The Proteolytic Substructure of Light Meromyosin

LOCALIZATION OF A REGION RESPONSIBLE FOR THE LOW IONIC STRENGTH INSOLUBILITY OF MYOSIN*

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Light meromyosin (LMM), prepared by limited tryptic digestion of myosin, usually contains several polypeptide chains, LMM-A, LMM-B, and LMM-C in decreasing order of molecular weight estimated from sodium dodecyl sulfate-gel electrophoresis. Further limited tryptic digestion of LMM produces well defined fragments (Balint, M., Szilagyi, L., Fekete, Gy., Blazso, M., and Biro, E. N. A. J. Mol. Biol. (1968) 37, 317-330). Fragments LF-1, LMM-D, LF-2, and LF-3, with chain masses equal to 63, 56, 47, and 30 kDa, respectively, have been isolated by column chromatography. Based on the time course of the changes in the sodium dodecyl sulfate-gel pattern of the digests, chain masses estimated from sodium dodecyl sulfate-gel electrophoresis, and the NH2- and COOH-terminal sequences of the isolated peptides, the following scheme can be deduced.

LMM-A → LMM-B → LMM-C → LF-1

N

LMM-D → LMM-C → LF-2 → LF-3

C and N over the arrows indicate removal of residues from the COOH and NH2 terminus, respectively.

The positions of the peptides along the myosin heavy chain have been established by comparison with the published primary structures of rabbit skeletal (Eliotzina, M., Behar, K., Walton, G., and Trus, B. L. (1980) Fed. Proc. 39, 1579) and nematode myosin (Melchlan, A. D., and Karn, J. (1982) Nature (Lond.) 299, 226-231). LMM and fragment LMM-D are insoluble, whereas LF-1, LF-2, and LF-3 are soluble at low ionic strength. Their solubility properties, in conjunction with their locations along the myosin heavy chain, suggest that a relatively small stretch of peptide (chain weight, 5,000 Da) located about 100 residues from the COOH terminus of myosin heavy chain is responsible for the insolubility of LMM at low ionic strength.

The myosin molecule consists of two heavy chains and four light chains. The NH2-terminal half of each heavy chain is folded into a globular head, while the remainder participates in a rod-like coiled-coil α-helical structure. Limited enzymatic proteolysis occurs chiefly in two regions, one at the head-rod joint and the other within the rod. Cleavage in the first region results in subfragment-1 (S-1) and rod, while cleavage in the second region produces HMM1 and LMM (1). Subfragment-2 (S-2), which connects S-1 and LMM, can be produced by further digestion of HMM or rod.

S-1 or HMM can be further degraded into well defined fragments whose connectivities have been established (2-5). Some functionally important amino acid residues and binding regions for actin, light chains, and an ATP-analog have been located in these fragments (6-15). However, little is known about the substructure of LMM. As previously reported, after prolonged tryptic digestion of LMM, three distinct bands, viz., LF-1, LF-2, and LF-3, appear on electrophoresis under nonelectrolyte conditions (16). It was thought that the three fragments had the same COOH terminus and differed at the NH2 termini (16). The present work defines the precise relation of the fragments to each other and determines their positions within the LMM structure by means of SDS-PAGE and NH2- and COOH-terminal analysis.

During this work we found that LMM preparations are often heterogeneous, containing peptides LMM-A, LMM-B, and LMM-C; they differ slightly in their Mr and COOH termini.

The abbreviations used are: HMM, heavy meromyosin; LMM, light meromyosin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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NH$_2$-terminal sequence determinations indicate that LMM-D is formed by the removal of residues from the NH$_2$ terminus of LMM while LF-1 is formed by clipping at the COOH terminus of LMM. LF-2 and LF-3 result from successive degradation at the COOH terminus of LMM-D. Therefore, a short segment near the COOH terminus of LMM appears responsible for the low ionic strength aggregation of LMM and it may also play a role in the assembly of myosin into filaments.

