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Primary Structure of Human J Chain: Isolation and Characterization of Tryptic and Chymotryptic Peptides of Human J Chain†

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ABSTRACT: Human J chain isolated from the plasma of a patient with Waldenstrom's macroglobulinemia was subjected to extended and limited digestion with trypsin and chymotrypsin. The digests were fractionated by combination of column chromatography and high voltage paper electrophoresis. Peptide purity was established by their amino acid analysis and a single amino terminal residue. All the necessary peptides which would provide the total primary structure of molecule were thus obtained.

Human J chain, a serum protein with a molecular weight of 15 600, is generally found to be covalently associated with polymeric immunoglobulins (Halpern and Koshland, 1970; Mestecky et al., 1971). The presence of a similar polypeptide in several animal species, distributed over the phylogenetic scale (Weinheimer et al., 1971; Kobayashi et al., 1973; Meinke and Spiegelberg, 1973; Klaus et al., 1971), suggests that it has an important biological role. Although the available data tend to substantiate the view that J chain plays some role in the polymerization of IgM and IgA, other biological functions have also been attributed to it (Brandtzaeg, 1976).

J chains, whether obtained from IgM or IgA molecules, are found to have similar amino acid compositions, peptide map patterns, immunological properties and other physicochemical characteristics. Elucidation of the complete primary structure of human J chain has been undertaken in order to provide a chemical basis for understanding its molecular organization in immunoglobulins, its genetic expression, and its appearance in the evolution of higher organisms. From this lab has previously been reported the sequence of the C-terminal 13 residues (Mole et al., 1974), the partial amino acid sequence of peptides obtained from limited acid hydrolysis of J chain (Bhown et al., 1976; Mole et al., 1977), and the sequence of N-terminal 17 residues (Mole et al., 1976). The present communication deals with the isolation, purification, and characterization of peptides obtained from enzymatic digestion of human J chain.

Materials and Methods

TPCK[1]-trypsin was purchased from Worthington Biochemical Corp. (Freehold, N.J.). TLCK-chymotrypsin was prepared as follows: chymotrypsin (three times crystallized, Worthington Biochemical) was incubated for 20 min at room temperature with TLCK (Sigma) dissolved in 0.2 M phosphate buffer (pH 7.0). The final concentrations were 6.66×10^{-5} M for chymotrypsin and 2.55×10^{-5} M TLCK. Dansyl chloride was obtained from Pierce Chemicals (Rockford, Ill.). Bio-Gel P-10 (200–400 mesh) and ion-exchange celluloses were purchased from Bio-Rad Labs (Richmond, Calif.) and Whatman Chemicals, Ltd. (Maidstone, Ky.), respectively. Analytical grade chemicals were obtained from Fisher Scientific Co. (Fair Lawn, N.J.) and were used without further purification. Fluram was the product of Hoffmann-La Roche (Nutley, N.J.).

Preparation of Human J Chain. S-Carboxyamidomethylated J chain was isolated from the plasma of a patient with Waldenstrom's macroglobulinemia as described previously (Mole et al., 1974).

Modification of Lysine Residues. S-Carboxyamidomethylated J chain was modified at lysine residues by reaction with citraconic anhydride in order to restrict the trypsin cleavage site to arginine residues only. The reaction was carried out in 0.2 M $NaHCO_3$ (pH 8.2) using a 25-fold excess of citraconic anhydride (Gibbons and Perham, 1970). The reaction mixture was dialyzed against 1% NH_4HCO_3 and lyophilized.

Enzymatic Cleavage with Trypsin. Citraconyl J chain was solubilized in 0.2 M NH_4HCO_3 and digested with TPCK-trypsin at 37 °C (i) using an enzyme:protein ratio of 1:50 for a total of 6 h, and (ii) using a 1:200 enzyme:protein ratio for 1 h. The latter will be referred to as limited trypsin digestion. The digests in either case were immediately subjected to column fractionation.

Unblocking of Citraconyl Peptides. The citraconyl peptides

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[1]Abbreviations used: Pth, phenylthiohydantoin; DEAE, diethylaminoethyl; CM, carboxymethyl; TPCK, 1-chloro-4-phenyl-3-tosylamido-2-butanone; TLCK, 1-chloro-3-tosylamido-7-amino-2-heptanone; dansyl chloride, 1-dimethylaminonaphthalene-5-sulfonyl chloride.

were deblocked by incubation at 40–42 °C in 0.2 M acetic acid for 5 h, as described by Habeeb and Atassi (1970).

Enzymatic Cleavage with Chymotrypsin. Modified human J chain solubilized in 0.2 M NH_4HCO_3 was subjected to cleavage with TLCK-chymotrypsin at 37 °C using an enzyme:protein ratio of (i) 1:50 for 3 h and (ii) 1:250 for 15 min; the latter is referred to as limited chymotryptic digestion. The digests in both cases were subjected to fractionation immediately.

