THE AMINO ACID SEQUENCE OF THE C- TERMINAL CYANOGEN BROMIDE PEPTIDE OF HUMAN J CHAIN

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SUMMARY: The carboxyl-terminal peptide obtained from human J chain treated with cyanogen bromide (CNBr)[1] has been isolated and the amino acid sequence determined as Val - Glx - Thr - Ala - Leu - Thr - Pro - Asx - Ala - CMCys - Tyr - Pro - Asx. Comparisons with the primary structures of human lambda, kappa, alpha, or mu chains failed to disclose analogous regions with these immunoglobulins.

Human J chain, molecular weight 15,600 (1) is a polypeptide containing approximately 130 amino acids and associated covalently with the polymeric forms of immunoglobulins, namely IgM, IgA, and S-IgA (2,3). The absence of J chain in all monomeric immunoglobulins examined, in addition to its stoichiometry of one chain per polymer (4,5) had initially implicated its function as joining the individual subunits of the polymeric immunoglobulins to form stable, viable, macromolecules (6). More recently, however, it has been suggested that the precise biological role of J chain may be to initiate and ultimately control the degree of polymerization of these proteins (7).

J chain synthesis parellels that of heavy and light chains in plasma cells secreting polymeric immunoglobulin (8-10). Since it had been adequately demonstrated that in these cells intracellular 7S IgM monomers were precusors of the secreted 19S pentamers (11) it was deduced that polymer assembly must transpire immediately before or at the time of secretion, perhaps in the cell membrane

[1] Abbreviations: CNBr, cyanogen bromide; NBS, N-bromosuccinimide

itself. Indeed, the data indicate that the availability of J chain is a major factor in controlling polymerization (12); that is, reassociation of partially reduced IgM in the presence of J chain led to an organized IgM pentamer, while reassembly in the absence of J chain induced aggregation (13). A satisfactory answer to the elusive question of why immunoglobulin assembly terminates in dimeric and tetrameric configurations for IgA, whereas pentamers are formed with IgM, has been lacking.

In order to provide information useful in the correlation of physical structure with biological function, we have undertaken studies to determine the primary sequence of human J chain.

This report presents part of our initial data, the elucidation of the carboxyl-terminal cyanogen bromide peptide.

MATERIALS AND METHODS

J Chain Purification. Monoclonal IgM obtained by repeated euglobulin precipitation from the plasma of a patient with Waldenstroms macroglobulinemia was used as a source of J chain. The isolation of J chain was carried out by the method of Kobayashi et al., (14) except that DEAE Sephadex (gradient from 0.03 M Tris-HCl containing 0.1 M NaCl, pH 9.0 to 0.03 M Tris-HCl containing 0.5 M NaCl, pH 9.0) and Sephadex G-200 chromatography in 5 M guanidine hydrochloride were substituted for the Sephadex G-100 step. The purified product demonstrated no antigenic determinants for human kappa, lambda, or mu chains when tested against appropriate antisera at a protein concentration of 10 mg/ml, but did react with an antisera specific for human J chain.

Sequence Analyses. The Edman-dansyl chloride technique described by Hartley (15) as scheme 3 was adopted except that benzene was substituted for n-butyl acetate, since the latter solvent resulted in significant peptide extraction. Successive amino acids were identified as their dansylated derivatives following thin-layer chromatography on polyamide sheets (16). Hydrazinolysis was performed as described (17).

Cyanogen Bromide Cleavage. Carboxyamidomethylated J chain was cleaved with

a 2-fold excess of cyanogen bromide in 70% formic acid for 4 hours. Gel chromatography through P-6 (Bio-Rad) in 0.1 M acetic acid - 0.1 M formic acid was employed to separate the C-terminal peptide from the remainder of the protein.

N-Bromosuccinimide (NBS) Cleavage. A one hour hydrolysis of the C-terminal peptide was performed in 50% acetic acid with a 3-fold excess of NBS (recrystallized twice from 2% acetone). Edman degradation and/or dansylation were performed directly following lyophilization.

Amino Acid Analyses. Acid hydrolyses were performed in 6N HCl at 108° for 20 hours prior to analyses on a Durrum amino acid analyzer using their single column high pressure system.