**MATERIALS AND METHODS**

Myosin was prepared as described previously (17) from rabbit white muscle except that the final column purification step was omitted. LMM was obtained by digesting myosin with trypsin (Sigma, Type III), at 20 °C in a solution containing 0.5 M KCl, 30 mM Tris-HCl, pH 8.2, 10 mM 2-mercaptoethanol, and 1 mM CaCl$_2$ for 10 min with an enzyme-to-substrate ratio of 1:500 by weight. LMM made by this procedure will be referred to as conventional LMM. Myosin concentration was about 25 mg/ml and the digestion was stopped by adding soybean trypsin inhibitor (Sigma, Type I-S), twice the weight of trypsin. After dialyzing overnight at 4 °C against 15 volumes of 10 mM Na phosphate, pH 6.5, the digests were centrifuged for 1 h at 75,000 × g. LMM was obtained from the pellets after ethanol treatment according to Szent-Gyorgyi et al. (18).

Myosin rod was prepared based on the procedure of Weeds and Pope (19). Myosin (25 mg/ml) was digested with chymotrypsin (Sigma, Type II) with an enzyme-to-substrate ratio of 1:400 by weight in 0.12 M NaCl, 10 mM Na phosphate, pH 7.0, 1 mM EDTA, and 10 mM 2-mercaptoethanol, at 20 °C for 20 min; the digestion was stopped with 0.5 mM phenylmethylsulfonyl fluoride. The digests were dialyzed against a solution containing 40 mM NaCl and 5 mM Na phosphate, pH 6.5, and centrifuged at 75,000 × g for 1 h. Rod was obtained from the pellets after ethanol treatment.

Fragments of LMM were produced by digesting a suspension of conventional LMM (6 mg/ml in 30 mM KCl, 30 mM Tris-HCl, pH 8.0, and 10 mM EDTA) with trypsin, using an enzyme-to-substrate ratio of 1:120, at 20 °C for 16 min, and stopping the digestion with soybean trypsin inhibitor. The digests were dialyzed overnight at 4 °C against 10 mM KCl and 20 mM Na phosphate, pH 6.5, and centrifuged at 75,000 × g for 1 h. The pellet, designated insoluble fraction, was washed twice with the dialysis buffer, redissolved in 0.5 M KCl, and lyophylized. The washings, combined with the supernatant, designated the soluble fraction, were lyophylized after the KCl concentra-

**Fig. 1.** SDS-PAGE of LMM and tryptic fragments of LMM. A, SDS-PAGE of different LMM preparations. LMM was prepared from myosin by digesting with trypsin at an enzyme-to-substrate ratio of 1:500 for 10 min (I); 1:1000 for 5 min (II); 1:1000 for 3 min (III). The digestion was carried out at 25 °C in a solution containing 0.5 M KCl, 30 mM Tris-HCl, pH 8.2, 10 mM 2-mercaptoethanol, and 1 mM CaCl$_2$, and LMM was purified as described under "Materials and Methods." B, SDS-PAGE of tryptic digests of a mixture of LMM-A and LMM-B. LMM was prepared as above (II) and digested with trypsin at an enzyme-to-substrate ratio of 1:120 in 20 mM Tris-HCl, pH 8.0, 30 mM KCl, and 10 mM EDTA at 25 °C. The amount of protein used for gel electrophoresis was 15 and 25 µg for A and B, respectively.
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Fig. 2. Time course of the tryptic digestion of LMM and rod. Rod or LMM (6 mg/ml) was digested with trypsin at an enzyme-to-substrate ratio of 1:120 (w/w), in a solution containing 30 mM KCl, 20 mM Tris-HCl, pH 8.0, and 10 mM EDTA, at 25 °C. Aliquots were taken at various times and 20 and 30 μg of protein were placed on the gel for LMM and rod digests, respectively.

Protein concentrations were determined by biuret (24) or microbiuret (25) method, using bovine serum albumin as the standard.

