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The amino acid sequence of rabbit J chain in secretory immunoglobulin A

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The primary structure of rabbit J chain, which occurs covalently bound to secretory IgA, was determined. J chain was isolated in its *S*-carboxymethylated form, in one step, by SDS/PAGE followed by electro-elution; 5 nmol of protein (approx. 75 μg), in all, was necessary for the determination of the complete sequence by the 'shot-gun' microsequencing technique: with the use of several site-specific endoproteinases, the various digests of *S*-carboxymethylated J chain were separated by micro-bore reverse-phase h.p.l.c. and the partial *N*-terminal sequences of all peptides were analysed. From the sequence alignment, gaps were filled by further extensive sequencing of the relevant overlapping fragments isolated from selected digests. Rabbit J chain comprises 136 amino acid residues, out of which eight are conserved cysteine residues, and is more closely similar to the human sequence (73.5 % identity) than to the mouse sequence (68 % identity). There is one unique glycosylation site at asparagine-48.

INTRODUCTION

J chain is an acidic 15 kDa polypeptide found exclusively in polymeric immunoglobulins (Igs), in covalent association with dimeric IgA and pentameric IgM molecules (Koshland, 1985). The transport of secretory IgM or IgA out of the epithelial cell is mediated by the polymeric Ig receptor or secretory component (SC) (Mostov & Simister, 1985). It has been suggested that binding of secretory IgA (sIgA) to SC may require the presence of J chain and that only J-chain-containing polymers would be transported through epithelial cells into exocrine fluids (Brandtzaeg & Prydz, 1984). J chain alone, however, does not constitute the SC-binding site, as purified dimeric J chain exhibits only marginal blocking of the SC–polymeric Ig complex (Brandtzaeg, 1975, 1985). Because J chain is disulphide-linked to two monomeric Ig subunits (Garcia-Pardo *et al.*, 1981), it may cause structural constraints in the Fc parts of polymeric Igs, thus determining a partial SC-binding site. In addition, direct non-covalent interactions between Ig-associated J chain and SC cannot be ruled out, despite the lack of disulphide bonding between them (Mestecky & McGhee, 1987). According to Koshland (1985), there is one molecule of J chain per molecule of Ig polymers, regardless of its size. This notion has been challenged by Brandtzaeg (1985), who found, on the basis of immunochemical estimates, two J chain molecules per IgA dimer and at least three per IgM pentamer.

Even though the primary structures of human (Max & Korsmeyer, 1985) and mouse (Matsuuchi *et al.*, 1986) J chains, as well as partial sequence data for J chain from bullfrog (Mikoryak *et al.*, 1988), are known, the three-dimensional structure of native J chain could not be determined because the standard reducing conditions used to release J chain from Ig polymers also reduced all intra-J-chain disulphide bridges (Mendez *et al.*, 1973; Elliott & Steiner, 1984; Mikoryak *et al.*, 1988). Two hypothetical models for the folding of J chain have been predicted from its amino acid sequence. One of these (Cann *et al.*, 1982) suggests a two-domain structure, whereas the other (Zikan *et al.*, 1985) predicts a single Ig-like β-barrel domain, in which all of the disulphide bridges are localized within exposed loops between the anti-parallel β-strands. The latter model appears to be more consistent with the finding of the unexpected lability of disulphide bridges of J chain (Mendez *et al.*, 1973; Elliott & Steiner, 1984; Mikoryak *et al.*, 1988).

In recent years our laboratory has been interested in domain–domain interactions between rabbit dimeric IgA, SC and J chain (Jaton *et al.*, 1988). We have shown that the isolated *N*-terminal domain of SC is necessary and sufficient for the non-covalent binding to sIgA of the g subclass (Frutiger *et al.*, 1986; Frutiger, 1987). In the course of a study of the possible non-covalent interactions between J chain, SC and α chains and the identification of the contacting regions between the three partners within the complex, we have isolated some cysteine-containing peptides that, unfortunately, could not be unambiguously assigned to known sequences of J chains (Max & Korsmeyer, 1985; Matsuuchi *et al.*, 1986; Mikoryak *et al.*, 1988), of α chains (Kabat *et al.*, 1987) or of SC (Mostov *et al.*, 1984; Eiffert *et al.*, 1984). This was unexpected in view of the high degree of sequence conservation around the cysteine residues in mammalian Ig-like proteins (Steiner, 1985). This prompted us to determine the complete amino acid sequence of rabbit J chain, including the site of attachment of asparagine-linked carbohydrate. The present paper describes the isolation of J chain from the sIgA complex by analytical SDS/PAGE, and its sequence determination with less than 5 nmol of protein by peptide isolation on micro-bore h.p.l.c. columns and micro-sequencing of 20–400 pmol of fragments generated from various site-specific proteolytic enzymes.

