Variability in the Amino Terminus of Myosin Light Chain 1†

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ABSTRACT: Three naturally occurring variants of myosin light chain 1, type I, II, and III from avian fast-twitch muscle, have been analyzed by reverse-phase HPLC peptide mapping and amino acid sequencing. Difference peptides were absent from accompanying digests of the related protein, myosin light chain 3, indicating that the heterogeneity was located in the N-terminal 50 residues unique to light chain 1. The type II variant possessed the previous published sequence for the protein [Nabeshima Y., Fujii-Kuriyama, Y., Muramatsu, M., & Ogata, K. (1984) *Nature (London) 308*, 333–338]. The type I variant, which migrates faster than the type II on SDS gene electrophoresis, contained a Pro → Ala substitution at residue 15, turning the Lys-Pro-$(Ala)_5$(Pro-Ala)$_7$ stretch in this region into Lys-Pro-$(Ala)_7$(Pro-Ala)$_6$. The type III variant, which migrates just faster than the type I, had an $(Ala)_2$ deletion in the $(Ala)_5$ run, yielding Lys-Pro-$(Ala)_3$-(Pro-Ala)$_7$. As indicated by the SDS gel migration rates, the type I and III variants are significantly shorter in length than the type II. The benign nature of the changes is consistent with a flexible arm function for the N-terminal region of light chain 1, with the structural changes in the variants occurring in the spacer region of the arm. The finding supports NMR studies identifying a moving arm function in which the positively charged N-terminus interacts with actin [Prince, H. P., Trayer, H. R., Henry, G. D., Trayer, I. P., Dalgarno, D. C., Levine, B. A., Cary, P. D., & Turner, C. (1981) *Eur. J. Biochem. 121*, 213–219; Henry, G. D., Winstanley, M. A., Dalgarno, D. C., Scott, G. M. M., Levine, B. A., & Trayer, I. P. (1985) *Biochim. Biophys. Acta 830*, 233–243; Bandhari, K. G., Levine, B. A., Trayer, I. P., & Yeadon, M. E. (1986) *Eur. J. Biochem. 160*, 349–356] and indicates that precise length of the arm is not crucial.

Specific functions for the myosin subunits light chain 1 (LC1)[1] and light chain 3 (LC3) are just beginning to emerge. The presence of LC1 and LC3 is unnecessary for expression of the ATPase activity of myosin, the major characteristics of which are determined by the heavy chain (Wagner & Giniger, 1981; Sivaramakrishnan & Burke, 1982). Differential modulation of the actin-activated ATPase by LC1 and LC3 was reported early on (Weeds & Taylor, 1975; Wagner & Weeds, 1977; Wagner et al., 1977). The relevance of this observation to the function of myosin was initially unclear since the differences were not present under physiological conditions. Trayer and Trayer (1985) have shown that associated differences in the binding of actin to myosin containing either LC1 or LC3 are maintained at physiological ionic strength provided that calcium is present and the actin is regulated. LC1 and LC3 thus appear to bring about interactions of slightly different intensities between the myosin head and actin during contraction. In addition, there must be functional significance in the fact that LC1 is present very early in muscle development while LC3 first appears around the time of birth or hatching (Dow & Stracher, 1971; Hoh, 1987).

LC1 and LC3 are transcribed from a single gene containing nine exons (Nabeshima et al., 1984; Robert et al., 1984). The C-terminal 141 amino acids of the two proteins are identical and are coded for by the last five exons. Exons 1 and 4 determine the N-terminal 50 residues specific to LC1, while exons 2 and 3 code for the N-terminal 8 residues of LC3. The major structural difference between the two proteins and the origin of functional differences is thus the extended sequence at the N-terminus of LC1.

We have identified three forms of myosin light chain 1 (LC1), type I, type II, and type III, in avian fast white muscle fibers (Rushbrook et al., 1982; Rushbrook & Somes, 1985). The variants, which differ in rates of electrophoretic migration on SDS gel electrophoresis, are not associated with disease or development. Their frequency of occurrence varies in different chicken strains, and the results of a genetic study are consistent with an allelic origin.

Detection of variants of LC1 but not LC3 suggested that the structural changes were located at the N-terminus of LC1. Further investigation was stimulated by the fact that a benign variability in this region of the protein would have implications for the particular function of LC1.