Fractionation and Purification of Peptides. Initial fractionation of peptides obtained by enzymatic cleavage of human J chain was achieved on two or three P-10 (Bio-Rad) columns attached in tandem, equilibrated with 1% NH_4HCO_3. Effluents were monitored both by absorption at 280 nm and/or by reaction with fluorescamine and fluorometric quantitation (Underfriend et al., 1972). Pooled fractions obtained from tryptic digestion were deblocked and lyophilized.

Electrophoresis on paper in pyridine–acetate buffer (pH 3.5) at 3000 V was adopted as an initial check for peptide purity. Further purification was achieved by ion-exchange chromatography on DEAE and/or CM-cellulose supports. Buffers which could be volatilized, such as NH_4HCO_3 or CH_3COONH_4, were employed using the following gradient:

gradient I: 0.025 M to 0.5 M NH_4HCO_3

gradient II: 0.025 M to 0.5 M CH_3COONH_4 (pH 4.5)

Purity of peptides was established by amino acid analysis and the presence of a single amino terminus following reaction with dansyl chloride.

Amino Acid Analysis. analyses were performed on samples hydrolyzed with constant boiling HCl, at 110 °C for 20 h using the Durrum D-500 amino acid analyzer (Benson, 1972).

N-Terminal Amino Acid Identification. Amino-terminal residues were determined by reacting peptides with dansyl chloride (Gray and Hartley, 1963) followed by thin-layer chromatography on polyamide sheets (Woods and Wang, 1967). In view of the fact that dansylproline is destroyed during 18 h of hydrolysis (Gray, 1967), dansylated samples were hydrolyzed for 4 and 18 h. Amino-terminal residues were also identified as the corresponding Pth-amino acid while performing subtractive Edman degradation.

Peptide Nomenclature. Peptides obtained from the trypsin digest of citraconylated J chain are designated by "T" and those obtained after limited trypsinization as "LTP". Letters "C" and "CH" are prefixed to designate those peptides obtained by chymotrypsin and limited chymotrypsin digestion, respectively. Each peptide in turn is then numbered in order of their elution from P-10 and ion-exchange columns. These numbers in no way denote their order or relative position in the J chain molecule.

Until ready for sequencing, all the peptides were kept lyophilized and stored at −20 °C.

Results

Isolation and Characterization of Tryptic Peptides. Peptides resulting from trypsin digestion of reduced and alkylated citraconyl J chain (160 mg) were chromatographed on two P-10 (Bio-Rad 200–400 mesh, 2.0 × 112 cm) columns attached in tandem. The elution profile is shown in Figure 1. Each fraction was examined for purity by high-voltage paper electrophoresis at pH 3.5 and was subsequently subjected to further purification by ion-exchange chromatography as summarized in Figure 2. The amino acid composition, number of residues, and the percent yield of each of the pure tryptic

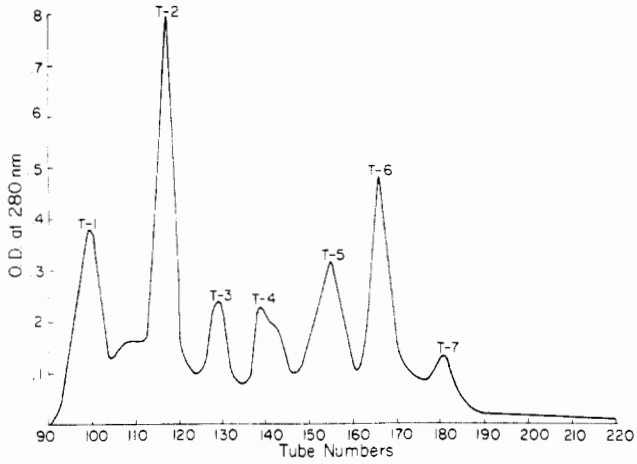

FIGURE 1: Gel filtration of tryptic digest of citraconylated J chain (160 mg) on two P-10 columns (Bio-Rad, 200–400 mesh, 2.0 × 112 cm) in 1% NH_4HCO_3 attached in tandem. Fractions (3.5 mL) were collected at a flow rate of 12 mL/h.

peptides are summarized in Table I. Treatment of all the seven fractions T-1 through T-7 from P-10 column is discussed below.

Fraction T-1. This fraction was chromatographed on a CM-cellulose column (1.5 × 42 cm) developed at 30 mL/h in 0.025 M CH_3COONH_4, pH 4.5, with gradient II. Two pure peptides, T-1-3 and T-1-5, were obtained in low yields but on amino acid analysis gave an integral value for each amino acid and showed a single amino-terminal residue. T-1-4, a tripeptide, isolated in high yield was found to have one residue of phenylalanine, and thus accounted for the single residue of that amino acid present in the whole molecule.