MATERIALS AND METHODS

Reagents

Acrylamide, *NN*-methylenebisacrylamide and urea were purchased from Bio-Rad Laboratories, Richmond, CA, U.S.A. Chymotrypsin, trypsin, endoproteinases Glu-C, Arg-C, Lys-C (sequencing grade) and Asp-N (sequencing grade) were from Boehringer, Mannheim, Germany. Dithiothreitol (Ultrol) was from Calbiochem Corp., San Diego, CA, U.S.A. Iodoacetic acid

Abbreviations used: SC, secretory component; sIgA, secretory immunoglobulin A; 1,5-I-AEDANS, *N*-(iodoacetaminoethyl)-1-naphthylamine-5-sulphonic acid.

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and solvents for h.p.l.c. were from Merck, Darmstadt, Germany. 4'-Dimethylaminoazobenzene-4-sulphonyl chloride, amino acid standard and carboxypeptidase Y were from Pierce Chemical Co., Rockford, IL, U.S.A. *N*-(Iodoacetaminoethyl)-1-naphthylamine-5-sulphonic acid (1,5-I-AEDANS) was from Fluka, Buchs, Switzerland. Immobilon membranes were from Millipore Corp., Bedford, MA, U.S.A. *N*-Glycanase [peptide-N^4-(*N*-acetyl-β-glucosamine)asparagine amidase, EC 3.5.1.52] was obtained from Genzyme Corp., Boston, MA, U.S.A. Solvents used for sequencing were from Fluka and further purified as described Hunkapiller *et al.* (1983). Water used for the preparation of buffers was obtained from a Milli-Q water system (Millipore Corp.). All other chemicals were analytical grade.

Purification of sIgA and J chain

Rabbit sIgAs (f and g subclasses) were purified from milk as described previously (Frutiger *et al.*, 1986); sIgA (2.5 mg) was incubated with a 5-fold molar excess of dithiothreitol over cysteine content for 2 h at room temperature in 75 mM-Tris/HCl buffer, pH 8.6 (0.2 ml/mg of protein), containing 6 M-guanidinium chloride. Following alkylation with a 2-fold molar excess over dithiothreitol with iodoacetate, the samples were extensively dialysed against 0.1 M-ammonium bicarbonate buffer, pH 8.5, and then dried by rotary evaporation *in vacuo*.

Electroblotting. A 1 nmol portion of reduced and 1,5-I-AEDANS-alkylated sIgA (Gorman, 1987) was loaded on a 12%-acrylamide analytical slab gel (Laemmli, 1970). After electrophoresis, the resolved bands were transferred on to Immobilon membranes by the method of Matsudaira (1987) with the modification that 20 mM-Tris/HCl/192 mM-glycine buffer, pH 8.3, was used as transfer buffer. The J chain band was located under u.v. light and sliced out. After careful washing with water, the filter was kept at −20 °C until *N*-terminal sequencing was performed.

Preparative SDS/PAGE. Reduced and carboxymethylated sIgA samples (2.5 mg each) were loaded on vertical slab gels (0.1 cm × 20 cm × 20 cm) that had been cast in accordance with Laemmli (1970). After electrophoresis, the bands were detected by negative staining with $ZnCl_2$ (Dzandu *et al.*, 1988). The J chain band was sliced out and electro-eluted at 200 V for 6 h in 20 mM-Tris/HCl/192 mM-glycine buffer, pH 8.3, containing 0.1% SDS. The purified J chain samples were then extensively dialysed against water with the use of Spectrapor membranes with a cut-off of 3.5 kDa and concentrated to 50 μl by rotary evaporation in a Speed-Vac instrument.

SDS removal. SDS was removed from the samples by repeated extractions (3 × 1 ml) with anhydrous acetone/triethylamine/acetic acid/water (17:1:1:1, by vol.) in accordance with Königsberg & Henderson (1983). After two washings with 1 ml portions of acetone, the precipitate was carefully dried under a gentle stream of N_2 and redissolved in 60 μl of deionized 8 M-urea.

