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PRODUCTION OF V_L AND C_L FRAGMENTS FROM HUMAN BENCE-JONES κ CHAINS*

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SUMMARY: The peptic digest of a Bence-Jones κ chain dimer in 4.5% formic acid yields two large fragments. These have been separated on Ultrogel AcA 54 in 1% NH_4HCO_3. Amino acid analysis, SDS gel electrophoresis and dansyl N-terminal analysis indicate that the larger fragment is the C_L dimer and the smaller one is V_L. Similar results have been obtained from a second κ chain.

Light chains of immunoglobulins comprise two compact domains, referred to as constant (C_L) and variable (V_L) regions, connected by a short stretch of residues, known as the switch region. The elucidation of the processes of folding of light chains and of interactions of light and heavy chains in the assembly of immunoglobulins requires investigation of the properties of the isolated domains, including studies to determine whether they are independently folded, and whether binding interactions of the separated domains are comparable to those observed in the intact chains.

Although the switch region between domains is relatively exposed, there appear to be other factors involved in proteolytic susceptibility which are not at present understood. The preparation of variable and constant region fragments of κ and λ immunoglobulin chains was reported in 1969 by Solomon & McLaughlin (1) and by Karlsson et al. (2). Both groups found that some proteins were resistant to proteolysis without prior reduction and alkylation. Subsequently, Seon et al. developed a procedure for isolating half-chain fragments which employed a two-step peptic digestion of κ chains (3). Digestion at 37°C yielded the variable regions while enzymatic action at 55°C produced the

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constant region. At each temperature, the other half of the κ chain was extensively degraded. Recent attempts in our laboratory to apply this procedure to a human κ_1 light chain failed to produce the constant region. In this paper, we report studies on proteolysis of two human κ chains, and the production of intact V_L and C_L fragments from these chains.

MATERIALS AND METHODS

Ammonium sulfate precipitates of the Bence-Jones proteins Fro and Sil were generously donated to us by Professor Elliott Osserman. The proteins were purified by extensive dialysis (2 weeks) vs. 0.1M Tris, 0.01M EDTA pH 8.1, followed by ion-exchange chromatography on DE-32 or DE-52, with a gradient consisting of one liter 0.02M Tris, 0.002M EDTA pH 8.1 and one liter of the same buffer containing 0.5M KCl, and gel filtration on Ultrogel AcA 54 (4). They were stored at -20°C prior to use.

Constant region was prepared from the covalent dimer (4). In large scale preparations approximately 50 mg protein were dissolved in 2 ml 4.5% HCOOH (Fisher Cert. 90% HCOOH diluted 1:20). A pepsin solution (Sigma P-7000; 1mg/ml in 4.5% HCOOH) was prepared and 500λ added to the immunoglobulin solution. Digestion was continued for 15-20 min., then stopped by the dropwise addition of concentrated NH_4OH until a pH of 7-8 was registered on pH paper. The alkaline mixture was applied to an equilibrated Ultrogel AcA 54 column (1.7 x 112 cm) in 1% NH_4HCO_3. Approximately 1.6 ml fractions were collected at a flow rate of 40ml/hr. The absorbance at 278nm of each fraction was read on a Cary 14 spectrophotometer. Fractions were pooled and lyophilized as noted. All column chromatogrphy was performed at room temperature.

The identification of amino terminal residues was achieved by dansylation as described by Gray (5) and Gros & Labouesse (6). DNS-substituted amino acids were identified on 5 x 5 cm thin layer plates following 4-5 hours hydrolysis at 110°C as described by Woods and Wang (7) and later modified by Hartley (8) and by Weiner et al. (9).

Amino acid analyses were performed on a Beckman 120C amino acid analyzer according to the modifications of Edelman et al. (10) of the Spackman et al. procedure (11).

Polyacrylamide gel electrophoresis in SDS was performed by the method of Weber et al. (12) using an ORTEC Model 4200 slab gel system. Samples were dissolved in 4mM sodium phosphate, pH 7.2, containing 1% SDS. A few crystals of iodoacetamide were added and the samples boiled 2-3 min. before adding glycerol or sucrose and the tracking dye, 0.01% bromphenol blue or malachite green. 10-20μl protein were loaded in each well of 12-well gels, 20-40μl in each well of 6-well runs. The gels containing 7.5% polyacrylamide and 0.2% bisacrylamide.

To show that the band corresponding to light chain monomer in molecular weight did, in fact, comprise 2 C_L fragments joined by S-S bonds, an aliquot of the suspected C_L dimer was reduced for 1 hr with a 150-fold excess of dithiothreitol (DTT:protein) in 1% NH_4HCO_3. The reduced protein was immediately alkylated for 20 min. in the dark by the addition of 315-fold excess of recrystallized iodoacetamide (Sigma). The alkylated samples were diluted 1:1 with sample buffer and run on gels with their unmodified counterparts. Both proteins, Fro and Sil, were subjected to this procedure.

Gels were stained for 1 hr with a 0.25% solution of Coomassie Brilliant Blue R-250 (Schwarz/Mann) in 45% methanol, 9% acetic acid (v/v) or overnight in a 10 times more dilute solution of the same dye in the same solvent. Destaining was accomplished by soaking the gel in 5% methanol, 7.5% acetic acid for a week or longer, with frequent changes of the destaining solution.