Fraction T-2. Chromatography of fraction T-2 on DEAE-cellulose column (1.5 × 55 cm) with gradient I resulted in four fractions T-2-1 through T-2-4. All except peptide T-2-1 were further chromatographed as summarized in Figure 2. T-2-1 on amino acid analysis was found to be an 11-residue glycopeptide isolated in good yield. T-2-2 was rechromatographed on a CM-cellulose column (1.5 × 40 cm), developed with gradient II (Figure 3a). Two pure peptides T-2-2-1 and T-2-2-2 were isolated and characterized (Table I). T-2-2-1 was a glycopeptide with an amino acid composition similar to that of T-2-1. T-2-2-2 was found to contain the only two glycine residues present in the molecule. The presence of lysine but no arginine in this peptide suggests cleavage due to incomplete blocking. Refractionation of T-2-3 on CM-cellulose column (1.5 × 40 cm), with a gradient of 0.05 M to 0.5 M CH_3COONH_4, pH 3.8, produced a single pure peptide (T-2-3-4). On amino acid analysis, this peptide was found to have methionine, which had been shown to be the 14th residue from the C-terminal end of J chain. Further purification of T-2-4 by chromatography on CM-cellulose column (1.5 × 40 cm) with a linear gradient of 0.05 M to 0.5 M CH_3COONH_4, pH 3.8, produced three pure peptides T-2-4-1, T-2-4-2, and T-2-4-3 (Figure 3b). T-2-4-1 and T-2-4-3 were both methionine containing peptides and have almost identical amino acid compositions except T-2-4-3 contains one extra residue each of lysine, threonine, glycine, and tyrosine. Peptide T-2-4-3, thus being more basic, elutes after T-2-4-1. T-2-4-2 emerged as an asymmetrical peak (Figure 3b), on two separate preparations, but on amino acid analysis was found to be homogeneous by stoichiometry and provided a single amino-terminal residue.

Fraction T-3. Further purification of this fraction was achieved on CM-cellulose column (1.5 × 18 cm) with gradient II followed by a 0.5 M CH_3COONH_4 wash (pH 6.1). Only

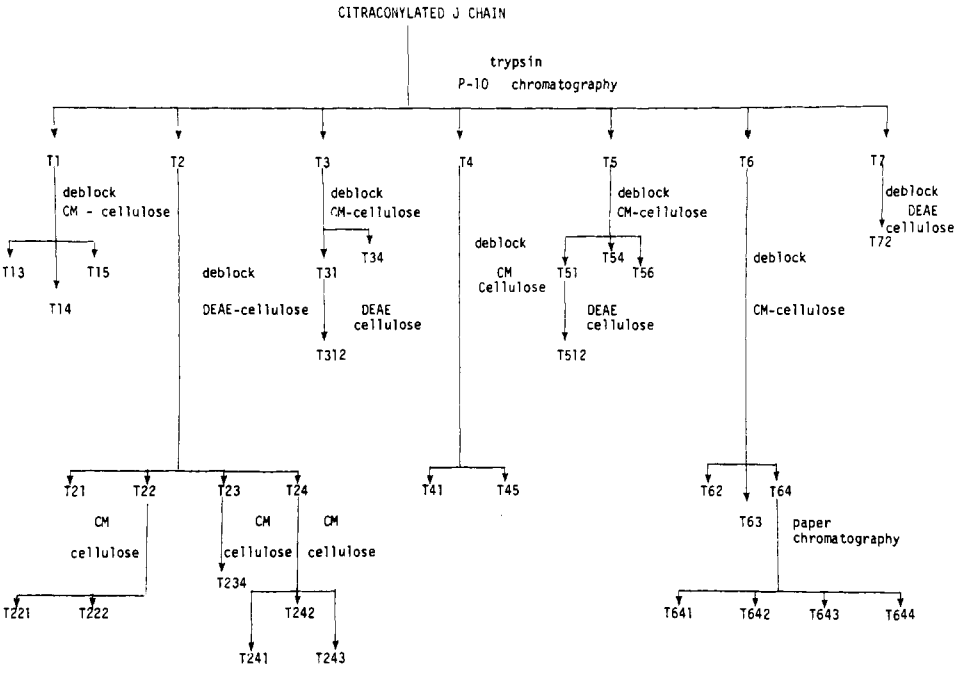

FIGURE 2: Schematic representation of the purification of peptides obtained from trypsin digest of citraconylated J chain.

two subfractions, T-3-1 and T-3-4, were further characterized. T-3-1 on rechromatography on DEAE-cellulose column (1.5 × 60 cm) with gradient I produced only one pure peptide, T-3-1-2, which was found to have a composition identical with T-2-4-2.

Fraction T-4. Cationic chromatography of T-4 on CM-cellulose column (1.5 × 1.8 cm) at a flow rate of 12 mL/h with gradient II produced two peptides, T-4-1, a six residue hydrophobic peptide, and T-4-5, a carboxymethylcysteine containing 10 residue peptide. Both the peptides were found to be pure and obtained in yields sufficient for further characterization.

Fraction T-5. CM-cellulose chromatography on a 1.5 × 17 cm column developed at 30 mL/h with gradient II produced six fractions, three (T-5-1, T-5-4, and T-5-6) were in high enough yields for further characterization. T-5-1 was refractionated on a DEAE-cellulose column (1.5 × 27 cm) with gradient I, yielding two subpeptides, T-5-1-1 and T-5-1-2. Only T-5-1-2 was found to be pure and in quantities sufficient for sequencing.

