

A MAJOR STRUCTURAL DIFFERENCE BETWEEN MEMBRANE-BOUND AND SECRETED IgM OF NORMAL MOUSE SPLEEN CELLS IS LOCATED IN THE C-TERMINAL REGION OF THEIR HEAVY CHAINS

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1. Introduction

The membrane μ chains from IgM molecules borne by normal polyclonal mouse B lymphocytes have an apparent molecular weight slightly larger than that of plasma cell secreted μ chains as revealed by SDS-PAGE [1,2]. The same observation is made in comparing unglycosylated μ_m and μ_s chains, synthesized in the presence of tunicamycin, which prevents the addition of asparagine-linked sugars [3,4]. We have shown that μ_m , but not μ_s chains, bind detergents, thus suggesting the presence of an hydrophobic region in the molecule [4]. We now show that these structural differences are, at least in part, located towards the C-terminal end of the μ chains.

2. Materials and methods

Membrane lymphocyte μ_m chains and plasma cell secreted μ_s chains were prepared from biosynthetically labeled purified mouse spleen lymphocytes, and from IgM polyclonal plasma cells, by immunoprecipitation followed by reduction, alkylation, and SDS-PAGE as detailed in [4–6]. μ Chains localized by radioautography were eluted from the gels by homogenization in 0.05% SDS–0.05 M NH_4HCO_3 buffer, lyophilized, and redissolved in 70% formic acid; they were desalted on Biogel P2 columns equilibrated in 70% formic acid and cleaved in the same solvent

Abbreviations: μ_s , secretory μ chains; μ_m , membrane-bound μ chains; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis; CNBr, cyanogen bromide; HPLC, high performance liquid chromatography; PTH, phenylthiohydantoin; amino acids are designated by the one-letter code

(1 ml) with 2.5 mg CNBr for 16 h at 4°C. The dried digests were run in 10–18% SDS–polyacrylamide gels, or in alkaline urea gels [7]. CNBr peptides were eluted from the gels and subjected to partial radioactive N-terminal sequence determination in a Beckman sequencer in the presence of 3 mg polybrene [8]. The same procedure was applied to CNBr peptide analysis of S-[2- ^{14}C]carboxamido-methylated μ chain from myeloma protein TEPC 183 IgM. Carboxypeptidase A digestions were done in 0.05% SDS–0.05 M NH_4HCO_3 buffer with 1 μg enzyme for 5–30 min at 37°C [9], and the identification of the released radioactive amino acids was carried out on an amino acid analyzer in the presence of a calibration mixture of cold amino acids.

3. Results and discussion

The reduced and alkylated chains obtained by immunoprecipitation of lysates of spleen small lymphocytes labeled under the pulse-chase conditions described (fig.1) show, by SDS-PAGE analysis, two μ chain bands. The slower-moving one represents μ_m chains since:

- (i) Its mobility in 1-D and 2-D gels is identical to that of surface radioiodinated ^{125}I - μ chains [6];
- (ii) It disappears selectively after pronase treatment of intact cells [3,6];
- (iii) It has an hydrophobic behaviour in the presence of detergents [4].

The kinetics of appearance of these two μ chains, as well as the peptide composition of the faster-moving μ chains, which is of the secretory type, are described in [6].

Biosynthetically labeled μ_m chains, localized by

radioautography (upper band in fig.1A), were eluted from preparative gels and compared to plasma cell μ_s chains. CNBr digests of these 2 types of chains were analyzed by SDS-PAGE (fig.1B) and by alkaline urea-PAGE (fig.2).

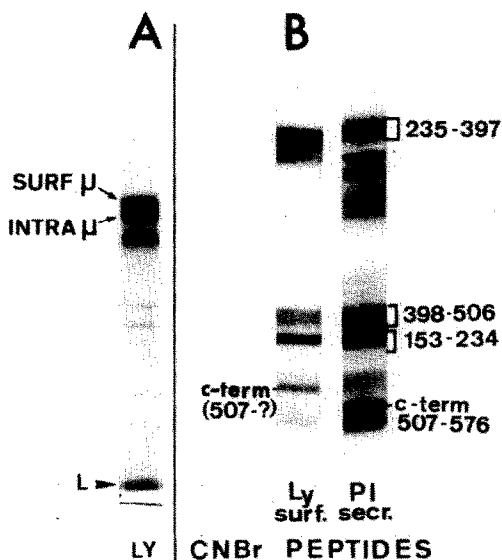


Fig.1. Autoradiographs of SDS-PAGE showing: (A) Reduced and alkylated anti-mouse Ig immunoprecipitate of a lysate of lymphocytes pulse-labeled for 2 h with [35 S]C and various [3 H]amino acids, then chased for 2 h. The chains migrating faster than the two μ chains are probably δ chains. L, light chains. (B) CNBr peptide patterns of biosynthetically labeled membrane (μ_m) and secreted (μ_s) chains on a 10–18% gradient SDS-PAGE. The numbered peptides were eluted and analyzed by partial N-terminal sequencing, followed by identification and quantitation of the radioactive PTH residues by HPLC analysis in the presence of a cold PTH calibration mixture. Note that μ_m C-terminal fragment (507-?) has an apparent molecular size larger than μ_s C-terminal peptide (507–576). The partial N-terminal sequence of the C-terminal peptide obtained from secretory myeloma MOPC 104E [10] and TEPC 183 μ chains is:

507
↓
PEPGAPGFYFTHSILT...

