Sequence Analysis of Human J Chain. Amino Terminal Location of a Disulfide Bond Linking the Immunoglobulin Heavy Chain

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Received September 10,1976

Summary

Carboxyamidomethylated J chain was shown to be an excellent substrate for the enzyme, pyrollidone carboxylyl peptidase, which specifically removed the cyclized amino terminal glutamyl residue. J chain lacking the "blocked" PCA group was subjected to automated Edman degradation and the amino terminal amino acid sequence determined as: PCA-Glu-Asp-Glu-Arg-Ile-Val-Leu-Val-Asp-Asn-Lys-CMCys-Lys-CMCys-Ala-Arg. Previous studies by others have identified a disulfide bridge between the heavy chain of immunoglobulins and a tripeptide identical in composition with the sequence at positions 15-17 in the J chain. These two sets of data locate the linkage of immunoglobulin heavy chain with Cys 15 of the J chain.

Introduction

Improvements in the technology of automatic sequence determination, coupled with enhanced sensitivity in the detection and identification of PTH[1] amino acids, has allowed the delineation of rather large sequences with limited quantities of initial material. These achievements have been exemplified in numerous publications where 40-50 cycles have been analyzed from a single sequencer run (1-3). Regrettably, elucidation of the amino acid sequences for peptides or proteins which possess a N-terminal PCA residue has been restricted, due to the absence of a free amino

[1]Abbreviations: PTH, phenylthiohydantoin; PITC, phenyl isothiocyanate; PCAase, pyrollidone carboxylyl peptidase; PCA, L-2-pyrollidone-5-carboxylic acid; EDTA·Na_2, disodium salt of ethylenediamine tetraacetic acid; TCA, trichloroacetic acid; TPCK, 1-chloro-4-phenyl-3-tosylamino-2-butanone; dansyl chloride, 1-dimethyl-aminonaphthalene 5-sulfonyl chloride.

group necessary for the coupling reaction with PITC. Moreover, enzymatic or chemical cleavage on the amino terminal side of glutaminyl residues frequently permits the acid-catalyzed conversion of glutamine to a PCA moiety.

Chemical treatment of peptides has been only partially successful in the removal of PCA (4-6). Indeed, no felicitous chemical method has been forthcoming that has not resulted in concommital injury to the polypeptide backbone structure.

Pyrollidone carboxylyl peptidase, a ubiquitous enzyme found in many bacteria (7-9) and to a diminished extent in plants and animals (10-11), is capable of selective cleavage of the PCA residue in both peptides and proteins. Enzymatic hydrolysis of the J chain, with PCAase, has allowed the unambiguous identification of the N-terminal amino acid sequence, thus establishing the sequence for a potentially difficult segment of its primary structure.

Materials and Methods

Pyrollidone Carboxylyl Peptidase Digestion of J Chain. The pyrollidone carboxylyl peptidase, a kind gift of Dr. R. F. Doolittle, was purified through Sephadex A-25 (12). One A-25 pellet was dissolved in 0.5 ml 50 mM potassium phosphate buffer, pH 7.3, containing 10 mM β-mercaptoethanol and 1 mM EDTA·Na_2 and dialyzed against this buffer at 4°C for 4 hours with hourly changes (13). Completely reduced and alkylated J chain (14) was separately dissolved in 0.5 ml of dialysis buffer prior to the addition of the peptidase. The mixture was capped and incubated at 30°C for 48 hours, dialyzed extensively against cold distilled water, and lyophilized.

Amino Terminal Identification. The procedure described by Percy and Buchwald (15) was employed with slight modification. PCAase-treated J chain (0.5 mg) in 0.5 ml of freshly deionized 8 M urea was mixed with 0.15 ml 0.4 M phosphate, pH 7.2, and 0.25 ml dimethylformamide. This was followed by 5 mg dansyl chloride (Sigma) in 100 ul acetonitrile. The tube was thoroughly vortexed and the reaction allowed to proceed at room temperature for 30 minutes. Following the addition of 10 ml of 10% TCA, the contents were set on ice for 1 hour. The precipitate was collected by centrifugation, washed twice with 5 ml portions of acetone, and dried in vacuo. The

dansylated product was hydrolyzed for 20 hours at 108°C and the dansyl amino acids identified by two-dimensional chromatography on polyamide sheets (16).