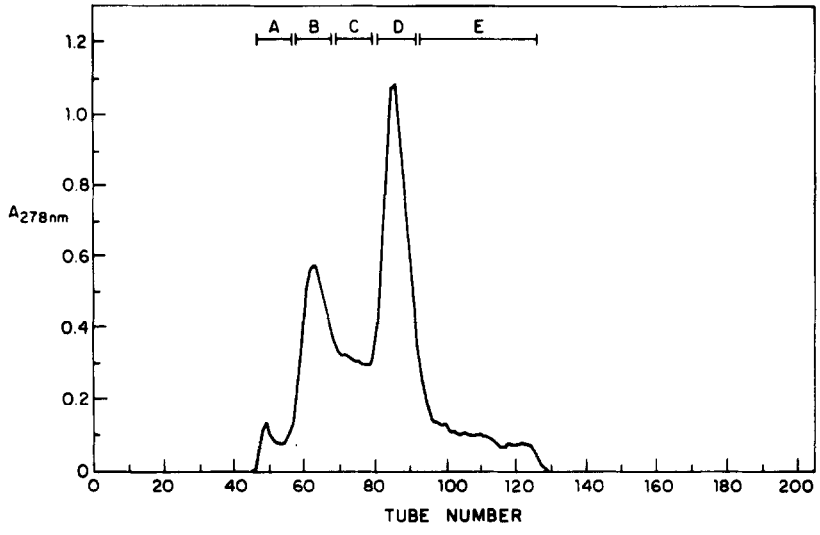

Figure 1. Fractionation of the peptic peptides of Fro Bence-Jones dimer by gel filtration on Ultrogel AcA 54 in 1% NH_4HCO_3. Column dimensions were 1.7 x 112 cm. The absorbance at 278nm is indicated by the solid line. Each tube contained 1.5 ml of the effluent.

RESULTS AND DISCUSSION

The column profile of a peptic digest of the Bence-Jones dimer is shown in Figure 1. 50 mg protein were digested and 42 mg recovered from lyophilization of the column effluent. Of this amount, 14.8 mg were isolated as V_L and 7.9 mg as C_L. Results were similar in a second experiment.

Polyacrylamide gel electrophoresis in SDS of a peptic digest is presented in Figure 2. The light chain monomer and V_L prepared by the method of Seon et al. (3) were run as standards. As expected, reduction and alkylation of the peptic digest yielded fragments of size equal to V_L. SDS gel electrophoresis of the material in peaks B and D of Figure 1, under identical conditions, showed that peak B had the same molecular weight as the light chain monomer while peak D was identical in weight to V_L.

The amino acid compositions of the effluent peaks of Figure 1 are listed in Table 1. Also recorded is analysis of C_L for κ chain (13) and V_L (computed by subtracting C_L from Fro L chain [4]).

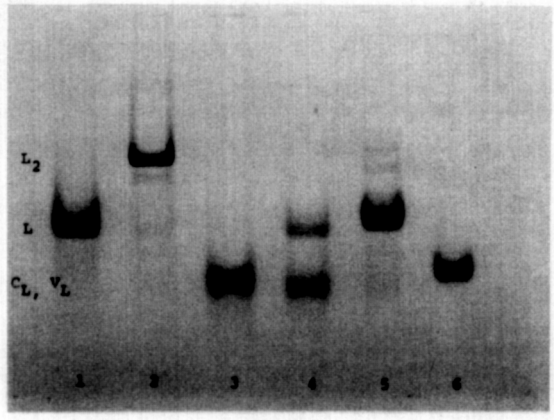

Figure 2. Polyacrylamide gel electrophoresis in SDS. First lane: reduced, alkylated BJ dimer; second lane: dimer prior to reduction and alkylation; third lane: pepsin digest following reduction and alkylation; fourth lane: peptic digest prior to reduction and alkylation; fifth lane: BJ monomer; sixth lane: V_L prepared by the method of Seon et al. (3). The 7.5% gel was run for about 2½ hours at 180 v using an ORTEC slab gel system. Reservoir buffer was 0.05M sodium phosphate, pH 7.2, 0.1% SDS. The gel was stained with 0.025% Coomassie brilliant blue and destained with 5% MeOH, 7.5% acetic acid.

Table 1

AMINO ACID COMPOSITIONS OF PEPTIC DIGEST FRAGMENTS OF BJ FRO DIMER[a]

Amino Acid	AcA 54B (C_L)	EuC_L (13)	AcA 54D (V_L)	Fro L (4,14)-C_L(13)
Lys	7.6	8	8.2	8
His	1.6	2	0.5	0
Arg	2.0	2	2.2	3
Asp	9.7	10	11.9	11
Thr	5.4	8	8.9	9
Ser	11.9	16	12.1	8
Glu	13	13	13	13
Pro	3.4	5	7.9	7
Gly	5.1	4	8.4	9
Ala	5.5	7	9.3	8
Cys*	1.7	3	1.6	2
Val	6.1	10	6.7	3
Met	0	0	0.9	1-2
Ile	1.4	1	6.9	6
Leu	6.9	8	10.4	10
Tyr	2.4	4	3.1	5
Phe	3.0	4	4.5	4

*Includes cysteic acid.