Fraction T-6. This fraction was purified on a 1.5 × 42 cm column of CM-cellulose at a flow rate of 20 mL/h. The column was initially developed with 0.025 M CH_3COONH_4 (pH 4.5) followed by a linear gradient of 0.025 M to 0.5 M CH_3COONH_4 (pH 4.5). Four fractions were separately pooled (Figure 3c) and labeled T-6-1 through T-6-4. T-6-2 was found to be similar to T-1-5 on amino acid analysis. T-6-3 is a four residue peptide containing arginine. T-6-4 was found to be impure and was purified further by paper chromatography (1-butanol–pyridine–glacial acetic acid–water, 90:60:18:72 v/v). The peptides were localized first on guide strips by spraying with 0.25% ninhydrin and numbered T-6-4-1 through T-6-4-4 in ascending order from the point of sample application. Spots corresponding to the ninhydrin-positive zones on guide strips were cut from unstained papers, eluted with 6 N HCl, and analyzed for amino acid composition.

Fraction T-7. Chromatography of fraction T-7 on a 1.5 × 61 cm column of DEAE-cellulose, developed with gradient I, produced two peptides T-7-1 and T-7-2. T-7-1, being present in low yields, was not characterized further. T-7-2 was a phenylalanine containing peptide with an amino acid composition identical with T-1-4.

Isolation and Characterization of Peptides Produced by Limited Trypsin Digestion. Histidine containing peptides were never reclaimed from a tryptic digest. In order to obtain a complete set of tryptic peptides, reduced and alkylated citraconyl J chain (60 mg) was digested with trypsin at a low concentration of enzyme for a short period of time (for details see Materials and Methods). The resultant peptide mixture was chromatographed on three P-10 columns (200–400 mesh, 2.0 × 112 cm) attached in tandem, and developed with 1% NH_4HCO_3 at 12 mL/h. Figure 4a shows the fractionation pattern. Eight fractions (LTP-1 through LTP-8) were obtained. Since the major purpose for limited trypsinization was to isolate and characterize histidine containing peptide(s), fractions LTP-1 through LTP-8 were first electrophoresed and strained with Pauli's reagent (Bennett, 1967). Only LTP-3 and LTP-4 were positive and were subsequently further characterized. On amino acid analysis only LTP-3 was found to contain histidine (Table I). This fraction was then further purified by column chromatography on DEAE-cellulose column (1.5 × 40 cm) developed initially with 200 mL of 0.025 M NH_4HCO_3 and then with gradient I (Figure 4b). Two peptides, LTP-3-1 and LTP-3-2, were obtained. Because of its very low yield, LTP-3-1 was not characterized further. Amino acid composition data on LTP-3-2, in addition to the presence of histidine, revealed the presence of 4 carboxymethylcysteine residues, which is half the total cysteine residues present in J chain.

Isolation and Characterization of Chymotryptic Peptides. In order to align and overlap the fragments obtained with extended and limited trypsin digestion of human J chain, the polypeptide was subjected to chymotrypsin digestion (see Materials and Methods). The soluble chymotryptic peptides were fractionated initially on two P-10 columns (2.0 × 120 cm, 200–400 mesh) in 1% NH_4HCO_3 attached in tandem. The elution profile is shown in Figure 5. The fractions were pooled as indicated and were examined for purity by high voltage electrophoresis at pH 3.5. The amino acid composition, yield, and total number of residues in each purified peptide are given

TABLE I: Amino Acid Composition of Peptides Obtained from Extended and Limited Trypsin Digestion of Human J Chain.

	T-1-3	T-1-4	T-1-5	T-2-1	T-2-2	T-2-3-4	T-2-4-1	T-2-4-2	T-2-4-3	T-3-1-2	T-3-4	T-4-1	T-4-5	T-5-1-2	T-5-4	T-5-6	T-6-2	T-6-3	T-6-4-1	T-6-4-2	T-6-4-3	T-6-4-4	T-7-2	LTP-3-2
Lys					1.0	1.0			1.0		1.8		1.0			1.0								1.9
His																								1.0
Arg			1.0	1.0	1.0					1.0	1.0				1.0		1.0	1.0	1.1	1.0	1.0	1.0		
CM-Cys						1.2	1.2	1.2		1.8	2.5		1.0		0.9				1.9					4.6
Asp	1.0		0.9	2.0	2.0	2.2	2.0	2.0	2.0	2.9	2.0		1.0		2.0	1.1	1.1				1.0			7.5
Thr	1.0		1.3	1.0	1.0	2.8	1.7	2.7				0.9	0.9				1.0	1.0		0.2				5.5
Ser				1.7	1.7			1.7		1.8								1.0						2.7
Glu	0.8			1.1	1.1	1.1	1.1	3.1	2.1	2.9														4.2
Pro				1.9	2.0	2.2	2.0	0.8	2.0	1.0		1.1	0.9	1.1	1.1									1.0
Gly									1.9															
Ala					2.0	2.1	2.0		2.1		1.0	0.9	0.9											1.9
Val	0.8	1.0				1.5	0.9	1.9	1.9	0.8	1.7	1.8	1.9	0.9	1.0							0.9		2.5
Met						0.9	0.8		0.9															
Ile				0.9	1.0			1.0		0.7	0.7			1.0	0.9			1.0						1.6
Leu				1.0	1.0		1.0		1.0		1.0	1.2	1.0	0.8	1.1									2.8
Tyr	1.2	1.0	1.0						1.7				1.0			1.1	0.9					1.0		2.8
Phe		1.0																				1.0		0.9
Carbohydrate				+	+																			
Residues	5	3	4	11	11	21	14	12	21	12	12	6	10	4	7	4	4	4	3	2	3	2	3	41–44
Yields %	7		6	22	13	37	43	38	22	43	26	23	15	9	15	21	13	32					17	42
N terminus	Val	Phe	Thr	Glu	Glu	Val	Met	Ser	Val	Ser	Ile	Thr	Asp	Ile	Ile	Asp	Thr	Ile					Phe	Phe