Amino Acid Sequence. Analyses were performed with the Beckman 890C sequencer, by the method of Edman and Begg (17), utilizing the slow protein - 1M quadrol program described by Beckman Instruments (022574). PTH amino acids were identified by gas-liquid chromatography (3), thin-layer chromatography (18-19), and as the free amino acids following hydrolysis at 130°C with 6 N HCl for 20 hours. All sequencer reagents were of sequenal grade and were purchased from Beckman.

Paper Chromatography and Electrophoresis. 10 mg of J chain was digested with TPCK-trypsin (1:100) in 0.2 M NH_4HCO_3 and one mg was spotted onto identical sheets of Whatman 3 MM chromatographic paper. Peptides were resolved by one dimensional chromatography in a system consisting of n-butanol/pyridine/acetic acid/water (300:200:60:240) and electrophoresed at a right angle to the direction of chromatography in pyridine/acetic acid/water (1:10:289), pH 3.65 (20). One sheet was dipped with ninhydrin reagent; the other was sprayed lightly with starch-iodine (21). The remaining 8 mg was applied to two preparative peptide maps and the position corresponding to the single ninhydrin-negative, starch iodine-positive peptide was eluted with 6 N HCL and analyzed for amino acid content.

Amino Acid Analysis. Samples for amino acid analysis were hydrolyzed in sealed, evacuated tubes at 110°C in 6 N HCl for 20 hours, and lyophilized. Amino acid separation and quantitation was conducted with a Durrum DC-500 high speed amino acid analyzer.

Results and Discussion

The occurence of PCA as the amino terminus of J chain has heretofore severely limited the procurement of sequence data, as the PCA moiety is not reactive towards PITC. However, after incubation with highly specified PCAase, J chain was successfully coupled with PITC or dansyl chloride. Thus, the amino acid sequence could be established directly with the Beckman sequencer on the intact protein. Table 1 summarizes results for the N-terminal 17 amino acids of human J chain. PCA release

Table 1

Amino Acid Sequence Data for Human J chain

Residue Number	Sequencer Cycle	Amino Acid	nmoles Recovered	% Yield
1		PCA	-	-
2	1	Glu	170	34
3	2	Asp	149	30
4	3	Glu	151	30
5	4	Arg	a	-
6	5	Ile	225	45
7	6	Val	194	39
8	7	Leu	235	47
9	8	Val	184	37
10	9	Asp	113	23
11	10	Asn	b	-
12	11	Lys	b	-
13	12	CMCys	b	-
14	13	Lys	b	-
15	14	CMCys	b	-
16	15	Ala	b	-
17	16	Arg	a	-

[a]qualitative identification with phenanthrenequinone (28).

[b]qualitative identification by TLC and acid hydrolysis.

was not specifically examined; nevertheless, it is apparent that hydrolysis of the J chain had occurred with the peptidase, since the yields of PTH amino acids are typical of most sequencer runs. The susceptibility of the J chain to PCAase further substantiated PCA as its N-terminus, as opposed to an acylated or formylated amino acid.

A half cysteine was not encountered within the first five amino acids. This contradicts the conclusions of two other laboratories (22-23) which reported the isolation of ninhydrin-negative peptides of J chain, with composition identical to the first five residues identified in this laboratory, but with cysteine substituted for one of the glutamic acid residues. To conform our findings with the sequencer, a ninhydrin-negative, starch iodine-positive peptide was isolated from two dimensional tryptic maps, and was found to contain only glutamate, aspartate and arginine. More recently, our laboratory has employed low temperature acid hydrolysis at

aspartylprolyl peptide bonds of the J chain to yield four easily separated peptides (unpublished). One of these, consisting of 25 amino acids, did not demonstrate an amino terminal proline, but did contain two moles of carboxymethylcysteine per mole, presumably those carboxymethylcysteines located in positions 13 and 15 of the J chain sequence.

Mendez et al. (25) successfully utilized the diagonal electrophoresis technique to isolate a peptide in the J chain which was disulfide bonded to the C-terminal octapeptide of one of the α chain in IgA. The amino acid composition of this peptide was reported as cys, ala, arg, and is identical with the sequence identified at positions 15-17 of the J chain. Moveover, our laboratory has recently elucidated the amino acid sequence of the entire J chain (25 and unpublished), and has encountered this particular sequence of amino acids only once. These observations, together with those of Mestecky et al. (26-27) therefore, disclose a physical association between the amino terminus of J chain and the carboxy terminus of polymeric immunoglobulins.

Acknowledgment

This work was supported by grants AI 09153, BMS 74-11522, and AM 03555. The authors express their thanks to Mr. Eddie Finch for his excellent technical assistance

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