[a]In computing the number of residues of each amino acid, it was assumed that each fragment contained 13 Glu. The composition of C_L was based on its sequence and that of Fro V_L was computed by subtracting C_L from the amino acid analysis of Fro L and rounding off to whole numbers of residues.

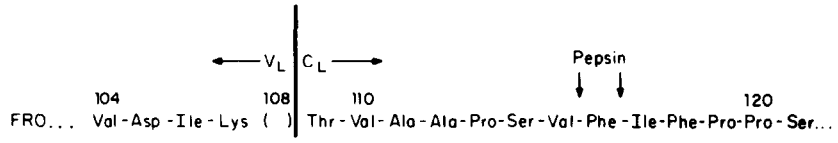

Figure 3. The probable site of peptic action on Fro L. The sequence of the V_L C-terminus was deduced from sequence homologies using the amino acid compositions of V_L tryptic peptides and is unconfirmed. Residue 108 was not isolated.

N-terminal residues of peaks B and D were determined using the techniques described above. Peak B showed several spots, one of which corresponded to Leu/Ile and a second, lighter one, to Phe. No Asp was observed. Peak D, on the other hand, showed a clear Asp endgroup.

From the results cited above, we conclude that peak B of Figure 1 corresponds to the constant region of the Fro κ chain and peak D to its variable region. The amino acid compositions listed in Table 1 show larger than expected amounts of Val and Ala for V_L and correspondingly smaller amounts of these amino acids in C_L. At the same time, the presence of Ile in C_L indicates that the pepsin must cleave to the left of residue 117 (Figure 3). These data, coupled with N-terminal analysis, point to residues 116-117 as the most likely site for enzymatic action. The sequence of residues 105-107 shown in Figure 3 was deduced from sequence homologies using the amino acid compositions of peptides (14) isolated from a tryptic digest of V_L. Reside 108 was not identified.

Figure 4 shows the results of a similar study carried out on the κ chain, Sil. Sil was found to have a Glu endgroup and is probably a member of the κ_3 subgroup of light chains. The 7.5% SDS gel compares the mobility of the peptic digest of Sil dimer with that of the unreacted dimer. To the left of each sample on the gel is an aliquot of the same sample taken after treatment with DTT and iodoacetamide as described above. Reduced, alkylated Fro IgG was run as a standard. The gel shows that enzymatic action results in the formation of one peptide with about the same molecular weight as the IgG light chain and a second peptide about half as large. Reduction and alkylation decrease the

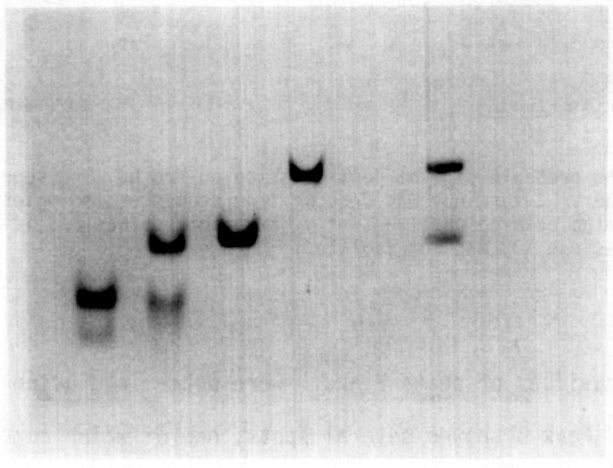

Figure 4. Polyacrylamide gel electrophoresis in SDS of the Sil peptic digest. Conditions for the gel are the same as described for Figure 1. Lane 1 contains the reduced, alkylated peptic digest of Sil dimer; lane 2 the dimer prior to reduction and alkylation; lane 3 contains reduced, alkylated Sil dimer and lane 4 the unmodified Sil dimer. In the extreme right lane is reduced, alkylated Fro IgG.

size of the larger peptide to that of the smaller one. These results are similar to those for Fro in Figure 2.

We have isolated V_L and C_L in substantial yield. The method avoids both the solubility problems encountered in the 55°C procedure and the complications of reduction and alkylation. Because the fragments are isolated from a single enzymatic digest in a single gel filtration step, the method is both rapid and economical in terms of the amount of protein consumed. Its application to a second κ chain, most probably from a different subgroup, indicates that its usefulness is not limited to Fro. Other immunoglobulin chains which are resistant to pepsin under the conditions given by the previous authors (1-3) might also be susceptible at a lower pH. Not only is the enzyme more active at pH 2 than at pH 3.5-4.5, but the immunoglobulin, itself, it likely to be slightly less compact and more amenable to proteolysis. Bence-Jones proteins which exist only as monomers or other immunoglobulin light chain monomers could be oxidized to the dimer form prior to peptic digestion.

Studies on the interaction of these fragments with heavy chains are in progress, as are investigations of rates of reoxidation of the reduced intra-domain cysteine residues.

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