Secreted μ chains were labeled with [35 S]C, [3 H]F, [3 H]Y, [3 H]A, and [3 H]P, and μ_m chains, with [35 S]C, [3 H]F, [3 H]Y, [3 H]I and [3 H]L. Edman degradation released the various radioactive PTH from both C-terminal fragments in the expected positions; i.e., P₅₀₇, P₅₀₉, A₅₁₁, P₅₁₂, F₅₁₄, Y₅₁₅, F₅₁₆ for μ_s , and F₅₁₄, Y₅₁₅, F₅₁₆, I₅₂₀, L₅₂₁ for μ_m . In the case of μ_s chains, the band moving faster than the C-terminal peptide also represents the same C-terminal peptide, starting at position 507, but in which the internal M residue located 8 residues from the C-terminus has been cleaved.

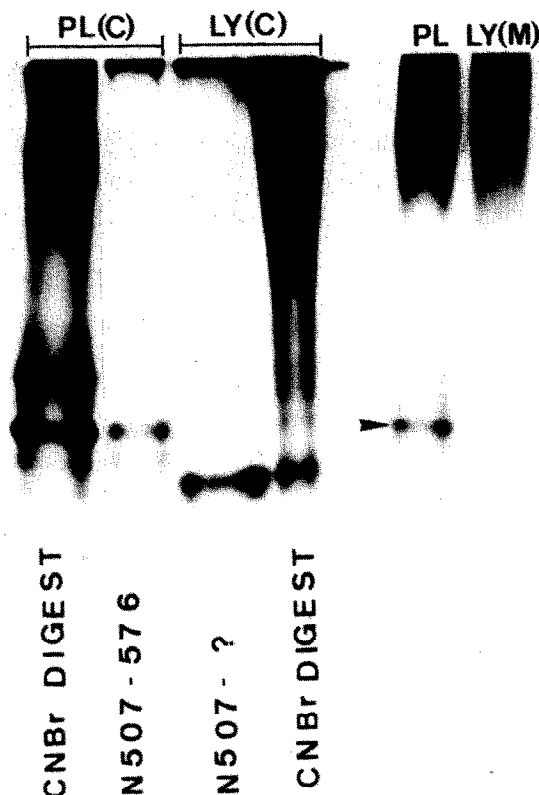


Fig.2. Autoradiography of alkaline urea-PAGE of the CNBr digests of secreted (PL) and membrane (LY) μ chains labeled with C or M. Slot 1,4, total CNBr digests of μ_s and μ_m chains, respectively; slot 2,3, the CNBr C-terminal peptides of these respective chains isolated from SDS gels as shown in fig.1B. Slots 5,6 correspond to the total CNBr digests of the 2 types of chains labeled with [35 S]M; μ_s C-terminal fragment is clearly labeled, but μ_m C-terminal peptide is not labeled.

On SDS gels, 5 major CNBr peptides are visible. They were eluted, characterized by partial N-terminal radioactive sequencing, and compared with the N-terminal amino acid sequences determined on comparable CNBr peptides obtained from mouse myeloma TEPC 183 μ chains. Their localization in the intact μ chain sequence was established on the basis of the known primary structure of mouse myeloma MOPC 104 E μ chain [10] (schematically numbered by their N- and C-terminal position in fig.1B). Two corresponding CNBr peptides from μ_m and μ_s chains display different mobilities: peptide 235–397, which we will not discuss further, and the C-terminal peptide, which in the case of μ_m has an apparent molecular weight higher than that of μ_s . Both fragments exhibit the same

N-terminal amino acid sequence and therefore span the end of the $C\mu_4$ domain (fig.1).

When analyzed on alkaline urea gels (fig.2), the CNBr digest of μ_s showed a distinct rapidly migrating peptide (slot 1) which, after elution, was identified by partial N-terminal sequence as the C-terminal peptide. This peptide comigrates with the C-terminal peptide isolated from SDS gradient gel and rerun in urea gel (slot 2, fig.2). The digest of μ_m showed a different pattern, with a faster migrating peptide (slot 4), which is the C-terminal peptide, since it exhibits an identical mobility with the μ_m C-terminal peptide eluted from SDS gels (slot 3); thus, the μ_m C-terminal fragment exhibits a higher net negative charge than that derived from μ_s , which could correspond to the observation that, on 2-D gels, the precursors of μ_m chains are found to be more acidic than those of μ_s chains [6].

Secreted μ chains contain a M residue located 8 positions from the C-terminus, which is partially cleaved by CNBr [10]. Indeed, CNBr digest of [^{35}S]M-labeled μ_s can be used to reveal the C-terminal peptide, both in SDS (not shown) or alkaline urea-PAGE (slot 5, fig.2). Conversely, the CNBr C-terminal peptide of [^{35}S]M-labeled μ_m is not detectable (slot 6, fig.2), indicating either that this peptide lacks the M residue, or that the C-terminal end of μ_m is cleaved by CNBr at a labile M residue. To explore this point, the C-terminal residue of this peptide was compared to that of intact μ chains by carboxypeptidase treatment.