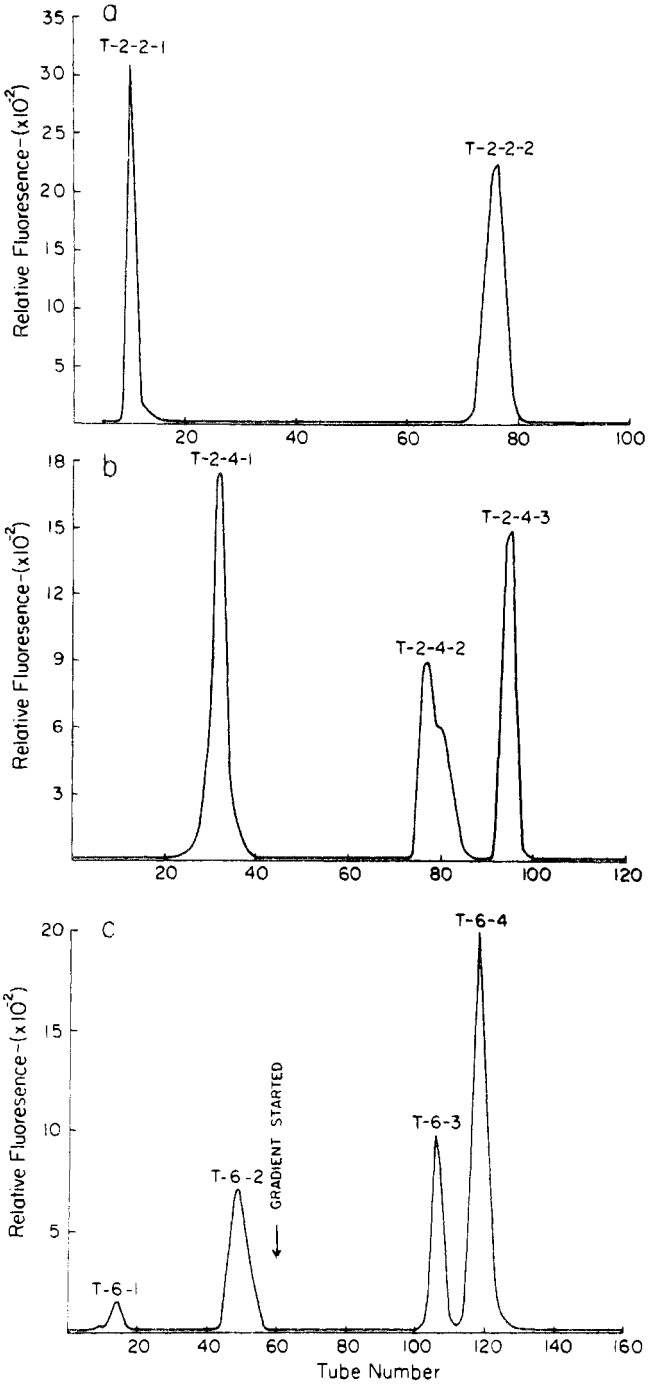

FIGURE 3: Further purification of tryptic fractions. (a) Fractionation of T-2-2 on CM-cellulose column (1.5 × 40 cm) developed with gradient II, and collected 3.5-mL fractions. (b) Fractionation of T-2-4. Experimental details are as "a" above. (c) Fractionation of T-6 on CM-cellulose column (1.5 × 42 cm) developed initially with 0.025 M CH_3COONH_4 (pH 4.5) followed by a gradient of 0.025–0.5 M CH_3COONH_4 (pH 4.5). Fractions (3.5 mL) were collected.

in Table II. The purification scheme is given in Figure 6. Fractions C-1, C-7, and C-8 were not further purified since they did not contain arginine and hence would not be expected to give an overlapping sequence.

Fraction C-2. Fraction C-2 was chromatographed on CM-cellulose column (1.5 × 40 cm), equilibrated with 0.025 M CH_3COONH_4, pH 3.8. Separation of peptides was achieved by employing first a gradient of CH_3COONH_4 from 0.025 M to 0.5 M (pH 3.8), followed by a pH gradient of 0.5 M ammonium acetate up to pH 4.5. Only C-2-1 and C-2-2

TABLE II: Amino Acid Composition of Peptides Obtained from Limited and Extended Chymotrypsin Digestion.