Proteolytic digestions. The purified J chain sample (2.4 nmol) was divided into six 10 μl aliquots before dilution of each to 50 μl with the appropriate digestion buffers: 0.1 M-ammonium bicarbonate buffer, pH 8.5, containing 0.1 mM-$CaCl_2$ for trypsin, chymotrypsin and endoproteinase Arg-C; 25 mM-Tris/HCl buffer, pH 8.5, for endoproteinase Lys-C; 50 mM-sodium phosphate buffer, pH 8.0, containing 20 mM-methylamine hydrochloride for endoproteinases Glu-C and Asp-N. A 0.2 μg portion of the appropriate enzyme was added to each aliquot and digestion was performed overnight at 37 °C.

Analytical procedures

Deglycosylation of J chain. A 0.5 nmol portion of purified J chain was dissolved in 20 μl of 250 mM-sodium acetate buffer, pH 5.5, and incubated overnight at 37 °C with or without 0.1 unit of *N*-Glycanase. Digested and control samples were loaded on a 10–22%-gradient SDS/polyacrylamide gel in order to assess the extent of deglycosylation.

Purification of peptides by reverse-phase h.p.l.c. Peptides derived from digestion with endoproteinases Asp-N, Glu-C, Arg-C and Lys-C and chymotrypsin were separated on an Aquapore RP300 column (1 mm × 250 mm) as detailed in the legend to Fig. 1. Tryptic peptides were initially fractionated on an Aquapore RP300 column (2.1 mm × 130 mm) at pH 7.0 and each peak was rechromatographed on an RP300 column (2.1 mm × 30 mm) at pH 2.0. For further details see the legend to Fig. 2.

Peptide characterization. *N*-Terminal sequence determination was carried out with a model 477A pulsed liquid-phase microsequencer from Applied Biosystems. Phenylthiohydantoin derivatives were analysed essentially as described by Fonck *et al.* (1986), except that an on-line microgradient system to the sequencer was used and that the 4 mm column was replaced with a 2 mm one. Carboxypeptidase Y digestions of *S*-carboxymethylated J chain were carried out at 24 °C for 10, 15 and 30 min (Frutiger *et al.*, 1986). Peptide hydrolysis was performed as reported previously (Frutiger *et al.*, 1986), and amino acid analyses were carried out by the procedure of Hughes *et al.* (1987).

RESULTS

Isolation of rabbit J chain

This was carried out by full reduction and alkylation of sIgA complex with iodoacetate followed by SDS/PAGE. After electroelution of the J chain band from two slab gels, SDS was removed by the procedure described in the Materials and methods section and about 5 nmol of *S*-carboxymethylated J chain (yield about 35%) was recovered, as assessed by amino acid analysis. The purity of the protein was better than 95%, as assessed by subjecting 300 pmol to automated Edman degradation.

Sequence determination

The following strategy was used: after digestion of *S*-carboxymethylated J chain with distinct endoproteinases that should specifically cleave at the amino or carboxy sides of aspartic acid, glutamic acid, lysine or arginine residues, peptides from the various digests were separated by micro-bore reverse-phase h.p.l.c. (results not shown) and rapidly screened by six to 14 Edman degradation cycles (two or three sequence determinations per day). From these 'shot-gun' experiments approx. 95% of the J chain sequence was determined. The remaining sequence gaps were filled by extended sequence determination of the endoproteinase-Asp-N-derived peptides (Fig. 1) and a thermolysin digest of the tryptic peptide T5 (Fig. 2). This was performed with the remaining 2 nmol of purified J chain and the results are reported in Table 1 and Fig 3. It should be emphasized that, in the present 'shot-gun' sequencing approach, the full characterization of each peptide (total length, *C*-terminal sequence and amino acid analysis) was omitted because three overlapping residues at least at each side of the peptides were clearly identified. The *N*-terminal sequence (after electroblotting) of unfragmented J chain was used to establish overlap between endoproteinase-Asp-N-derived peptides A1 and A4 (Fig. 1), and the *C*-terminal

