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Site of J Chain Attachment to Human Polymeric IgA

(immunoglobulin structure/polypeptide chain assembly)

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ABSTRACT **A fragment containing J chain was released from human polymeric myeloma IgA protein by cyanogen bromide cleavage. The identity of the fragment was determined by its electrophoretic mobility and antigenic determinants. After purification by gel filtrations and DEAE-Sephadex chromatography, this fraction appeared similar (with respect to its amino acid and carbohydrate compositions and its peptide maps) to the J chain isolated from this IgA protein; the molecular weight was 17,000 ± 100. Upon reduction and alkylation, with subsequent separation of peptides by gel filtration, three components were obtained: the largest component (molecular weight 13,400) corresponded to the N-terminal segment of J chain and contained a homoserine residue, the second corresponded to the C-terminal part of J chain with 13–18 amino acid residues, and the third corresponded to the C-terminal octapeptide of the α chain. The data indicate that J chain is attached to α chain(s) through the penultimate cysteine residue of the C-terminal octapeptide.**

In addition to heavy and light chains, human and animal polymeric immunoglobulins contain a polypeptide termed J chain (1–7) with a molecular weight of 15,600 ± 200 (5, 8, 9). Studies of immunofluorescence and biosynthesis indicated that J chain was produced in plasma cells that synthesized IgA and IgM molecules (10–12). On the basis of the absence of J chain in monomeric and its presence in all polymeric immunoglobulins examined, its relatively large content of cysteine residues, and its disulfide bond attachment to immunoglobulin molecules, it was suggested that J chain joins the monomeric units of IgA and IgM to form, in an undefined manner, the respective polymeric molecules (13). Although the precise location of the disulfide linkages of J chain to IgA and IgM molecules has not been elucidated, studies on the products of proteolysis of these immunoglobulins strongly suggested an attachment in the Fc region of the heavy chain; the Fab and $F(ab)_2$ fragments were devoid of J chain (14–16).

It has been shown that J chain contains one methionine residue near the carboxy terminus (17). Therefore, part of the J chain linked by disulfide bonds to one or more heavy chain fragments should be present after cleavage with CNBr. This approach was applied for identification of the α-chain portion involved in the binding of J chain in a polymeric myeloma IgA. In this communication we present evidence which indicates that J chain is linked to the penultimate cysteine residue of the α chain(s).

MATERIAL AND METHODS

Purification and Characteristics of Polymeric IgA. Blood plasma from a patient (Fel) with IgA multiple myeloma was recalcified to remove fibrinogen, and a crude gamma globulin fraction was obtained by precipitation with ammonium sulfate to 50% saturation. The precipitate was collected by centrifugation and dialyzed against saline buffered with Tris·HCl at pH 7.4 and subsequently gel filtered through Sephadex G-200 and Sepharose 6-B as described in detail in a previous communication (18). The resultant fraction, which contained IgA, was dialyzed against 0.01 M sodium phosphate buffer (pH 7.5) with 0.1 M NaCl, and applied to a DEAE-Sephadex A-25 column that was equilibrated with the same buffer. The protein eluted from the column under these conditions was desalted on Sephadex G-25 in 1% ammonium bicarbonate and then lyophilized. Upon immunoelectrophoresis at 2% protein concentration it reacted by forming one strong precipitin line with γ1 electrophoretic mobility when tested against polyvalent antisera to whole human serum (produced in horse, Hyland Laboratories, Los Angeles, Calif., and Behring-Hoechst Pharmaceutical Co., Kansas City, Mo.). The protein belonged to the IgA2 subclass, with λ chains covalently linked to heavy chains, and had a sedimentation constant of 9.5 S, as characterized previously (19).

After cleavage of disulfide bonds by reduction and alkylation or oxidative sulfitolysis (18), J chain was detectable as an anodically moving protein upon examination by immunoelectrophoresis with the use of a monospecific anti-J-chain serum (20) and by disc electrophoresis under alkaline pH, in the presence of 10 M urea (21).

Preparation of Polypeptide Chains and CNBr Fragments. To obtain α and L chains, totally reduced and alkylated (iodoacetamide) (18) IgA was gel filtered through a Sephadex G-200 column (2.6 × 100 cm) in 5 M guanidine·HCl. The J chain was found in the L-chain fraction when examined by alkaline urea disc electrophoresis (21) and immunoelectrophoresis (20); light and J chains were separated by DEAE-Sephadex chromatography under conditions used in previous experiments (20).