	C-2-1	C-2-2	C-3-2-2	C-3-4	C-3-6	C-4-6	C-4-9	C-5-5	C-6-4	CH-1-3	CH-4	CH-7
Lys				1.0	1.7	1.2	0.9				1.6	
His											0.7	
Arg	1.1	1.0					1.1	1.2	1.2		1.1	1.6
CM-Cys					2.3		1.3				3.5	
Asp	4.1	4.1	1.2		2.1		1.8				5.0	
Thr	0.9	1.0	0.8	1.9		1.0		0.8	0.9	0.8	3.8	1.2
Ser	1.5	1.3									2.1	
Glu	1.2	1.3	2.9	2.3		1.1					4.8	
Pro	2.0	1.9								0.9	1.4	
Gly				1.9		1.5						
Ala								0.8		1.0	2.1	
Val				1.0	1.2					2.9	2.5	
Met				0.9		0.8						
Ile	1.1	1.1						1.2			1.8	
Leu	1.1	1.0								1.2	2.5	
Tyr							0.9			1.0	1.3	
Phe									0.9			0.9
Carbohydrate	+	+										
Residues	12-13	12	5	9	7	6	6	4	3	8	33-36	4
Yields %	21		13	12	21	23	21	21	10	18	14	18
N-terminus	Asp			Gly	Val		Asp	Ala	Thr	Thr		Arg

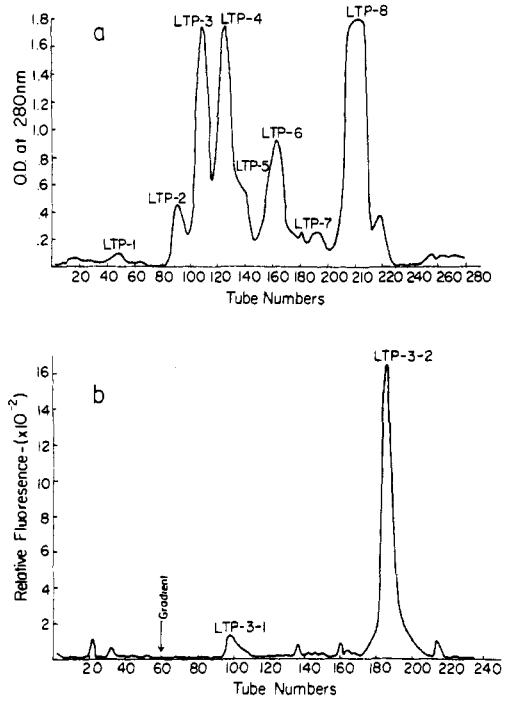

FIGURE 4: Separation of peptides obtained with limited trypsin digestion of human J chain. (a) Initial fractionation on three P-10 columns (Bio-Rad, 200-400 mesh, 2.0 × 112 cm) in 1% NH_4HCO_3 attached in tandem; fractions of 3.5 mL were collected. (b) Fractionation of LTP-3 on DEAE-cellulose column (1.5 × 40 cm) developed first with 0.025 M NH_4HCO_3 followed by gradient I, collecting fractions of 3.5 mL each.

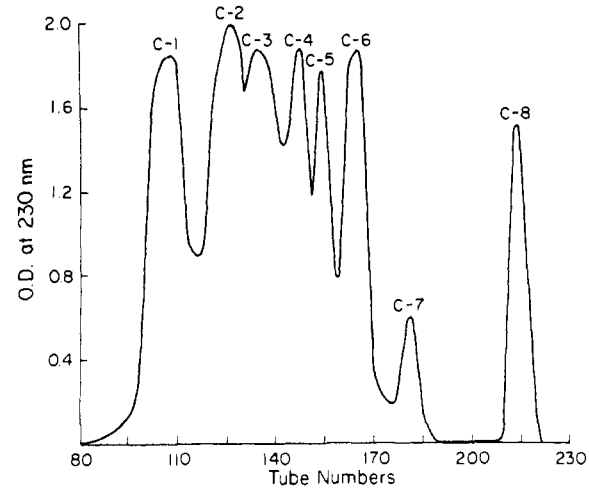

FIGURE 5: Initial fractionation of chymotryptic digest of human J chain on two P-10 (Bio-Rad, 200-400 mesh, 2.0 × 120 cm) columns in 1% NH_4HCO_3 attached in tandem. Fractions of 3.5 mL were collected at a flow rate 12 mL/h.