RESULTS

Heterogeneity of LMM—SDS-gel electrophoresis of conventional LMM shows two bands, LMM-B and LMM-C, 72 and 68 kDa subunit mass, respectively (Fig. 1A). Even when myosin is digested with trypsin under very mild conditions, at an enzyme-to-substrate ratio of 1:1000, two bands appear on SDS-PAGE: the band of 72 kDa, which is obviously equivalent to a LMM-B chain, and the band of 78 kDa, designated LMM-A; the latter is not present if the digestion is carried out at an enzyme-to-substrate ratio of 1:500 (Fig. 1A, channels II and III). LMM-A, together with LMM-B and LMM-C.

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LMM-C, is also seen after the rod is digested with trypsin for 2 min (Fig. 2). LMM-A may be regarded as the longest form of LMM. As shown by the sequential appearance of LMM-B and LMM-C on digesting an LMM preparation, LMM-A is the precursor of LMM-B and LMM-C (Fig. 1B).

Tryptic Digestion of LMM and Rod—LMM-A present in the rod preparation quickly disappears as LMM-B and LMM-C are formed when trypsin is added (Fig. 2). Changes with time in the band pattern of tryp tic digests of LMM indicate that LF-1 and LMM-D are formed first, and are then further degraded into smaller fragments, viz. LF-2 and LF-3. The fragments in tryp tic digests of LMM and rod, LF-1, LMM-D, LF-2, and LF-3 have chain masses of 63, 56, 47, and 30 kDa, respectively (Fig. 2). Dimers of LF-1, LF-2, and LF-3 would have masses corresponding to those of the fragments previously studied under nondenaturing conditions and identified as LF-1, LF-2, and LF-3 (16). Fragments studied under nondenaturing conditions and identified as LF-1, LF-2, and LF-3 have chain masses of 63, 56, 47, and 30 kDa, respectively, appearing only in the digests of rod, must be derived from the S-2 segment.

Solubility Properties of the LMM Fragments—After dialysis against a solution of low ionic strength (see “Material and Methods”), the supernatant of an 8-min tryp tic digest of LMM contains the fragments LF-1, LF-2, and LF-3 (Fig. 3), while the precipitate contains LMM-D, undegraded LMM-B and LMM-C, and some LF-1 (Fig. 3). Since purified LF-1 is fully soluble at low ionic strength, we suggest that the appearance of LF-1 in the precipitate is due to the formation of hybrid dimers (see “Discussion”).

The Behavior of the LMM Fragments under Nondenaturing Conditions—Gel filtration on a Sephadex G-200 column led to the separation of three fragments from the soluble fraction (Fig. 4A, in Miniprint), viz. LF-1, LF-2, and LF-3, each containing a single peptide (Fig. 5, A and B, channels 1, 3, and 4). LMM-D was purified by gel filtration of the insoluble fraction (Fig. 4B, in miniprint); it is also apparently homogenous under both nondenaturing and denaturing conditions (Fig. 5, A and B, channel 2). On SDS-PAGE, the migration velocities, in increasing order, are LMM-B, LMM-C, LF-1, LMM-D, LF-2, and LF-3. Under non-denaturing conditions, the mobility of LMM-D is reduced: it becomes slower than LF-1 (Fig. 5, A and B, channel 6) and co-migrates with LMM-C (Fig. 5, A and B, channel 7). It should be noted that conventional LMM appears as a single band under nondenaturing conditions, although on SDS-gel electrophoresis, two peptides can be distinguished (Fig. 1 and Fig. 5, A and B, channel 5).