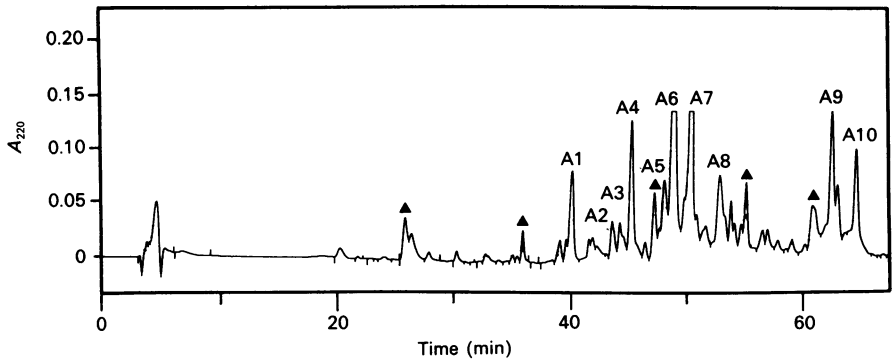

Fig. 1. Separation of the endoproteinase Asp-N digest derived from rabbit J chain on a micro-bore reverse-phase h.p.l.c. column

The fully reduced and *S*-carboxymethylated J chain was purified by electro-elution. After the removal of SDS, 400 pmol of the material was incubated overnight with 0.2 μg of endoproteinase Asp-N at 37 °C. The digest was applied to an RP300 column (1 mm × 250 mm) equilibrated with 0.1 % (v/v) trifluoracetic acid. Elution was performed at room temperature with a linear gradient of 0–30 % (v/v) acetonitrile in 60 min at a flow rate of 50 μl/min. Detection was at 220 nm. Each peak was subjected to Edman degradation, and the locations of the *N*-termini of the peptides (in parentheses) within the J chain sequence are as follows: A1 (1), A2 (79), A3 (9), A4 (9), A5 (104), A6 (104), A7 (79), A8 (31), A9 (51), A10 (31). No useful sequence information was obtained from the fractions symbolized with triangles (▲).

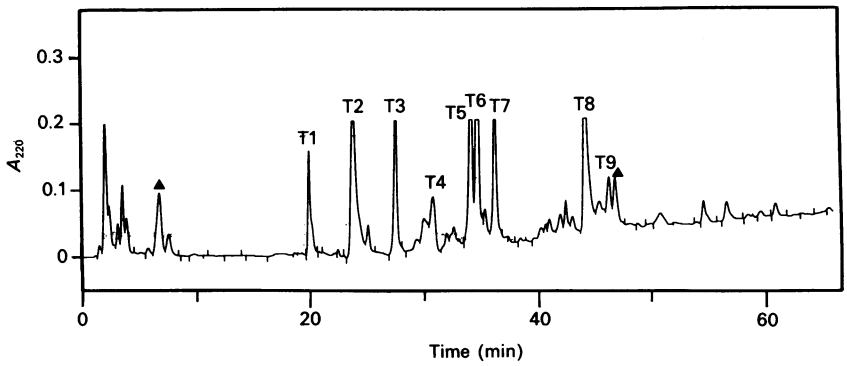

Fig. 2. Elution profile of the tryptic peptides of the J chain

A 400 pmol portion of fully reduced and *S*-carboxymethylated J chain was digested overnight at 37 °C with 0.2 μg of trypsin and applied to an RP300 column (2.1 mm × 130 mm) equilibrated with 20 mM-sodium phosphate buffer, pH 7.0, containing 10 mM-$NaClO_4$. Elution was with a gradient of increasing acetonitrile concentration [0–30 % (v/v) in 60 min]. The flow rate was 300 μl/min and the temperature was ambient. Each fraction was rechromatographed to remove salts on an RP300 column (2.1 mm × 30 mm) equilibrated with 0.1 % (v/v) trifluoroacetic acid. Elution was performed with linear gradient of 0–60 % (v/v) acetonitrile in 30 min at a flow of 300 μl/min. Each peak from this second chromatography was subjected to Edman degradation, and the locations of the *N*-termini of the peptides (in parentheses) within the J chain sequence are as follows: T1 (1 and 12), T2 (126), T3 (21), T4 (47), T5 (70), T6 (71), T7 (62), T8 (108), T9 (39). No sequence information was obtained from the fractions symbolized with triangles (▲).

sequence of *S*-carboxymethylated J chain was determined by carboxypeptidase Y digestion to be -Pro-Asp. On the other hand, the *C*-terminal tryptic peptide T2 (residues 126–136, Fig. 2), identical with peptide C4 (Fig. 3), was shown by amino acid analysis to contain two aspartic acid residues per peptide, thus confirming aspartic acid as the *C*-terminal residue of rabbit J chain.

The validity of the data obtained for the various digests was confirmed by amino acid analysis of isolated tryptic peptides (Fig. 2) that encompass 116 of the 136 amino acid residues in J chain. Table 2 provides convincing evidence that the present strategy is sufficient to warrant reliable unequivocal sequence determination.