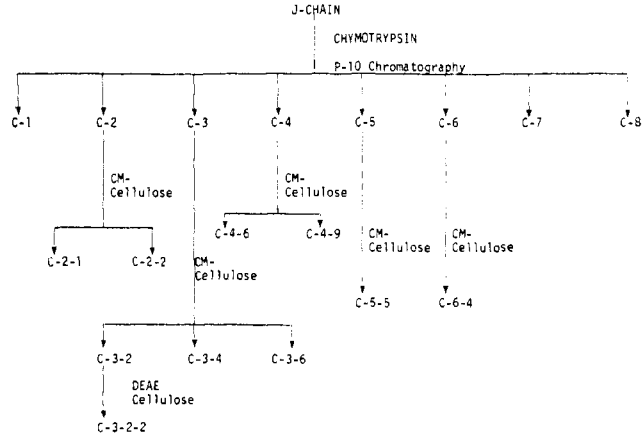

FIGURE 6: Schematic representation of the purification of peptides obtained after chymotryptic digestion of J chain.

were further analyzed. On the basis of amino acid composition, glycopeptides C-2-1 and C-2-2 were indistinguishable, but they elute as discrete peaks on cation chromatography, probably due to differences in the carbohydrate moiety. The amino acid compositions of these glycopeptides indicate their relationship to T-2-1 and T-2-2-1.

Fraction C-3. Chromatography of this pool on a CM-cellulose column (1.5 × 40 cm) produced two pure peptides, C-3-4 and C-3-6. The amino acid analysis of C-3-4 revealed that it contained methionine, and thus must have originated from the C terminus of J chain.

Fraction C-4. Purification of C-4 on a CM-cellulose column (1.5 × 40 cm) in 0.05 M CH_3COONH_4 (pH 3.9) yielded only two pure peptides, C-4-6 and C-4-9. A linear gradient of 0.05

M CH_3COONH_4 (pH 3.9) to 0.5 M CH_3COONH_4 (pH 3.9) was used. On amino acid analysis, C-4-6 was found to be analogous to C-3-4 except it had one residue less of threonine, glutamic acid, and valine. C-4-9 was a six residue peptide containing arginine.

Fraction C-5. When chromatographed on CM-cellulose column (1.5 × 40 cm) using the gradient as described for C-2, only one pure peptide (C-5-5) containing arginine was obtained (Table II).

Fraction C-6. Fraction C-6, when chromatographed on a CM-cellulose column (1.5 × 40 cm) using the gradient as described for C-2, produced only one pure peptide, C-6-4. On amino acid analysis it was found to contain one residue each of arginine, threonine, and phenylalanine.

Isolation and Characterization of Peptides from Limited Chymotryptic Digest. Proteolysis of J chain with chymotrypsin failed to produce sufficient peptides to provide unambiguous overlap. So as to have the complete set of peptides, which would provide the overlap information needed to order tryptic fragments, limited chymotrypsin digestion was investigated. Peptides resulting from limited chymotrypsin digest of human J chain were initially fractionated on a CM-cellulose column (1.5 × 60 cm), equilibrated with 0.05 M CH_3COONH_4, pH 3.8. A concentration gradient coupled with the pH gradient described for C-2 (as above) was employed. The fractions were cut and pooled as described above. Only fractions CH-1, CH-4, and CH-7 were further characterized.

Fraction CH-1. On DEAE-cellulose column (1.5 × 40 cm) chromatography with a linear gradient of NH_4HCO_3 from 0.025 M to 0.5 M, only one pure peptide (CH-1-3) was obtained. This was further characterized and the results of amino acid analysis are given in Table II.

Fraction CH-4. High voltage paper electrophoresis and N-terminal determination indicate CH-4 to be a pure peptide. Amino acid composition of CH-4 established a resemblance with LTP-3-2, in containing histidine and four carboxymethylcysteine residues.

Fraction CH-7. When analyzed this fraction was found to be a pure tetrapeptide, containing phenylalanine.

Discussion

The development of our strategy for determination of the primary structure of J chain was somewhat dictated by certain properties of the polypeptide. First, J chain has a blocked glutamic acid as its amino terminus and, therefore, is not susceptible to direct Edman degradation on the intact molecule. Second, it contains only one methionine located 14 residues from the C terminus, so cyanogen bromide fragmentation is not too helpful. Third, the molecule is extremely susceptible to proteolytic cleavage. The latter procedure, utilizing trypsin and chymotrypsin, was disappointing in that digestion often resulted in many small peptides, which had only limited use in sequence determination. These intrinsic properties of the molecule necessitated the utilization of several fragmentation approaches, so that adequate peptide representation, accounting for the entire molecule, could be obtained.

Human J chain contains 129 amino acid residues including nine arginine and five lysine. Cleavage with trypsin, after reversibly blocking the lysine residues, resulted in more fragments than expected. Sequence and amino acid composition data indicate this is due to incomplete blockade and nonspecific cleavages. The latter is rather conspicuous and is exemplified by such peptides as T-6-4-1 and T-5-1-2. These two peptides were judged to be subfragments of T-5-4 based on their composition and sequence data of the latter (see accompanying paper). Similarly T-4-5 was subfragmented into T-5-6 and T-4-1. Identification of a peptide containing the single histidine was made only following limited trypsin and chymotrypsin digestion. Remarkable similarity in the amino acid composition between histidine containing peptides LTP-3-2 and CH-4 though obtained as a result of limited digestion with trypsin and chymotrypsin, respectively, suggests that they originate from the same region of the molecule. Our failure to identify a histidine containing peptide from extended trypsin and chymotrypsin digestion indicates the extreme susceptibility of this central region of the molecule to enzymatic degradation.