NH2-terminal and COOH-terminal Sequence Determination of LMMs and the Fragments—To throw further light on the relationship among the various kinds of peptides, NH2- and COOH-terminal sequence analyses were carried out. The same NH2-terminal sequence, viz. Gly-Lys-Gln-Ala-Phe-Thr-Gln-Gln-Ile-Glu-Glu-Leu-Lys-Arg-Gln . . . , was obtained (Table I) for all our LMM preparations, whether they contained mostly LMM-A (Fig. 1, channel III) or a mixture of LMM-B and LMM-C (Fig. 1, channel I). The same NH2-terminal sequence was found for LMM-C isolated from the insoluble fraction of the tryp tic digests of LMM. These results indicate that the NH2-terminus of LMM-A remains intact and it is the residues in the COOH-terminal region that are removed as LMM-A is degraded to LMM-B and finally to LMM-C. The NH2-terminal region of LF-1 is identical with that of LMM which shows that a segment is removed from the COOH-terminal region of LMM-C when LF-1 is formed. LMM-D, LF-2, and LF-3 have the same NH2-terminal sequence, Asn-Phe-Asp-Lys-Ile-Leu-Ala-Glu-Trp-Lys-His-Lys-Tyr-Glu-Glu . . . , which however differs from that of LMM. Thus, these fragments are formed as a result of clipping both from the NH2-terminal and the COOH-terminal regions of LMM.

When a preparation of LMM containing mostly LMM-A was treated with CpA, no amino acid was released. However, Arg was released when CpB was used and Leu and Lys were also released when both CpA and CpB were used. Thus, the COOH-terminal sequence of LMM-A is . . . Lys-Leu-Arg.

![Fig. 3. Solubility of LMM fragments in low ionic strength buffer.](image)

**Table I**

| Peptides  | NH2-terminal sequence | COOH terminus |
|-----------|-----------------------|---------------|
| LMM-A     | G-K-Q-A-F-T-Q-Q-I-E   | K-L-R         |
| LMM-B*    | G-K-Q-A-F-T-Q-Q-I-E   | L-K           |
| LMM-C     | G-K-Q-A-F-T-Q-Q-I-E-L-K-R-Q-L-E-E   | K               |
| LF-1      | G-K-Q-A-F-T-Q-Q-I-E   | R             |
| LMM-D     | N-F-D-K-I-L-A-E-W-K-H-K   | A-E-L         |
| LF-2      | N-F-D-K-I-L-A-E-W-K-H-K-Y-E-E-T-H   | R             |
| LF-3      | N-F-D-K-I-L-A-E-W-K-H-K-Y-E-E-T-H   | A-E-L-E-A     |

*The NH2-terminal sequence determination of LMM-B was carried out on a LMM preparation containing LMM-B and LMM-C.*
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Using the same technique, . . . Leu-Lys was identified as the COOH terminus of LMM-C; only Lys was released from LF-1 or LF-2 and Arg from LMM-D or LF-3 (Table I).

**DISCUSSION**

Some peptides in tryptic digests of the rod and LMM are identical. Additional ones in the digests of rod correspond to the S-2 which is at the NH$_2$-terminal portion of the rod, and its fragments. Thus the 58-kDa peptide is presumably the long S-2 (19, 26, 27), while the 37-kDa peptide can be identified as the short form of S-2 (28, 29). It has been shown that it is the COOH terminal end of long S-2 that is removed when short S-2 is formed (30). The fact that each LMM fragment, viz. LF-1, LF-2, LF-3, and LMM-D, isolated by column chromatography under nondissociating conditions shows only one band on SDS-PAGE suggests that the two chains that make up LMM fragments are very similar, if not identical. The molecular weights of the dimers of LF-1, LF-2, and LF-3 calculated from the apparent chain weights based on the mobilities on SDS-PAGE are 126,000, 94,000, and 60,000, which agrees with the previously reported values for LF-1, LF-2, and LF-3, respectively (Table II, in Miniprint).