Purified intact IgA and totally reduced and alkylated α and J chains were dissolved in 70% formic acid at 2% protein concentration and subjected to CNBr cleavage. Two parts CNBr (Eastman Kodak) were added to one part protein (weight/weight), and the reaction was allowed to proceed at room temperature for 4 hr. The samples were then diluted with 10 volumes of distilled water and lyophilized. The products of CNBr cleavage of intact IgA were applied on a Sephadex G-200 column (2.6 × 100 cm, upward flow) in 5 M guanidine·HCl and those products from α and J chains were fractionated

on a Sephadex G-25 fine column (1.6 × 34 cm or 1.6 × 60 cm); each column was equilibrated in 5 M guanidine·HCl. Desalting was performed on Sephadex G-10 in 1% ammonium bicarbonate. Purity of the isolated polypeptides was ascertained by electrophoresis in the presence of sodium dodecyl sulfate (22).

Peptide Maps, Amino Acid and Carbohydrate Analyses. Isolated polypeptide chains were treated with performic acid (23) prior to tryptic digestion, which was carried out in 0.2 M ammonium bicarbonate buffer, pH 8.5, for 4 hr at 37° at a trypsin–protein ratio of 1:50. Chromatography was performed with a system of *n*-butanol, pyridine, glacial acetic acid, and distilled water (15:10:3:12); subsequent high-voltage electrophoresis was performed in pyridine–acetate buffer, pH 3.6 (24).

After 24-hr hydrolysis of the peptides in constant-boiling HCl at 108°, amino acid analyses were performed on a Beckman 120 C amino acid analyzer modified for single-column, high-speed analyses. Corrections due to losses of threonine and serine during hydrolysis were 5 and 10%, respectively (20). Cysteine was determined as cysteic acid after performic acid oxidation (23). Carbohydrate analyses were done by gas chromatography of alditol acetates of neutral and amino sugars. The conditions and equipment used for the determinations were described (25).

Ultracentrifugation. Molecular weight values were determined by sedimentation equilibrium in a Beckman model E ultracentrifuge equipped with an electronic speed control, absorption optics, and a photoelectric scanner (8). An An-F rotor and 12-mm cells were used in all experiments. The light source monochromator was set at 280 nm and the temperature was regulated at 20°. The sample column height was 3 mm. Equilibrium was judged to be established when there was no change in the slope of the logarithm of the absorbance (A) plotted against the square of the distance from the center of rotation (r) over a minimum of 4 hr. Prior to analyses, the proteins were dialyzed into 5 M guanidine·HCl. The partial specific volume used in calculating apparent molecular weights of proteins containing J chain was assumed to be the same as that of purified J chain (0.705 ml/g). This value was calculated from its amino acid and carbohydrate composition (8).

RESULTS

Fragments derived from IgA by CNBr cleavage were resolved into three fractions when subjected to gel filtration on Sephadex G-200 in 5 M guanidine·HCl (Fig. 1). Material with the electrophoretic mobility and antigenic determinants of J chain was present in fraction II (Fig. 1) but absent from the first fraction, which lacked polypeptide with J-chain properties, whether untreated or reduced and alkylated. Other peptides detected in fraction II by both sodium dodecyl sulfate and alkaline urea disc electrophoreses were removed by chromatography on DEAE-Sephadex (20). The protein obtained at the elution position of J chain was desalted, lyophilized, and rechromatographed on Sephadex G-25 in 5 M guanidine·HCl. A single symmetrical elution peak was observed (fraction A1, Fig. 2*A*). The latter appeared as a single component by electrophoreses in sodium dodecyl sulfate and pH 9.4 (in 10 M urea). Sedimentation equilibrium studies were performed on duplicate samples of fraction A1 at two speeds (20,000 and 24,000 rpm). The samples were homogenous, as indicated by linear plots of log A versus r^2 (Fig. 3), and essentially identical values were obtained at both speeds. The average molecular weight, and its standard deviation, as determined in this series of four experiments was 17,000 ± 100. This value was significantly higher than the value of 15,600 ± 200 determined for J chain that was purified from the polymeric myeloma IgA used in this investigation and examined under the same conditions (8). Peptide maps of J chain and fraction A1 were identical except for the presence of an additional peptide with slow electrophoretic mobility in fraction A1 (Fig. 4). The amino acid and carbohydrate compositions of fraction A1 strongly resembled those of isolated J chain (Table 1); however, levels of aspartic acid, threonine, glutamic acid, glycine, and alanine were consistently slightly higher in fraction A1 than in J chain. The amounts and proportions of individual carbohydrates in both samples were similar.

