglobulin, and to determine whether these substitutions will prove to be allelic or isotypic. Previous studies have reported that three of the four possible Kern-Oz combinations exist but no Kern(+) and Oz(+) λ chain has as yet been detected. Five substitutions in the constant region would permit a total of 32 different isomers. The possibility of some or all of these substitutions being linked must also be considered. Investigations of this type require studies of the λ chains of both the normal IgG fraction of single individuals and of monoclonal IgG's. To date five other λ chains possessing the substitutions at 116, 118, and 167 and the Oz(−) and Kern(+) specificities have been found. The preliminary results thus indicate that the Mcg L chain is a product of a specific λ-chain gene. Extensions of this work which will include studies of normal IgG will permit elucidation of the frequency of occurrence of this protein.

Another facet of these studies will be to determine if the above noted λ-chain constant region substitutions will bear any relationship to the C_1 region of the H chain with which it interacts. Extensional studies of this type have been initiated and it is hoped that they will not only provide answers to the above questions but also expose other areas related to the structural aspects of the constant region of L chains.

Acknowledgments

The authors have benefited from discussions with Dr. Allen Edmundson and Dr. K. D. Lin during the course of this investigation. They also wish to thank Mrs. Patricia Moore for performing the amino acid analyses and automated Edman degradations.

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Phospholipid Metabolism in the Eggs and Embryos of the Sea Urchin *Arbacia punctulata*†

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ABSTRACT: The incorporation of labeled phospholipid precursors into the phospholipids of *Arbacia punctulata* eggs and of embryos prior to the first cell cleavage has been investigated. Incorporation of [^{3}H]choline into phosphatidylcholine was not detected in either eggs or embryos although fertilization resulted in a fourfold stimulation of [^{3}H]choline uptake into the cells. Both eggs and embryos, when incubated with [^{3}H]ethanolamine, incorporated radioactivity into phosphatidylethanolamine.The incorporation by unfertilized eggs was three to four times greater than that observed in embryos. However, when eggs were preincubated with [^{3}H]ethanolamine and subsequently fertilized, the resultant preloaded eggs and embryos behaved identically in the incorporation of ethanolamine into phosphatidylethanolamine. In contrast, when $^{32}P_i$ or [^{3}H]inositol was used as a lipid precursor, embryos were markedly more active than eggs in incorporation of these compounds into phospholipids. With both precursors the major reason for the marked difference in incorporation between eggs and embryos was related to an increased ability of the embryos to transport the labeled precursor. Incorporation of $^{32}P_i$ into phospholipids was highly specific in both eggs and embryos. Ninety-five per cent of the $^{32}P_i$ incorporated into phospholipid was found in the phosphoinositides: phosphatidylinositol, diphosphoinositide, and triphosphoinositide. The most highly labeled lipid was diphosphoinositide. When eggs and embryos were incubated in [^{3}H]inositol, radioactivity was incorporated into the three phosphoinositides. Although in both cases the major inositol-labeled lipid was phosphatidylinositol, when eggs were preloaded with [^{3}H]inositol and then fertilized, an increase in the proportion of label in the polyphosphoinositides was detected in the resultant embryos, when compared to the unfertilized eggs.

In recent years a wealth of information has accumulated on the many changes that occur in the eggs of the sea urchin upon fertilization (reviewed by Giudice, 1973). In general terms, these changes may be classified into two categories. One category includes changes in metabolic activities within the cytoplasm, such as an increase in respiration and a markedly enhanced rate of protein synthesis. The other category encompasses changes that are likely to be related to alterations in the properties of the plasma membrane of the egg. These changes include: (a) a marked change in the membrane potential; (b) extrusion of H^+; (c) uptake of Ca^{2+}; (d) activation of a Na^+-dependent transport system for several amino acids; (e) development of a K^+-dependent membrane potential; and (f) activation of a transport system for inorganic phosphate (reviewed by Giudice, 1973; Epel *et al.*, 1969).

A unique aspect of a number of these membrane-associated changes is that once the alteration has been triggered by fertilization it remains a permanent cell property. For example, P_i uptake is markedly enhanced in embryos and is linear throughout several cell cleavages (Brooks and Chambers, 1954). Amino acid and nucleoside transport behave similarly, at least until the first cell cleavage (Giudice, 1973). Thus, the plasma membrane of the embryo is locked in an active form, whereas the plasma membrane of the egg remains in an inactive state. This unique situation should prove convenient for studying the chemical and enzymatic differences in the membrane that may be brought about by fertilization of the egg cell.

As a prelude to such a comparative study of the two membranes, we have studied the incorporation of a variety of lipid precursors into membrane phospholipids of the egg and the embryo prior to the first cleavage. These studies revealed marked differences in the efficacy of incorporation of different lipid precursors into egg and embryo lipids. In addition, a pro-

† From the Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205. *Received April 17, 1974.* This work was supported by U. S. Public Health Service Grant A1-06888-09 and a grant from the Rockefeller Foundation. E. S. is a Public Health Service Trainee supported by Training Grant GM-00184-15.