Materials and Methods

Chemicals. Chemicals used for routine analytical and

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[1] Abbreviations: LC1, LC2, and LC3, myosin light chains 1, 2, and 3; HPLC, high-performance liquid chromatography; SDS, sodium dodecyl sulfate; S-1, myosin subfragment 1; TLCK, N^{α}-*p*-tosyllysine chloromethyl ketone; TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone; TFA, trifluoroacetic acid; PTH, phenylthiohydantoin.

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preparative work were of reagent grade or better. Chemicals used for HPLC were those described previously (Rushbrook, 1985).

Chicken Strains. Numerous chicken strains were used during the study. White Leghorn, Rhode Island Red, New Hampshire (normal line 412 and dystrophic line 413), Connecticut dystrophic, and birds bred for a genetic study were obtained from sources described previously (Rushbrook et al., 1982; Rushbrook & Somes, 1985). Inbred White Leghorn (normal line 03 and dystrophic line 433) and inbred New Hampshire (normal line 400 and dystrophic line 443) birds were obtained from the University of California at Davis.

Myosin. Myosin was prepared from the pectoralis major muscle of adult chickens (6 months or older) as described (Rushbrook et al., 1981), employing an extraction buffer:tissue ratio of 3:1 and a 35–50% saturation ammonium sulfate cut. The red strip (Gauthier & Lowey, 1977) was omitted.

Purification of Alkylated LC1 and LC3. This was carried out as described previously (Rushbrook, 1985). Briefly, myosin was alkylated in 6 M guanidine hydrochloride and the heavy chain subunit precipitated by dilution. The soluble light chain fraction was dialyzed against 0.5% acetic acid, and LC1 and LC3 were purified by reverse-phase HPLC using a trifluoroacetic acid/acetonitrile elution system.

Protein Determination. The protein concentrations of myosin and soluble light chain fraction solutions were obtained by the procedure of Lowry et al. (1951) with bovine serum albumin as standard. Protein concentrations of purified LC1 and LC3 solutions were calculated from the areas of peaks in the preparative HPLC chromatograms.

SDS Gel Electrophoresis and SDS Gel Peptide Mapping. SDS gel electrophoresis and peptide mapping (using *Staphylococcus aureus* V8 protease) were carried out as described previously (Rushbrook et al., 1982).

Reverse-Phase HPLC Peptide Mapping of Thermolysin Digests of LC1 and LC3. Lyophilized, purified, alkylated LC1 and LC3 were solubilized at 0.3–1.0 mg/mL in 0.05 M NH_4HCO_3/1.0 mM $CaCl_2$, pH 7.8, and digested with thermolysin (0.02 mg/mL) for 30 min at 37 °C. The reaction was terminated by the addition of acetic acid to 4.5%, and the samples were dried on a Savant evaporator. Prior to chromatography the samples were solubilized in 0.5% acetic acid. Reverse-phase HPLC was carried out on a Brownlee C8 RP-300 column and the HPLC equipment described previously (Rushbrook, 1985). Buffer A was comprised of 0.25% H_3PO_4 with triethanolamine added to bring the pH to 2.00; buffer B consisted of CH_3CN. Elution conditions are given in the figure legends.

Tryptic Peptide Mapping of the Major Difference Peak in the Thermolysin Digests of the LC1 Variants. The thermolysin digests of the three LC1 variants were chromatographed on a Brownlee RP-300 C8 column with a trifluoroacetic acid (TFA) elution system (Rushbrook, 1985), and the major difference peptide was collected. Though lacking the fine resolution of the H_3PO_4/triethanolamine/CH_3CN system used for the peptide mapping analyses, this elution system gave a base-line resolution of the major difference peptide similar to that of the H_3PO_4 system while permitting ready removal of the solvent by vacuum centrifugation. Purified peptide from 0.5 mg of LC1 was solubilized in 0.6 mL of 0.1 M NH_4HCO_3, pH 7.9, and digested with 8 μg of *N*-tosylphenylalanine chloromethyl ketone (TPCK) treated trypsin at 37 °C for 24 h. The reaction was terminated with the addition of *N*-tosyllysine chloromethyl ketone (TLCK) to 0.2 mM. The digests were chromatographed in the H_3PO_4 elution system described for the thermolysin digests. For sequencing, the peaks were collected, the CH_3CN level was reduced partially by vacuum centrifugation or lyophilization, and H_2O added. The peaks were then rechromatographed to remove the nonvolatile buffer on the same type of column with buffer A (0.1% TFA in H_2O) and buffer B (0.1% TFA in 60% H_2O/40% CH_3CN). The gradient was 1–100% B in 20 min; the flow rate was 1 mL/min.