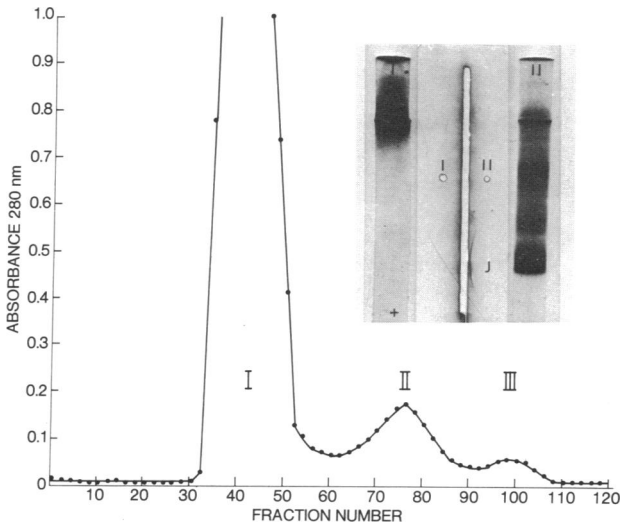

FIG. 1. Gel filtration of CNBr-treated IgA (250 mg) through a Sephadex G-200 column (2.6 × 100 cm, upward flow) in 5 M guanidine·HCl. *Insert,* disc electrophoresis in alkaline urea–polyacrylamide gel (21) and immunoelectrophoresis with anti-J-chain serum (20). As indicated by both techniques, J chain (J) was present in fraction II.

Fraction A1 was totally reduced and alkylated and then subjected to gel filtration through Sephadex G-25; three fractions were obtained (Fig. 2*B*). Sedimentation equilibrium analysis of the first and largest fraction (B1) in 5 M guanidine·HCl at 20,000 rpm indicated the presence of a small quantity of heterogeneous material with higher molecular weight. The plot of log A versus r^2 demonstrated slight upward curvature near the bottom of the cell. A molecular weight of 13,400 was calculated from the linear portion of the plot for each of three samples having an initial absorbance of 0.62 at 280 nm. The peptide map of this fraction was comparable to those of A1 and J chain except for the absence of peptides that exhibited slow electrophoretic mobility at pH 3.6 (Fig. 4). The amino acid and carbohydrate compositions of fraction B1 resembled those in J chain (Table 1). One homoserine residue was detected per mol of this fraction. This polypeptide, therefore, represents a large N-terminal fragment of J chain.

The two peptides (B2 and B3) with low molecular weights were pooled from several runs and further separated by re-