The interpretation of the digestion pattern of LMM appears, at first glance, difficult because of the existence of heterogeneity in LMM preparations, their homogeneity on a nondenaturing gel notwithstanding. We have shown here that the chains denoted as LMM-B and LMM-C result from the sequential degradations of LMM-A at the carboxyl terminus. On the basis of information on the NH$_2$- and COOH-terminal sequences alone, the following relationship among the fragments can be deduced: LMM-A → LMM-B → LMM-C → LF-1 → LF-2 → LF-3 and LMM-D → LF-2 → LF-3. The amino acid sequence of the rod portion of nematode myosin has recently been deduced from the DNA sequence (31). Based on the homology between these two species, the common NH$_2$ terminus of LMM-A, LMM-B, LMM-C, and LF-1 is placed at residue number 467 which would give 466 and 632 amino acids for S-2 and LMM, respectively (Fig. 6). The common NH$_2$ terminus of LMM-D, LF-2, and LF-3 is placed at residue number 611, suggesting a stretch of about 144 residues between the NH$_2$ termini of the two groups of peptides. The peptides are aligned with the nematode sequence so as to obtain the greatest number of identical residues. It should be emphasized that the identity of the NH$_2$-terminal sequences within each of the two groups of fragments and the differences between the two groups of fragments rest on direct sequence data and are independent of the homology assignment between rabbit and nematode myosin. If one takes into account the molecular weight difference between LMM-B and LMM-D and the temporal changes in the intensities of the peptide bands in digests of rod and LMM (Fig. 2), it appears that LMM-D is derived from LMM-B directly by the removal of a stretch of amino acids from the NH$_2$ terminus. The same applies to the relationship between LF-1 and LF-2; the fact...
that LF-1 and LF-2 differ about 17 kDa in their chain weights suggests that only a small segment, if any, was removed at the COOH terminus (Fig. 7). The most likely relationship among the fragments is represented by the following scheme.

\[
\text{LMM-A} \rightarrow \text{LMM-B} \rightarrow \text{LMM-C} \rightarrow \text{LF-1}
\]

\[
\text{LMM-D} \rightarrow \text{LMM-C} \rightarrow \text{LF-2} \rightarrow \text{LF-3}
\]

C and N over the arrows indicate removal of residues from the COOH and NH₂ termini, respectively.

Comparison of the COOH-terminal sequence of LMM-A with the recently published sequence of the COOH-terminal region of the rabbit heavy chain (32, 33) indicates that even the longest form of LMM has lost 14 amino acids from the original COOH terminus of myosin heavy chain (Fig. 7). Elzinga et al. (32) have reported that in the LMM preparation they used, 38 residues from the COOH terminus of heavy chain were missing. The COOH-terminal sequence of LMM-C, Leu-Lys, taken in conjunction with the molecular weight of the peptide and the known sequence of the LMM portion (32, 33), suggests that it has lost 129 residues from the COOH terminus of rod. The fact that one of the cleavage sites, that between 969 and 970 (COOH terminus of LMM-C), is located at a region where a stable coiled-coil α-helical structure is interrupted (31) suggests that susceptibility to proteolytic enzyme is related to the conformational characteristics.

The fact that LMM-C is insoluble, while LF-1, whose NH₂-terminal sequence is identical to that of LMM-C, but lacks a segment at the COOH terminus, is soluble at low ionic strength leads to the conclusion that the proteolytic removal of a peptide at the COOH terminus, following the formation of LMM-C, renders the fragments soluble. Or, conversely, the presence of a small region (5 kDa, the difference in chain mass of LMM-C and LF-1) close to the COOH terminus of the myosin heavy chain is responsible for the self-association property of the myosin rod and LMM (Fig. 7), and, by inference, for filament formation in vivo. (The same conclusion can be drawn based on the analogous situation: LMM-D and LF-2 have identical NH₂-terminal sequence, however, only LF-2 which lacks a segment at the COOH terminus is soluble at low ionic strength). This is further borne out by the fact that the smaller fragment, LMM-D, that contains this COOH-terminal region is insoluble, while LF-1, which contains a larger portion of the rod but lacks the segment near the COOH terminus, is soluble at low ionic strength. Although some LF-1 is found in the insoluble fraction, it can be explained by postulating the existence of "hybrid" LMM-C/LF-1 dimers or LMM molecules with one of the chains nicked. The existence of such hybrids would suggest that the presence of the relevant region in one of the two chains is sufficient to produce the insolubility at low ionic strength.