Intact μ_s chains and μ_s C-terminal peptide sequentially released Y and C residues (fig.3), as expected from the known μ chain sequence [10]; however, μ_m released Y but not C residue. In control experiments, the faster-moving μ chains extracted from gels as shown in fig.1A, which corresponds to lymphocytic μ_s [6], released Y and C like plasma cell μ_s . This suggests that intact μ_m does not share the same C-terminal ... C-Y sequence as μ_s . The same observation was made with unglycosylated μ_m and μ_s obtained under tunicamycin treatment (not shown).

In contrast, the μ_m C-terminal CNBr peptide released neither C nor Y residues. This suggests that μ_m might contain a labile M residue close to the C-terminus, which was cleaved during CNBr digestion.

These data conflict with reports suggesting that μ_m of mouse B lymphoma cells end, like μ_s , with the same C-Y sequence [11-13]. This might result from a confusion between μ_m and μ_s chains and their precursors made by the same cell line, a mistake we made [3]

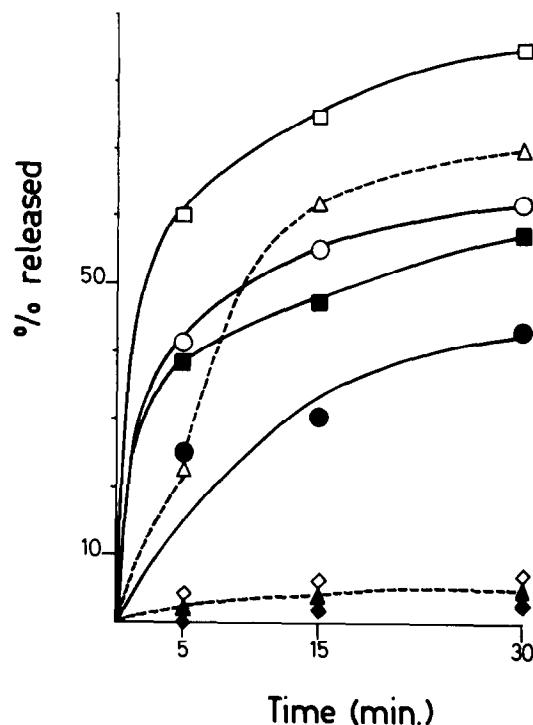


Fig.3. Carboxy-terminal tyrosine and cysteine released by carboxypeptidase A digestion from reduced and carboxamidomethyl- [^{35}S]C, [^3H]Y labeled μ_m , μ_s , and from the corresponding C-terminal CNBr fragments: (Open symbols) Y release; (full symbols) C release; (Δ , \blacktriangle) μ_m ; (\circ , \bullet) μ_s ; (\square , \blacksquare) μ_s C-terminal CNBr peptide; (\diamond , \blacklozenge) μ_m C-terminal CNBr peptide. C was identified as carboxamidomethyl- [^{35}S]C residue which elutes between D and S. The amount of radioactivity released by carboxypeptidase A ranged from 3–10-times above the background radioactivity found in control experiments run in parallel without enzyme. The yields of Y were computed on the basis of 16 Y residues/molecule of μ chains and 4 Y residues/C-terminal peptides. Yields for C release were expressed on the basis of 14 C residues/ μ chain and 2 C residues/C-terminal fragment.

before realizing that normal lymphocytes also synthesize the 2 chain types [4,6]. In a human lymphoma cell line [14] μ_m ended with a different C-terminal structure than that of μ_s . μ_m synthesized by a mouse lymphoma cell line ends with the sequence ... K-V-K (L. Hood, personal communication). This prompted us to examine if [^3H]K-labeled μ_m isolated from normal lymphocytes would release this residue under carboxypeptidase B treatment; this was found not to be the case (<2% of the expected release assuming that lysine was C-terminal). This observation is of

major interest since it suggests not only that μ_m differ from μ_s in their C-terminal regions, but that the genetic mechanisms responsible for this difference are not necessarily operating in an identical way in normal polyclonal lymphocytes and monoclonal B lymphoma cells. One possibility is that these mechanisms might be altered in some way in some B lymphoma cell lines. Alternatively, a variety of hydrophobic C-terminal tails of μ_m might exist, and be expressed in polyclonal normal B lymphocytes in contrast to monoclonal B lymphoma cells; however, this hypothesis appears less likely, in view of the quantitative kinetics of amino acids release shown in fig.3: here Y release from μ_s chains, which all end by Y, is closely comparable to that observed from μ_m chains, strongly suggesting that most, if not all polyclonal μ_m chains have a tyrosine residue at, or very close to the C-terminus.

Further studies have shown extensive peptide differences in the C-terminal half of μ_s and μ_m C-terminal CNBr fragments after proteolytic treatment, and will be reported elsewhere.

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