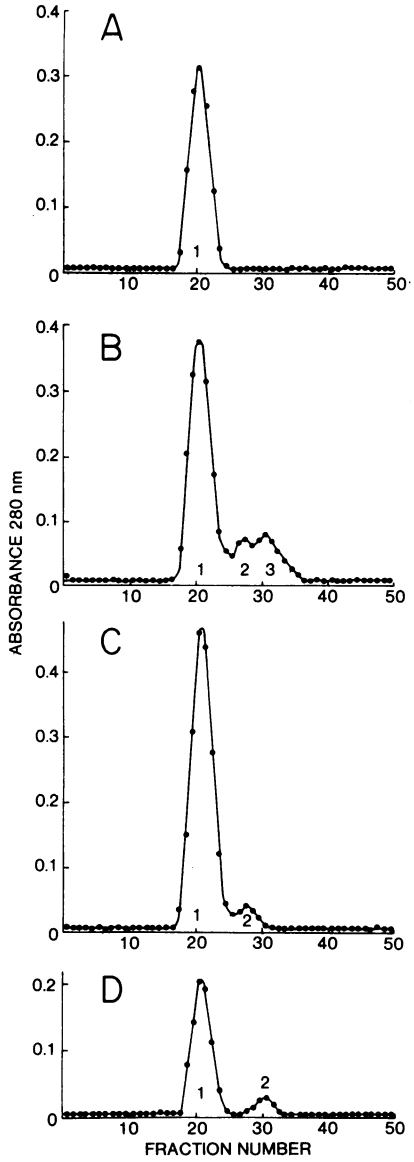

FIG. 2. Gel filtration through a Sephadex G-25 fine column (1.6 × 35 cm) in 5 M guanidine·HCl. (*A*) Purified J-chain-containing fragment (molecular weight 17,000) released by CNBr treatment of IgA. (*B*) Totally reduced and alkylated fraction A1. The molecular weight of B1 was 13,400. (*C*) Totally reduced, alkylated and CNBr-cleaved J chain. (*D*) Rechromatography of low-molecular-weight peptides, released by CNBr treatment, from totally reduced and alkylated α chain.

chromatography on a longer Sephadex G-25 fine column (1.6 × 60 cm) equilibrated in 5 M guanidine·HCl. The amino acid composition of B2 revealed that homoserine was absent from this fraction; the probable numbers of residues are listed in Table 1. The composition of the fraction B3 revealed the presence of eight amino acids in approximately equimolar amounts, with the exception of tyrosine (Table 1); homoserine was also absent from this fraction. The composition of fraction B3 strongly resembled that of the C-terminal octapeptide of the α chain (26). To verify the possibility that fraction B3 was derived from the C-terminal octapeptide of α chain, and to determine the origin of peptides present in fractions B3 and B2, the following steps were taken: α and J chains were totally reduced and alkylated, cleaved with

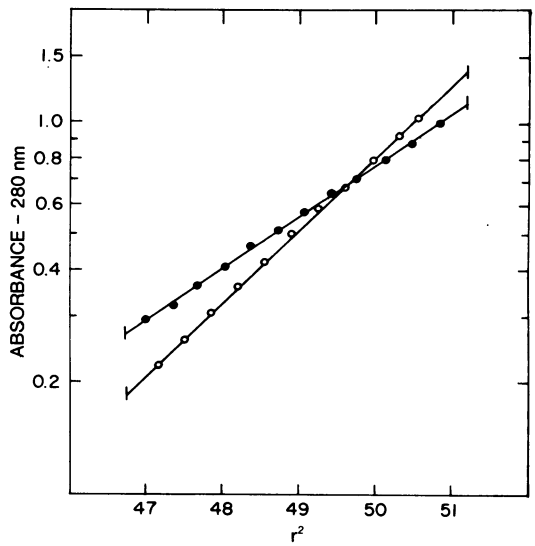

FIG. 3. Distribution at sedimentation equilibrium of fraction A1 in 5 M guanidine·HCl. The sample concentration was 0.61 absorbance unit at 280 nm. Linear plots of log *A* as function of r^2 (at two rotor speeds: 20,000 rpm ●——● and 24,000 rpm ○——○) were observed.

CNBr, and then subjected to gel filtration on Sephadex G-25, to amino acid analysis, and to high-voltage electrophoresis. The small peptide that was released by CNBr cleavage of totally reduced and alkylated J chain (fraction C2—Fig. 2*C*) was shown to be the same as that from fraction B2, since both peptides were eluted at the same position on Sephadex G-25, had similar amino acid compositions, including the lack of homoserine, and displayed identical electrophoretic mobility. Thus, both fraction B2 and C2 are derived from the C-terminus of J chain, and contain approximately 18 amino acids; however, this number might be as low as 13 amino acids, contingent upon the determination of the molecular weight (Table 1). Peptides of low molecular weights from several consecutive gel filtrations of CNBr-cleaved α chain were pooled and then refiltered through the same column of Sephadex G-25 (Fig. 2*D*). Fractions D2 and B3 were eluted at the same position, had an amino acid composition (Table 1) essentially identical to each other and to the C-terminal octapeptide of the α chain described by Prahl *et al.* (26). Homoserine was absent from both fractions. By high-voltage electrophoreresis, the mobility of the peptide that was present in both fractions B3 and D2 was identical.