We have noted in comparing the order of migration velocities of the fragments in nondenaturing and SDS gel, that LMM-D (56 kDa) migrates more slowly than LF-1 (63 kDa) under nondenaturing conditions. The reversed order of mobilities suggests that the COOH-terminal region responsible for the insolubility has a lower density of net negative charge.

At the time this paper was completed, a report appeared (34) in which the authors also reached the conclusion that a relatively small stretch of the heavy chain is responsible for the insolubility of LMM fragments. They, however, have not been able to decide whether the location of the solubility determining region was close to the S-2/LMM joint or to the COOH terminus of the heavy chain. Our results clearly show it is located near the COOH terminus.

Acknowledgments—We wish to thank Dr. Ed Morris for helping us to use the computer to search for homology between the primary sequence (31).

Alignments were established by using a computer program to search for maximum homology. This residue corresponds to number 467 in nematode sequence; a the NH₂ terminus of LMM and LF-1; b this residue corresponds to number 611 in nematode sequence; c the NH₂ terminus of LMM-D, LF-2, and LF-3.

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FIG. 7. The partial NH₂-terminal sequence of LMM and its fragment aligned with nematode myosin sequence (31). Alignments were established by using a computer program to search for maximum homology. This residue corresponds to number 467 in nematode sequence; a the NH₂ terminus of LMM and LF-1; b this residue corresponds to number 611 in nematode sequence; c the NH₂ terminus of LMM-D, LF-2, and LF-3.

FIG. 6. The partial NH₂-terminal sequence of LMM and its fragment aligned with nematode myosin sequence (31). Alignments were established by using a computer program to search for maximum homology. This residue corresponds to number 467 in nematode sequence; a the NH₂ terminus of LMM and LF-1; b this residue corresponds to number 611 in nematode sequence; c the NH₂ terminus of LMM-D, LF-2, and LF-3.
structure of rabbit skeletal and nematode myosin. We also thank Katalin Kurucz Varadi for excellent technical assistance.

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Table III. Amino Acid Compositions of LM-6 and LM-8 Fragments

| Amino Acid | LM-6 | LM-8 |
|------------|------|------|
| Asp        | 3.2  | 3.3  |
| Glu        | 3.3  | 3.0  |
| Lys        | 3.8  | 3.7  |
| Thr        | 2.1  | 2.4  |
| Ile        | 2.0  | 2.1  |
| Val        | 2.3  | 2.3  |
| Met        | 2.1  | 2.2  |
| Leu        | 3.9  | 4.0  |
| Phe        | 3.8  | 3.8  |
| Tyr        | 2.9  | 2.9  |
| Ser        | 3.6  | 3.6  |
| Thr        | 1.7  | 1.7  |
| His        | 1.1  | 1.2  |
| Lys        | 1.9  | 1.0  |
| Gln        | 1.5  | 1.5  |
| Trp        | 0.6  | 0.6  |
| Pro        | 9.8  | 9.2  |
| Cys        | 5.6  | 5.6  |
| Asn        | 1.5  | 1.5  |
| Arg        | 1.0  | 1.0  |
| Gly        | 11.0 | 11.5 |
| Ala        | 11.5 | 11.1 |
| Thr        | 11.0 | 11.0 |
| Val        | 10.9 | 10.5 |
| Ser        | 10.9 | 10.4 |
| Leu        | 10.4 | 10.4 |
| Phe        | 10.4 | 10.4 |
| Tyr        | 10.4 | 10.4 |
| Thr        | 10.4 | 10.4 |
| His        | 10.4 | 10.4 |
| Lys        | 10.4 | 10.4 |
| Gln        | 10.4 | 10.4 |
| Trp        | 10.4 | 10.4 |
| Pro        | 10.4 | 10.4 |
| Asn        | 10.4 | 10.4 |
| Arg        | 10.4 | 10.4 |