Short-Term Tryptic Digestion of Intact LC1 Variants. In preliminary experiments, LC1 type II and LC3 were digested with trypsin at 37 °C for times ranging from 3 to 60 min. The digests were chromatographed on a TFA/H_2O/CH_3CN system and peaks present in the LC1 and not the LC3 profile examined by N-terminal amino acid sequencing for peptides beginning prior to the $(Ala)_5$ stretch. Two such peaks were identified, each with a major component cleaved after Lys-7. The purified LC1 variants and LC3 at 1 mg/mL were then digested for 30 min at 37 °C with TPCK-treated trypsin at 0.02 mg/mL. The reaction was terminated by the addition of TLCK to 0.2 mM. The digests were chromatographed on the reverse-phase column described above with buffer A (0.1% TFA in H_2O) and buffer B (0.1% TFA in CH_3CN) with a gradient of 1–51% B in 25 min and a flow rate of 1 mL/min. The two peaks of interest were collected and further purified by cation-exchange chromatography on a Brownlee CX-300 column, 4.6 × 30 mm, in buffer A (0.005 M potassium phosphate, pH 6) and buffer B (0.5 M potassium phosphate, pH 6) at a flow rate of 1.0 mL/min with a gradient of 0–20% B in 20 min. The major peak eluting after the injection peak was collected, run through a TFA/CH_3CN/H_2O reverse-phase HPLC system to remove the nonvolatile potassium phosphate, and subjected to amino acid sequencing.

Amino Acid Analysis and Sequencing. Amino acid analysis was carried out as previously described (Huszar et al., 1985). Amino acid sequencing at Brookhaven National Laboratory was carried out on a Beckman 890C sequencer (0.1 M Quadrol program) with amino acid phenylthiohydantoin detection as previously described (Huszar et al., 1985) and also on an Applied Biosystems Model 470A gas-phase sequencer. Amino acid sequencing at SUNY Health Science Center at Brooklyn was carried out on the latter instrument online to a microbore HPLC PTH-amino acid analyzer (Model 120A) from the same company (Applied Biosystems, Foster City, CA) as previously described (Rushbrook et al., 1987).

Results and Discussion

Identification of Myosins Homozygous for the Type I, II, and III LC1 Variants for Use in Structural Studies. SDS gel electrophoresis readily identified myosins homozygous for type I or type II LC1 in the commercial chicken population and in strains available from the University of California at Davis (Rushbrook et al., 1982). On SDS gels the type III/III homozygote, however, closely resembled the type I/III heterozygote, requiring a comparison of the type shown in Figure 1 for unequivocal identification prior to structural studies. We have found the type III variant, in its homozygous form, only in the F_2 generation of a genetic study involving all three variants (Rushbrook & Somes, 1985).

Comparison of the LC1 Variants by SDS Gel Peptide Mapping. The three LC1 variants were compared by SDS gel peptide mapping using *S. aureus V8* protease (Figure S1 of supplementary material; see paragraph at end of paper regarding supplementary material). In agreement with previous results (Rushbrook et al., 1982), the type I and II species differed in digestion pattern at several positions. No differences were apparent between the type I and type III forms,

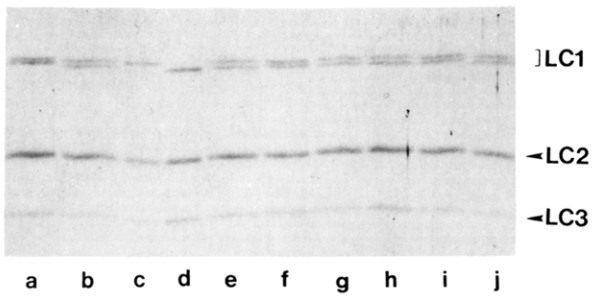

FIGURE 1: Identification by SDS gel electrophoresis of a myosin homozygous for type III LC1. Light chain region only of gel shown. Tracks: (a) control, myosin containing type I and II LC1; (b) control, myosin containing type II and III LC1; (c) control, myosin containing type II LC1; (d) LC1 type to be evaluated (X); (e) mixture of equal amounts of myosins containing type II and X LC1. The size of the gap between the LC1 species in (e) is to be compared with the gap in (f) which contains LC1 from a mixture of the myosins in (a) and (b), i.e., type I, II, and III LC1, and in (g) which contains LC1 from myosin in (b), i.e., type II and III LC1 only. Tracks h–j repeat tracks e–g. The results indicate that the chicken possessing the myosin in track d is homozygous for the type III LC1 species and not heterozygous for the type I and III forms. 2–4 μg of myosin/track.

despite the SDS gel migration rate differences in the intact variants.