Identification of the asparagine-linked carbohydrate

S-Carboxymethylated J chain was subjected to *N*-Glycanase digestion and analysed by SDS/PAGE before and after treatment (results not shown). A decrease in the apparent molecular mass from 25 kDa to 21 kDa was taken as evidence for the presence of carbohydrate in J chain. On the other hand, the lack of identification of an amino acid phenylthiohydantoin derivative for the residue at position 48 (Fig. 3) prompted us to treat peptide T4 (Fig. 2) with *N*-Glycanase and to sequence it after treatment: a clear aspartic acid residue was identified in position 2 of peptide T4, a finding that positively identifies an *N*-linked carbohydrate at position 48 in the consensus sequence Asn-Ile-Ser (Fig. 3). No other *N*-linked sugars were detected in the J chain sequence.

DISCUSSION

The possible biological roles for J chain are far from being understood. J chain, which is synthesized by plasma cells (Kaji & Parkhouse, 1974), appears to be disulphide-linked to the penultimate half-cysteine residue of two α chains in IgA (Garcia-Pardo *et al.*, 1981) and of at least one μ chain in IgM (Mestecky & Schrohenloher, 1974). Although J chain was found exclusively associated with secreted polymeric Igs, it does not appear to be

Table 1. Partial *N*-terminal sequence analysis of peptides derived from J chain by proteolysis

Yields are expressed in pmol of the amino acid phenylthiohydantoin derivative recovered from each degradation cycle. Cysteine was determined as the phenylthiohydantoin derivative of *S*-carboxymethylcysteine. R, A, G, K, C and T indicate peptides derived from endoproteinases Arg-C, Asp-N, Glu-C and Lys-C, chymotrypsin and trypsin respectively; NH2J indicates the *S*-carboxymethylated intact J chain; T5Th indicates the thermolysin-derived peptide from tryptic peptide T5. CHO indicates asparagine-linked carbohydrate at position 48.

Cycle no.	Amino acid	Recovery (pmol)				Cycle no.	Amino acid	Recovery (pmol)			
		A1	NH2J								
1	Glu	119	234			69	Lys	4.8	68	T5	
2	Asp	138	156			70	Lys	8.0	91	48	
3	Glu	66	135			71	Cys	6.9	112	35	
4	Ser	29	24			72	Asp		124	59	
5	Thr	30	33			73	Pro		68	35	
6	Val	3.1	51			74	Thr		54	19	
7	Leu		66			75	Glu			32	
8	Val	A4	21			76	Ile			22	
9	Asp	180	5.2			77	Glu		G2	13	
10	Asn	137	6.2			78	Leu	A7	214	22	
11	Lys	104	5.3			79	Asp	139	74	13	
12	Cys	86	5.5			80	Asn	101	55	13	
13	Gln	72				81	Gln	83	43		
14	Cys	111				82	Val	107	41		
15	Val	119				83	Phe	113	59		
16	Arg	10				84	Thr	55	53		
17	Ile	107				85	Ala	68	18		
18	Thr	63				86	Ser	32	22		
19	Ser	32				87	Gln	53	9		
20	Arg	5	T3			88	Ser	32			
21	Ile	74	218			89	Asn	37	T5Th		
22	Ile	83	229			90	Ile	49	124		
23	Arg	3	20			91	Cys	26	98		
24	Asp	36	227			92	Pro	39	110		
25	Pro	29	215			93	Asp	15	68		
26	Asp	27	137			94	Asp	22	72		
27	Asn	2.7	128			95	Asp	25	55		
28	Pro	3.2	125			96	Tyr	2.7	74		
29	Ser		51			97	Ser	2.7	18		
30	Glu	A8	54			98	Glu	1.4	57	G1	
31	Asp	130	58			99	Thr		10	27	
32	Ile	171	53			100	Cys		15	24	
33	Val	114	18			101	Tyr		22	20	C1
34	Glu	66	22			102	Thr			16	18
35	Arg	2.6				103	Tyr	A6		12	15
36	Asn	74				104	Asp	66		8	25
37	Ile	93				105	Arg	22			3.2
38	Arg	2.3				106	Asn	45			19
39	Ile	57				107	Lys	30		K3	
40	Ile	80				108	Cys	24		393	
41	Val	64				109	Tyr	44		490	
42	Pro	57				110	Thr	36		386	
43	Leu	45		C3		111	Thr	37		404	
44	Asn	33		108		112	Leu	31		495	
45	Thr	28		58		113	Val	26		229	
46	Arg	2.1	T4	29		114	Pro	23		377	
47	Glu	9.5	131	107		115	Ile	21		470	
48	Asn–CHO					116	Thr	15		301	
49	Ile	2.8	132	94		117	His	7.3		42	
50	Ser	A9	23	20		118	Arg	0.5		84	
51	Asp	113	25			119	Gly	9.4		187	
52	Pro	120	26			120	Val	6.0		41	
53	Thr	95	9.7			121	Thr	5.4		93	
54	Ser	56				122	Arg	0.4	R3		
55	Pro	70				123	Met	2.7	384		
56	Leu	63				124	Val	0.5	213		
57	Arg	4.6				125	Lys	0.3	444	C4	
58	Thr	45				126	Ala	0.8	282	88	
59	Glu	34	G4			127	Thr	1.1	140	46	
60	Phe	36	368			128	Leu	1.4	380	93	
61	Lys	13	190			129	Thr		110	34	
62	Tyr	33	332			130	Pro		216	42	
63	Asn	17	384			131	Asp		114	35	
64	Leu	27	405			132	Ser		36	12.6	
65	Ala	21	322			133	Cys		31	8	
66	Asn	19	248			134	Tyr		25	6.9	
67	Leu	25	282			135	Pro		4.8	2.7	
68	Cys	6.5	179			136	Asp				