N-Terminal Sequence Determination

For each peptide subjected to Edman degradation, 2 mg of the Fmoc-amino acids were used for quantitation by high-performance liquid chromatography (HPLC). The rate of the reaction was calculated in moles of peptide released per hr, and the rate of reaction was determined. No corrections were made for the recovery rate of each Fmoc-amino acid during hydrolysis. The reaction was stopped by adding 2 M guanidine hydrochloride and 2 M TCA, which were used as internal standards for the amino acid analysis after the reaction was stopped. In Exp. 2, 10 µg of tryptophan was added to 2 ml of solution containing 2 mg of LM-6, 0.5 M N-ethylmaleimide, pH 7.5, and 0.5 M NaCl, which were used as control standards for the amino acid analysis after the reaction was stopped. The reaction was stopped after 3 hr by acidification to pH 2 or 3. All samples were used for amino acid analysis. The final weight of the sample was 10 mg. 2 µg of amino acids released are equivalent to 0.5 µmol.

Table IV. Sequencing Data for LM-C (50 cycles)

| Residue Identified | Sequence Number | Fraction Number |
|--------------------|-----------------|-----------------|
| 1                  | Ala             | 19.5            |
| 2                  | Gly             | 19.9            |
| 3                  | Val             | 20.3            |
| 4                  | Glu             | 20.5            |
| 5                  | Glu             | 20.8            |
| 6                  | Glu             | 21.0            |
| 7                  | Ala             | 21.2            |
| 8                  | Ala             | 21.4            |
| 9                  | Ala             | 21.6            |
| 10                 | Ala             | 21.8            |
| 11                 | Ala             | 22.0            |
| 12                 | Ala             | 22.2            |
| 13                 | Ala             | 22.4            |
| 14                 | Ala             | 22.6            |
| 15                 | Ala             | 22.8            |
| 16                 | Ala             | 23.0            |
| 17                 | Ala             | 23.2            |
| 18                 | Ala             | 23.4            |

Table V. Sequencing Data for LM-C (50 cycles)

| Residue Identified | Sequence Number | Fraction Number |
|--------------------|-----------------|-----------------|
| 1                  | Ala             | 19.5            |
| 2                  | Gly             | 19.9            |
| 3                  | Val             | 20.3            |
| 4                  | Glu             | 20.5            |
| 5                  | Glu             | 20.8            |
| 6                  | Glu             | 20.8            |
| 7                  | Ala             | 20.8            |
| 8                  | Ala             | 20.8            |
| 9                  | Ala             | 20.8            |
| 10                 | Ala             | 20.8            |
| 11                 | Ala             | 20.8            |
| 12                 | Ala             | 20.8            |
| 13                 | Ala             | 20.8            |
| 14                 | Ala             | 20.8            |
| 15                 | Ala             | 20.8            |
| 16                 | Ala             | 20.8            |
| 17                 | Ala             | 20.8            |
| 18                 | Ala             | 20.8            |

Table VII. Amino Acids Released from the Cationic of LM-C

| Exp. | Cation | Amino Acid | Released (%) | Control | Released (%) |
|------|--------|------------|--------------|---------|--------------|
| 1    | NaCl   | Leu        | 11.0         |         | 10.0         |
| 2    | Qb+0.8 | Leu        | 10.8         |         |              |

Table VIII. Amino Acids Released from the Cation of LM-C

| Exp. | Cation | Amino Acid | Released (%) | Control | Released (%) |
|------|--------|------------|--------------|---------|--------------|
| 1    | NaCl   | Leu        | 11.0         |         | 10.0         |
| 2    | Qb+0.8 | Leu        | 10.8         |         |              |
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