DISCUSSION

On the basis of the properties of peptides present in a fragment released from a polymeric myeloma IgA by CNBr cleavage, it was concluded that this fraction (A1) contained J chain attached by disulfide bonds to the C-terminal octapeptide(s) of the α chain. A plausible arrangement of the peptides that compose fraction A1 is shown in Fig. 5. The C-terminal (B2 and C2) and the N-terminal (B1) peptides of J chain were separated as a consequence of reduction, indicating that the single cysteine residue of B2 fraction participated in the intra-J-chain disulfide bond, rather than being involved in a disulfide bond linking J chain to α chain. Of note is the presence of three proline residues in this fraction. The C-terminal amino acid has been identified as aspartic acid (Zikan, J., unpublished observation). The molecular weight

of approximately 2000 for B2 was calculated by the subtraction of the molecular weight of fragment B1 (13,400) from the molecular weight of the original J chain (15,600). It probably included residues 107–124 from the N-terminus. The methionine residue can thus be placed in the vicinity of position 106. However, the number of amino acid residues expected might be as low as 13, when amino acid compositions of B2 and C2 are considered as a base. These two peptides would contain the following residues: 2 aspartic acid, 2 threonine, 1 glutamic acid, 2 proline, 2 alanine, 1 valine, 1 leucine, 1 tyrosine, and 1 cysteine. The carbohydrate moiety of J chain is found in the large N-terminal CNBr fragment (B1). The N-terminal residue is blocked (27, 28).

The third peptide (B3) that was released from A1 upon cleavage of disulfide bonds was eluted from Sephadex G-25 at the same position as the C-terminal octapeptide of the α chain (D2); the electrophoretic mobility and amino acid composition of both fractions were identical. The amino acid composition and sequence of the C-terminal octapeptide of the α chain were previously reported by others (26, 29). The amount of C-terminal tyrosine was lower than anticipated. This observation was confirmed in our investigation. The only

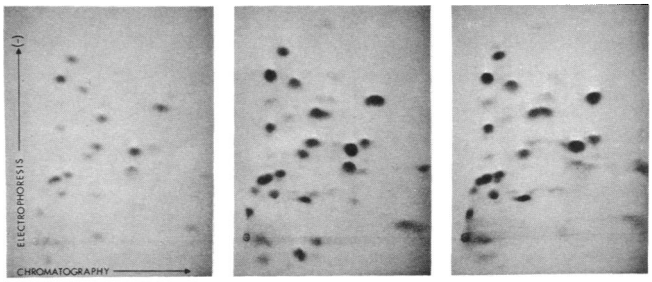

FIG. 4. Peptide maps of J chain, fraction A1, and fraction B1. Cathode is at the top.

TABLE 1. *Comparison of amino acid* and carbohydrate compositions of J chain and fractions A1, B1, B2, B3, C2, and D2 (Fig. 2). Amino acids are expressed in residues per 100 (%) and in number of residues per mole (m) after the subtraction of molecular weights of carbohydrates present in each fraction*

	J Chain		A1		B1		B2		C2		B3		D2	
Amino acid	%	m	%	m	%	m	%	m	%	m	%	m	%	m
Aspartic Acid	16.90	20.8	16.98	23.7	17.25	18.3	16.7	3.1	16.7	3.0	13.9	1.1	13.1	1.0
Threonine	9.53	11.7	9.73	13.6	9.38	9.9	15.1	2.8	15.8	2.8	13.8	1.1	12.5	1.0
Serine	7.25	8.9	6.78	9.5	6.71	7.1								
Glutamic acid	12.05	14.8	12.92	18.1	13.07	13.8	9.9	1.8	6.3	1.1	15.5	1.2	14.7	1.2
Proline	6.23	7.7	5.91	8.3	5.4	5.7	13.9	2.6	16.7	3.0				
Glycine	1.96	2.4	2.74	3.8	2.08	2.2	3.8	0.7	0.8	0.1	15.9	1.3	15.3	1.2
Alanine	4.82	5.9	5.03	7.0	4.30	4.5	16.5	3.1	16.4	3.0	10.0	0.8	14.7	1.2
Valine	7.38	9.1	6.94	9.7	6.86	7.3	6.2	1.2	6.0	1.1	13.2	1.1	13.2	1.1
Methionine	0.64	0.8												
Isoleucine	4.70	5.8	4.25	5.9	4.25	4.5								
Leucine	6.18	7.6	6.01	8.4	6.35	6.7	7.7	1.4	8.4	1.5				
Tyrosine	4.12	5.2	3.38	4.7	3.84	4.1	4.9	0.9	7.5	1.4	9.0	0.7	5.8	0.5
Phenylalanine	0.88	1.1	0.81	1.1	1.04	1.1								
Lysine	4.08	5.0	4.01	5.6	4.75	5.0								
Histidine	0.88	1.1	0.90	1.3	1.18	1.2								
Arginine	7.45	9.2	7.20	10.1	7.54	8.0								
Cysteine†	4.95	6.1	5.53	7.7	4.96	5.3	5.3	1.0	5.4	1.0	8.7	0.7	10.7	0.8
Tryptophan‡	0.00	0												
Homoserine			0.87	1.2	1.03	1.1								
Probable number of amino acids per chain	124		140		106		18–19¶		18¶		8		8	
Carbohydrates§														
Fucose	0.34	1	0.40	1	0.17	1								
Mannose	2.13	2	2.84	2–3	2.25	2								
Galactose	1.27	1	1.27	1	0.80	1								
Glucosamine	2.72	3	2.48	2–3	1.97	2								
Galactosamine	0.00	0	0.00	0	0.00	0								
Sialic acid	1.11	1												
Total	7.6	8												
Molecular weight														
Determined	15,600 ± 200		17,000 ± 100		13,400									
Calculated							2,000		1,900		860		860	