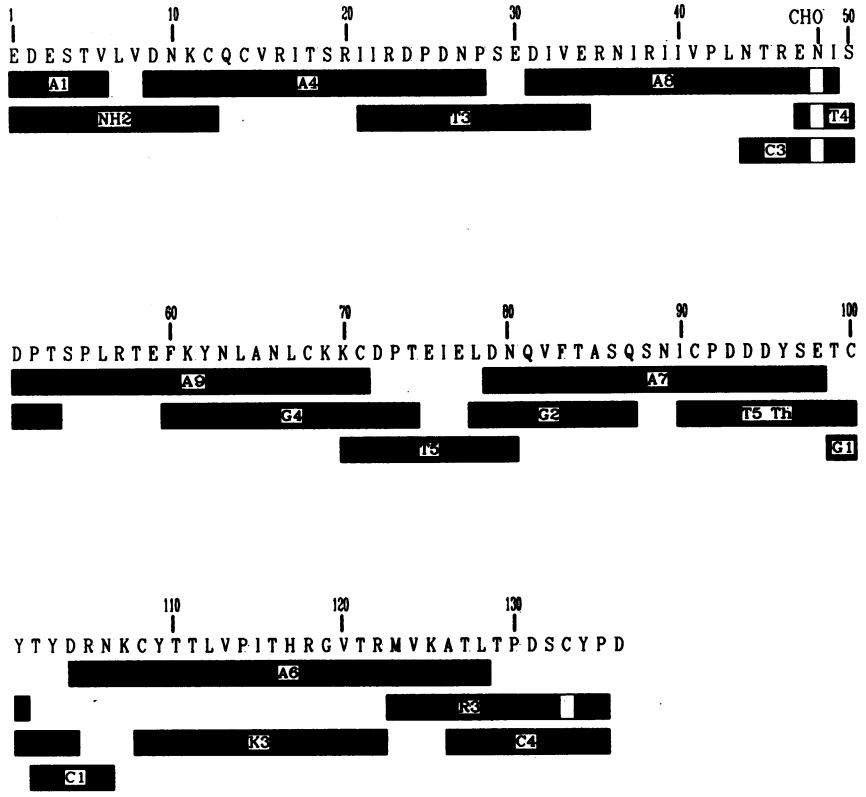

Fig. 3. Complete amino acid sequence of rabbit J chain

Representative overlapping peptides from various proteolytic digests were used to establish the full sequence of J chain. Peptide abbreviations are listed in Table 1. Small open rectangles indicate the lack of identification of residue at that position. Note that the black rectangles represent the extent of the sequence determined but not the length of the relevant peptides. CHO indicates asparagine-linked carbohydrate at position 48. See the text and Table 1 for details.

Table 2. Amino acid compositions of J-chain-derived tryptic peptides

Selected tryptic peptides purified as described in Fig. 2 legend were hydrolysed and subjected to amino acid analysis (see the Materials and methods section). The results are uncorrected for losses during the 20 h hydrolysis time. In peptides T3 and T9, cleavage of Ile–Ile peptide bond was not optimized by extending hydrolysis to 72 h. Data are expressed taking Arg or Lys as 1.0, except for peptide T2 in which Ala was taken as 1.0. Numbers in parentheses are the values expected on the basis of sequence data.