At no point were differences apparent in the LC3 species accompanying the LC1 variants, either by SDS gel electrophoresis of the intact proteins or by SDS gel peptide mapping of the proteins (not shown).

Reverse-Phase HPLC Peptide Mapping of the Variants. Subsequent structural studies were carried out on alkylated LC1 variants and LC3 purified by reverse-phase HPLC (Rushbrook, 1985). The intact LC1 variants were not differentiated by the reverse-phase HPLC preparatory procedure.

Reverse-phase HPLC of timed digestions of the LC1 variants and LC3 with several proteases revealed a simple and generally stable pattern of fragments in the early stages: 30-min digestions with thermolysin are shown in Figure 2. Arrowheads in the LC1 and LC3 digests indicate peaks unique to LC1 and LC3, respectively, and locate fragments characteristic of the N-terminal sequence of each protein.

Comparison of the chromatograms of the three LC1 variants reveals differences at two positions (brackets 1 and 2). Each of these positions is also that of a peak unique to the LC1 pattern, indicating that the differences in the LC1 variants are located in the N-terminal sequence.

The region under bracket 1 contains small peaks which lacked stability in the early stages of the digestion. Bracket 2 locates the most prominent peak in the digest. This peak was consistently present after 30-min digestion and was selected for further study. Retention time differences, though small, are significant when compared with those of adjacent peaks. Upon prolonged digestion (24 h), this peak disappeared, and peaks at the earlier eluting position of difference became prominent (J. I. Rushbrook, unpublished observation), suggesting that the two positions of difference involved the same sequence.

The retention time differences of the prominent peak in Figure 2 were confirmed when digests of the type I and II variants and of the type I and III variants were cochromatographed (Figure S2). The digest of Figure S2 is slightly less advanced than that of Figure 2. For example, the peak heights of late-eluting peaks are higher, those of early-eluting peaks are lower, and the early-eluting position of difference (bracket 1 of Figure 2) is not yet present. In general, however, peaks may be readily correlated with those of Figure 2. The major difference peak among the three variants (bracket) is located