* Values are the average of five determinations (for J chain and B1), four (for A1), and three (for B2, B3, C2, and D2).

† Cysteine was determined as cysteic acid (23).

‡ Tryptophan was determined by magnetic circular dichroism spectra (8).

§ Values are the average of nine analyses of three preparations of J chain (Fel) (25) and two analyses of fraction A1 and B1. In the case of carbohydrates, % indicates the amount of carbohydrates per total J chain mass.

¶ The number might be 13 (for explanation see *Discussion*).

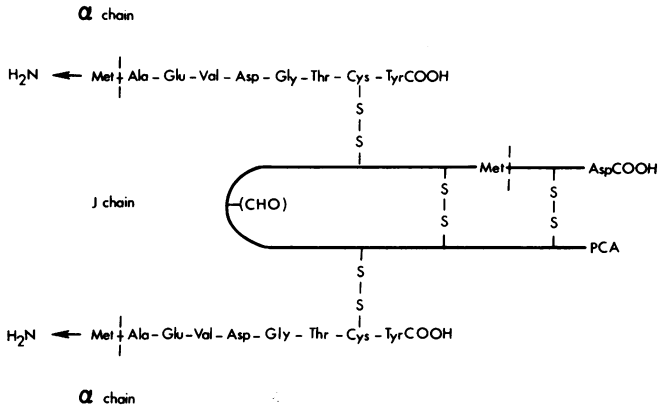

FIG. 5. Proposed model of peptide arrangement in fraction A1. The distribution of cysteine residues in J chain is arbitrary except for cysteine in the C-terminal part of J chain that is involved in the intra-J-chain disulfide bridge. Vertical broken lines indicate the site of CNBr cleavage. *CHO* indicates the carbohydrate moiety and *PCA*, pyrrolidone carboxylic acid.

cysteine residue available for the formation of a disulfide bridge with J chain occurs in the penultimate position. Prahl *et al.* (26) reported that cleavage of disulfide bonds, in addition to the CNBr treatment, was necessary for the release of C-terminal octapeptide from monomeric or polymeric IgA. It was suggested that the penultimate cysteine might be involved in the formation of an asymmetric intra- or inter-heavy chain disulfide bond. The association of polymeric IgA with J chain might also prevent the spontaneous release of the C-terminal octapeptide. The number of C-terminal octapeptides found in fraction A1 has not been positively established; however, according to the amino acid composition (Table 1) and molecular weights of A1, B1, B2, and B3 fragments, the A1 fraction would most probably include two C-terminal octapeptides of the α chain. Likewise, the location has not been established for the remaining cysteine residues involved in either the intra- or inter-chain bonding of fraction B1.

The C-terminal octapeptide of α chain is a part of the 19 amino acid residues that extend beyond the carboxy-terminal residue of the γ and ϵ chains (29–31). The μ chain also contains an additional 19 residues (29, 32), with the last four amino acids of α and μ chains in the same sequence. It is important to establish whether the penultimate cysteine residue of μ chain that is homologous to α chain is also involved in J-chain-binding of IgM. If so, this might clarify the reason for the extended length of α and μ chains of polymeric over γ and ϵ chains of monomeric immunoglobulins. The process of final assembly of intracellular monomeric subunits of IgM and IgA into corresponding polymers and the role played by J chain remains to be clarified.

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