	Amino acid composition (mol of residue/mol)									
Peptide...	T1a	T1b	T3	T9	T4	T7	T5	T6	T8	T2
Position...	1–11	12–17	21–35	39–46	47–57	62–69	70–105	71–105	108–118	126–136
Amino acid										
Asp	3.2 (3)		4.0 (4)	0.8 (1)	1.7 (2)	2.1 (2)	8.2 (8)	7.8 (8)		1.9 (2)
Glu	1.6 (2)	1.1 (1)	1.9 (2)		1.0 (1)		5.0 (5)	4.8 (5)		
Cys*		1.7 (2)				0.8 (1)	2.7 (3)	2.6 (3)	0.6 (1)	0.7 (1)
Ser	0.8 (1)		0.8 (1)		1.7 (2)		2.7 (3)	2.7 (3)		0.7 (1)
Thr	0.9 (1)			0.8 (1)	0.9 (1)		3.9 (4)	3.5 (4)	2.6 (3)	1.7 (2)
Ala						1.2 (1)	1.2 (1)	1.4 (1)		1.0 (1)
Arg		1.0 (1)	2.2 (2)	1.0 (1)	1.0 (1)		1.0 (1)	1.0 (1)	1.0 (1)	
Pro			1.8 (2)	1.0 (1)	2.2 (2)		2.4 (2)	2.1 (2)	0.9 (1)	2.3 (2)
Val	2.1 (2)	0.9 (1)	0.7 (1)	0.9 (1)			1.1 (1)	1.2 (1)	0.9 (1)	
Ile			2.3 (3)	1.5 (2)	1.2 (1)		2.1 (2)	2.0 (2)	0.8 (1)	
Leu	1.0 (1)			1.1 (1)	1.1 (1)	2.0 (2)	1.2 (1)	1.3 (1)	0.9 (1)	1.1 (1)
Phe							1.1 (1)	1.1 (1)		
Lys	1.0 (1)					1.0 (1)	1.0 (1)			
His									0.9 (1)	
Tyr						0.9 (1)	2.8 (3)	2.6 (3)	0.8 (1)	0.9 (1)

* Determined as *S*-carboxymethylcysteine.

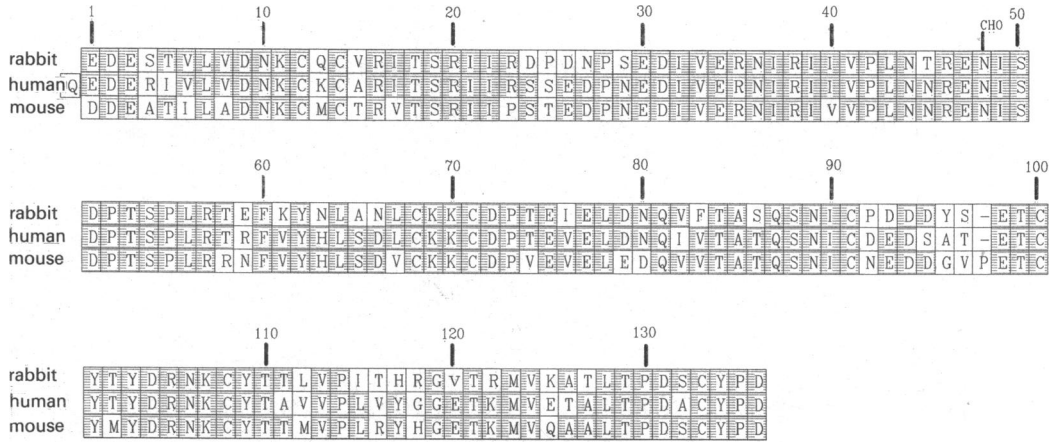

Fig. 4. Comparison of J chain sequences from rabbit, human and mouse

The human and mouse J chain sequences were taken from cDNA clones (Max & Korsmeyer, 1985; Matsuuchi *et al.* 1986). Amino acid substitutions between the sequences are illustrated at non-shaded positions. CHO indicates asparagine-linked carbohydrate at position 48. Numbering is according to the rabbit sequence.