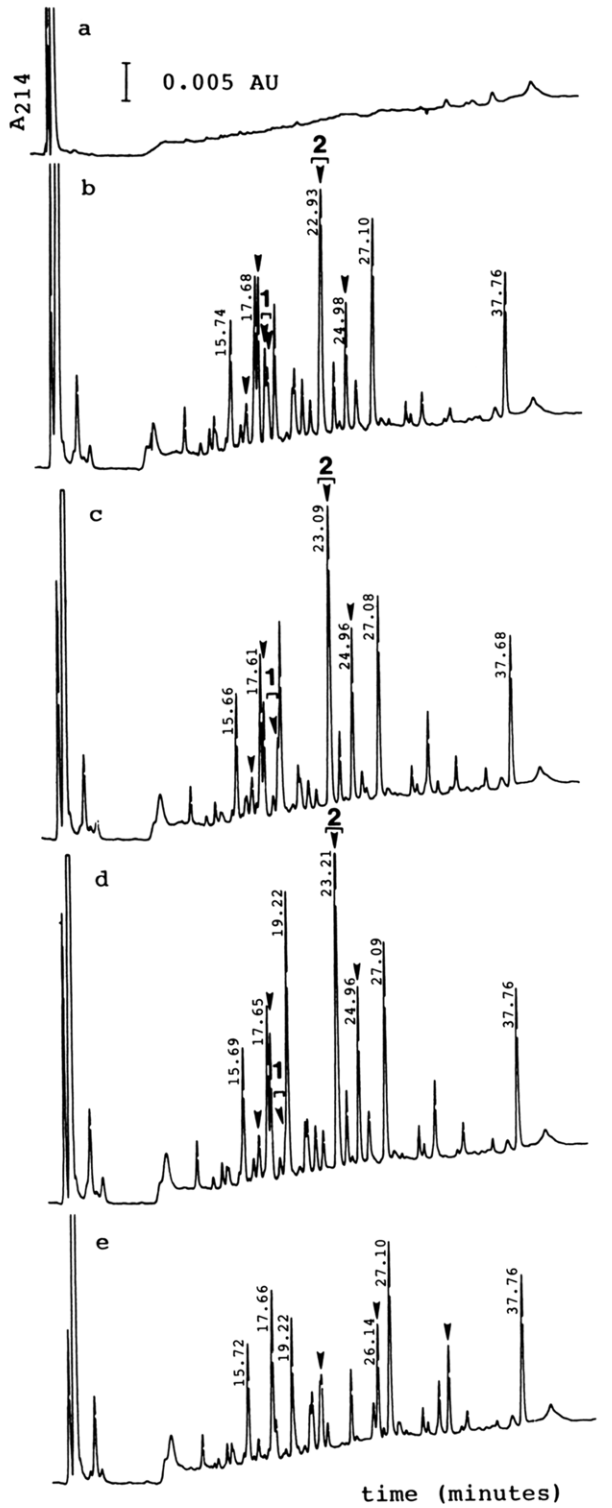

FIGURE 2: Comparison of thermolysin digests of the LC1 variants and LC3 by reverse-phase HPLC chromatography. A Brownlee RP-300 C8 column was equilibrated with buffer A and eluted with a linear gradient to 50% buffer B in 50 min at 1.0 mL/min (buffer A, 0.25% H_3PO_4 with triethanolamine added to give pH 2.0; buffer B, acetonitrile). (a) Control, enzyme with no substrate; (b) type I LC1; (c) type II LC1; (d) type III LC1; (e) LC3 from the type I LC1 myosin. Arrows indicate peaks unique to either the LC1 or LC3 digests. Numbered brackets indicate positions of difference in the LC1 maps. 14 μg of digested light chain chromatographed per run.

by the absence of a peak at this position in the accompanying LC3 digest (Figure S2d). The major peak is clearly a doublet in each of the LC1 mixtures, the separation in the chromatogram of the type I and III digests (Figure S2c) being greater than that of the type I and II digests (Figure S2b), consistent

Table I: Amino Acid Sequences of Selected Tryptic Peptides in Figure 3

	a 1 10
Published sequence:	(TM)Ala-Pro-Lys-Lys-Asp-Val-Lys-Lys-Pro-Ala-Ala-Ala-Ala-Ala-
LC1 I (Peak 1)	
(Peak 2)	Ala-
(Peak 3)	Ala-Ala-
LC1 II (Peak 1)	Ala-Ala-
LC1 III (Peak 2)	Ala-Ala-Ala-
	20
Published sequence:	Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-
LC1 I (Peak 1)	Ala-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-
(Peak 2)	Ala-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-
(Peak 3)	Ala-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-
LC1 II (Peak 1)	Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-
LC1 III (Peak 2)	Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-
	30 40
Published sequence:	Lys-Pro-Lys-Glu-Pro-Ala-Ile-Asp-Leu-Lys-Ser-Ile
	b
LC1 I (Peak 1)	Lys-Pro(Lys)
(Peak 2)	Lys-Pro-Lys
(Peak 3)	(Lys)Pro(Lys)
LC1 II (Peak 1)	Lys-Pro-Lys
LC1 III (Peak 2)	Lys-Pro(Lys)
	32 38
All variants (Peak B)	Glu-Pro-Ala-Ile-Asp-Leu-Lys

[a] Published sequence is taken from Nabeshima et al. (1984), Matsuda et al. (1981), Henry et al. (1982). [b] Parentheses indicate assignment from published sequence.

with the retention time differences in Figure 2. Mixtures of the type II and III difference peptides were not resolved by this procedure.

Preliminary amino acid composition and sequence analysis of the major difference peptides of the variants revealed a sequence beginning within the $(Ala)_5$ run of the N-terminal published sequence and extending through the subsequent Pro-Ala repeat region to terminate most probably at Ser-39 (for the published N-terminal sequence, see Table I). Specific sequence assignment after the initial Ala residues tended to be difficult, suggesting a ragged cleavage by thermolysin within the $(Ala)_5$ region.

The major difference peptide of each variant was collected and cleaved further with trypsin, and the three digests were analyzed by reverse-phase HPLC (Figure 3). Profiles typical of each variant were obtained. These were additive when heterozygotes were taken through the procedure.

Selected components from each chromatogram in Figure 3 were subjected to amino acid sequencing (Table I). The final peak in each of the chromatograms (peak B) had the same sequence, -Glu-Pro-Ala-Ile-Asp-Leu-Lys-, that of residues 32–38 of the published sequence.