Peptide data obtained on extended and limited enzymatic digestions accounted for all the residues in J chain, except for a five-residue N-terminal segment which escaped our detection because of the blocked amino terminus. The isolation, characterization, and sequence of this amino-terminal region have already been reported earlier (Mole et al., 1976). Thus, by utilizing various methods of proteolytic fragmentation, we were able to procure the necessary peptides to provide the total primary structure of J chain. These data may be found in the following paper (Mole et al., 1977b).

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Primary Structure of Human J Chain: Alignment of Peptides from Chemical and Enzymatic Hydrolyses†

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ABSTRACT: The primary structure of the J chain from a human Waldenströms IgM protein has been determined using a combination of automated and conventional Edman degradative procedures. Eighty-five percent of the sequence was established with peptides isolated from tryptic digests of carboxyamidomethylated and citraconylated J chain, many of which were sequenced completely. Alignment of the tryptic fragments was achieved with peptides generated by chymotrypsin and limited acid hydrolyses. The J chain consists of 129 amino acids and a single oligosaccharide structure linked to asparagine at position 43 of the sequence. The molecular weight, including 7.5% carbohydrate by weight, is 16 422. The location and arrangement of three half-cystines could be deduced from previous studies, whereas the pairing of the remaining five disulfide bonds still needs to be clarified.

Immunoglobulin M has been shown to consist of five 7S subunits which are joined by disulfide bonds into a complex macromolecule. Similarly, IgA may be organized into polymeric patterns, although a monomeric form of this immunoglobulin also exists in serum. Associated covalently with the polymeric forms of IgA and IgM is a polypeptide, the J chain, which is distinct from both heavy and light chains of immunoglobulins. The importance of this additional polypeptide chain to the integrity of polymeric immunoglobulin structures has been well documented. Furthermore, the presence of the J chain in myeloma cells secreting monomeric IgG (Kaji and Parkhouse, 1974) and in normal IgD and IgG producing cells (Brandtzaeg, 1974) serves to enhance speculation that the J chain has a dominant biological role in humoral immunity and also may be important in the activation of B lymphocytes. Indeed, recent studies have shown a parallel synthesis between J chain and immunoglobulin heavy and light chains during mitogen-induced differentiation of B lymphocytes (Mestecky et al., 1977). In light of these observations we have undertaken a study to elucidate the complete amino acid sequence of J chain, isolated from a human Waldenströms IgM protein. The results described in this and the preceding article provide a complete covalent structure of human J chain.

Materials and Methods

J chain used in these studies was isolated from a Waldenströms IgM macroglobulin (Gray) as previously reported (Mole et al., 1974). Procedures for enzymatic digestion, column chromatography, fractionation of peptides, and amino acid analysis have been described in the preceding paper (Bhown et al., 1977).

Edman Degradations. Automated Edman degradations were performed in a Beckman Model 890C sequencer employing the 1 M Quadrol program developed by Beckman Instruments (022574) and the 0.1 M Quadrol program of Brauer et al. (1975). The latter system was modified slightly for our instrument. The Quadrol concentration was increased to 0.5 M which enhanced the coupling efficiency with PITC.[1] Sequencer reagents and solvents were obtained from Beckman Instruments or Pierce Chemical Co. Dithioerythritol (15 mg/L) was added to the 1-chlorobutane to improve the recoveries of Pth-Ser and Pth-Thr. Repetitive yields for peptides were generally between 92 and 97%.

Manual Edman degradations were conducted using modifications of the three-stage method of Edman (1960). Peptide (10-40 nmol) was first lyophilized in a series of 2.5-mL graduated centrifuge tubes, one for each cycle of the degradation. Coupling was performed in 200 μL of 50% aqueous pyridine (v/v) using 5 μL of PITC. The coupling reaction was carried out under a nitrogen atmosphere, for 30 min, at 50 °C. The aqueous phase, containing the PTC peptide, was then dried, in a vacuum desiccator, over P_2O_5 for 30 min.

Cleavage was performed, under nitrogen, using 200 μL of TFA, for 10 min at 50 °C. If Gln was suspected as the penultimate amino-terminal amino acid, heating was omitted. TFA was then quickly evaporated under vacuum, in the presence of damp NaOH pellets. Following cleavage the residue was redissolved in 200 μL of distilled water and two 1.0-mL butyl acetate extractions were performed and the organic phases discarded.

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[1] Abbreviations used: PITC, phenyl isothiocyanate; PTC, phenylthiocarbamoyl; TFA, trifluoroacetic acid; Pth, phenylthiohydantoin; butyl-PBD, 2-(4′-*tert*-butylphenyl)-5-(4″-biphenyl)-1,3,4-oxidazole; PCA, 2-pyrrolidone-5-carboxylic acid; PCAase, pyrrolidonecarboxylyl peptidase; DEAE, diethylaminoethyl; TPCK, 1-chloro-4-phenyl-3-tosylamido-2-butanone; CPA, carboxypeptidase A; CPB, carboxypeptidase B; dansyl chloride, 1-dimethylaminonaphthalene-5-sulfonyl chloride; TLC, thin-layer chromatography; GLC, gas-liquid chromatography.