RESULTS

The elution profile obtained on P-6 fractionation of the cyanogen bromide digest of J chain is illustrated in Figure 1. The first peak which eluted near the void volume of the column had very nearly the same mobility as intact J chain on SDS polyacrylamide gels. In addition, its amino acid composition closely resembled that of J chain and possessed both homoserine and homoserine

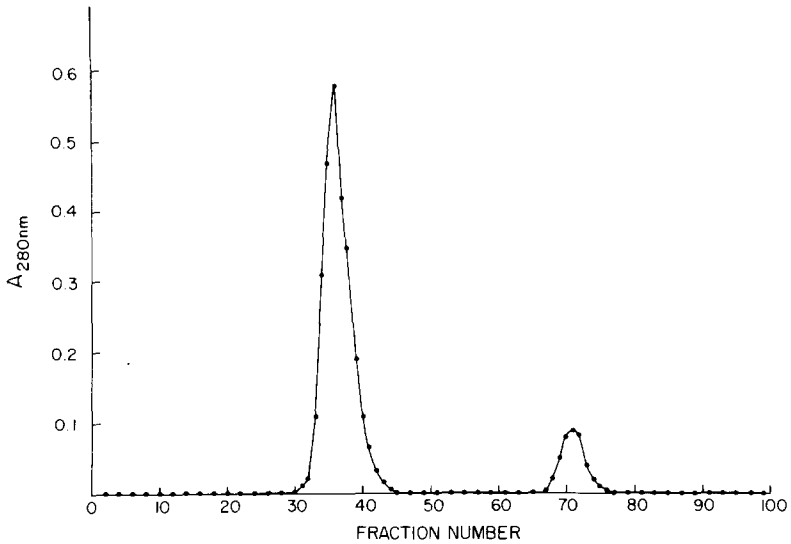

Figure 1. Gel chromatography of cyanogen bromide-treated J chain. Column dimensions were 1.7 x 110 cm and 3.3 ml fractions were collected.

lactone, indicating cleavage had occurred proximal to the carboxyl terminus of the protein. Acid hydrolysis of the second peak revealed two residues each of aspartate, threonine, alanine, and proline and one each of glutamate, valine, leucine, tyrosine, and carboxymethylcysteine.

Prolonged incubation of the small cyanogen bromide peptide with carboxypeptidase A (37^{o}, pH 7.5) failed to release a carboxyl-terminal amino acid as was the case with intact J chain. However, after hydrazinolysis a C-terminal aspartic acid could be identified.

Utilizing the manual Edman-dansyl chloride technique in which the N-terminal amino acid is removed and the newly generated N terminus is labeled with dansyl chloride, hydrolyzed and identified on polyamide sheets as the dansyl derivative, we have ascertained the sequence of the first eleven amino acid residues in the small cyanogen bromide peptide. Furthermore, subtractive degradation of this peptide after one complete cycle revealed the loss of valine, whereas the other amino acids remained essentially unchanged.

Selective cleavage of the peptide at tyrosine with NBS produced two peptides, one containing eleven amino acids and the other two residues. These could be degraded simultaneously to complete the sequence of the C-terminal peptide. The following sequence was determined: Val - Glx - Thr - Ala - Leu - Thr - Pro - Asx - Ala - CMCys - Tyr - Pro - Asx.

DISCUSSION

The interest generated in J chain from a biological viewpoint has advocated the initiation of experiments in our laboratory pertaining to the primary structure. Moreover, the ease with which the carboxyl-terminal peptide could be obtained motivated us to commence these studies with this peptide. Amino acid compositional analyses for the cysteine containing peptides (18) and for a glycopeptide (19) of J chain have been reported. These were shown to be clearly distinct from both the heavy and light chain-derived cysteine and carbohydrate peptides from IgA and IgM. From the sequence data above it is evident that the C-terminal peptide also has a unique sequence not previously found in any sector of the immunoglobulins.

Recent developments pertaining to J chain bonding to immunoglobulin have revealed disulfide linkage via a cysteinyl residue in the C-terminal octapeptide of the heavy chain in both IgA (20) and IgM (21). The striking homology of the penultimate octapeptides of the α and μ chains induced the authors to attribute those regions of the heavy chains with a tendency of the immunoglobulin monomers to polymerize. Lack of analagous regions in IgG and IgE could explain, at least in part, the existence of these classes of immunoglobulins in the monomeric state.

Although the physical mechanisms which pilot the assembly process have not yet been elucidated, procedures might be forthcoming through an application of primary structure data to disclose the interactions between J chain and its immunoglobulin partners.

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