required for IgM polymerization as such (Cattaneo & Neuberger, 1987; Davis *et al.*, 1988), since mutants [Ser-414]IgM and [Ser-575]IgM are assembled as circular pentamers and hexamers that lack J chain (Davis *et al.*, 1988). Similarly, J chain does not seem to be needed for Ig secretion (Cattaneo & Neuberger, 1987; Davis *et al.*, 1988), nor is it required for complement activation by polymeric Ig molecules (Kownatzki & Drescher, 1973; Davis & Shulman, 1989). It has been suggested (Brandtzaeg & Prydz, 1984) that only J-chain-containing Ig polymers could be transported through secretory epithelia into exocrine fluids. The use of J-chain-containing polymeric IgMs and of corresponding mutant IgM polymers that lack covalently attached J chain should possibly help resolve this question (Davis & Shulman, 1989). The understanding of the functions of J chain also requires the knowledge of its three-dimensional folding and of its exact covalent and non-covalent association within Ig polymers (Koshland, 1985). With this in mind, we report here the complete primary structure of the rabbit J chain derived from IgA.

Rabbit J chain sequence is aligned with its human and mouse counterparts in Fig. 4. It comprises 136 amino acid residues, compared with which the mouse sequence has an insertion of a proline residue between positions 97 and 98, and human J chain exhibits an extra *N*-terminal glutamine residue. One asparagine-linked carbohydrate unit only was found at position 48. The direct sequence comparison confirms the high degree of conservation in mammalian species, as well as in amphibians, whose partial sequence data have also been reported (Mikoryak *et al.*, 1988). Rabbit J chain is more closely similar to the human (73.5% identity) than to the mouse sequence (68% identity), whereas human–mouse comparison indicates 79% identity. Amino acid substitutions are clustered in four regions at positions 24–29, 59–66, 92–97 and scattered in positions 112–127. We used the computer program of Novotny & Auffray (1984) to predict the secondary structure of rabbit J chain; the series of sequence profiles (not shown) describing hydrophobicity, electrical charges, reverse-turn and β-propensities were found to be almost superimposable on those cumulative profiles illustrated for human and mouse J chains (Zikan *et al.*, 1985). Such profiles were consistent with the suggestion that J chain consists of eight β-strands organized into two anti-parallel sheets and folded as a single Ig-like β-barrel (Koshland, 1985). This model is attractive because the eight cysteine residues in J chain are located within the loops connecting both sheets, and thus are exposed to the solvent. This may explain why all cysteine residues are highly susceptible to mild reduction (in the absence of denaturing solvents). Indeed, we have found that mild reduction of rabbit sIgA with 20 mM-2-mercaptoethanol (which is less potent than dithiothreitol) for 30 min at 25 °C sufficed to cleave all disulphide bonds in rabbit J chain; a 5 mM concentration of 2-mercaptoethanol appeared to be the lowest one necessary for the release of J chain from the sIgA complex, as assessed by SDS/PAGE after alkylation with 1,5-I-AEDANS reagent. Further experiments are required to test whether such conditions may be selective enough for the reduction of only two putative inter-chain disulphide bonds linking J chain to α chains. The outcome is of critical importance because Cys-12 and Cys-14 have been postulated to form inter-chain disulphide bonding with α chains (Zikan *et al.*, 1985) in the β-barrel model (Koshland, 1985), whereas Cys-14 and Cys-68 would become the possible candidates in the two-domain model proposed by Cann *et al.* (1982).

The microsequencing strategy used to establish the rabbit J chain sequence deserves some technical considerations, imposed by the relatively small amount of starting material readily available. The J chain was electro-eluted from SDS/polyacrylamide gels, a procedure that obviates the tedious column separations used in the past (Mole *et al.*, 1977; Mikoryak *et al.*, 1988). From two such SDS/polyacrylamide gels 5 nmol of *S*-carboxymethylated J chain was recovered and this proved to be sufficient for the determination of the complete sequence. After the removal of SDS, 2.4 nmol of the material was divided into six aliquots and each was digested with one of the following proteinases: endoproteinases Asp-N, Glu-C, Arg-C and Lys-C, chymotrypsin, and trypsin. The resulting peptides were separated on a 1 mm-internal-diameter reverse-phase column and each one was subjected to partial sequence determination.

The information obtained from this first series of digests (95% of the full sequence) allowed us to focus our attention on those peptides that, when extensively sequenced, would permit rapid completion of the sequence determination.

We consider that the 'shot-gun' sequencing approach from a large number of peptides generated by several different proteolytic digests offers, for a small-size protein, a good, fast and reliable alternative to the corresponding gene-cloning strategy.

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