Table I reveals that tryptic peaks 1–3 from LC1 type I (Figure 3b) follow the progression

-Ala-Ala-$(Pro\text{-}Ala)_6$Lys-Pro-( )
-Ala-Ala-Ala-$(Pro\text{-}Ala)_6$Lys-Pro-Lys
-Ala-Ala-Ala-Ala-$(Pro\text{-}Ala)_6$( )-Pro-( )

These sequences may be compared with residues 10–31 of the published sequence, -Ala-Ala-Ala-Ala-Ala-$(Pro\text{-}Ala)_7$Lys-Pro-Lys, and confirm initial ragged cleavage by thermolysin within the $(Ala)_5$ region. The sequence of peak B indicates that trypsin cleavages of the thermolysin peptide mixture occur at Lys-31 and -38 (Table I). Of note is the presence of $(Pro\text{-}Ala)_6$ in the type I variant, rather than $(Pro\text{-}Ala)_7$ found in the published sequence.

Peak 1 of the *LC1 type II* chromatogram (Figure 3c) contained the sequence -Ala-Ala-$(Pro\text{-}Ala)_7$Lys-Pro-Lys (Table I).

Peak 2 of the *LC1 type III* profile (Figure 3d) possessed a similar sequence, but three rather than two Ala residues preceded the $(Pro\text{-}Ala)_7$ run. This peak eluted with a similar retention time to peak 2 of the type II chromatogram which presumably had a similar sequence.

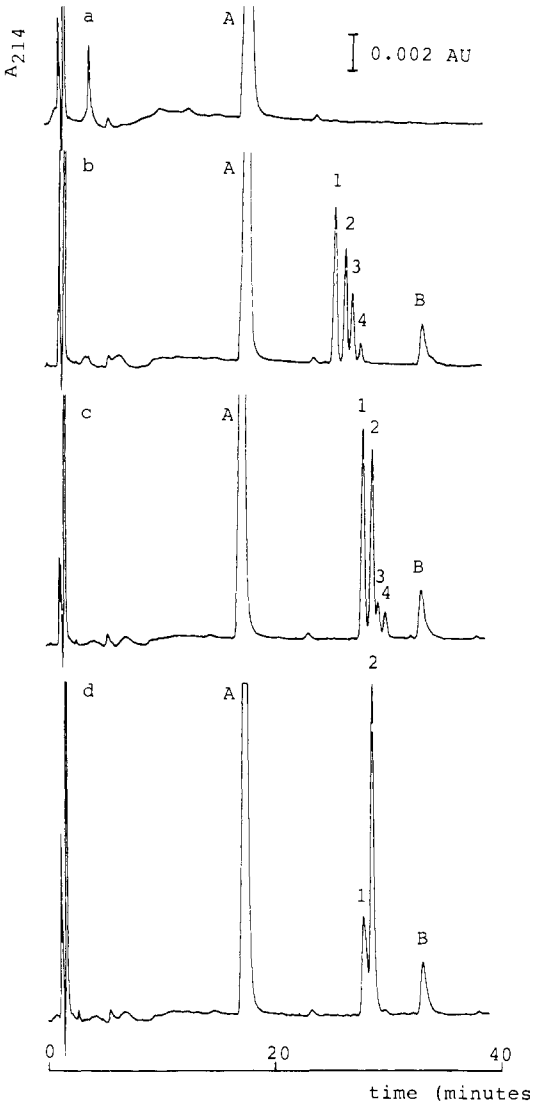

FIGURE 3: Tryptic peptide mapping of the major difference peak in the thermolysin digests of the three LC1 variants. The column described in Figure 2 was equilibrated with 99% buffer A/1% buffer B and eluted with a linear gradient to 16% buffer B in 45 min at a flow rate of 1 mL/min (buffer A and buffer B as in Figure 2). (a) Control, enzyme with no substrate. The very large peak (A) in this and the other chromatograms is the TLCK used to stop the reaction. (b) Digested difference peptide from type I LC1. (c) Digested difference peptide from type II LC1. (d) Digested difference peptide from type III LC1. Peak B is discussed in the text. Digests of difference peptide from 12.5 μg of LC1 applied in each chromatogram.

The results of the above analyses together with the preliminary work on the uncleaved thermolysin difference peptides indicated that thermolysin had generated a series of peptides in each LC1 varying in starting position within the repetitive alanine run and terminating at Ser-39 or shortly thereafter. Trypsin subsequently cleaved these peptides at Lys-31 and -38 of the published sequence.

The type I variant is unique in possessing $(Pro\text{-}Ala)_6$ rather than the $(Pro\text{-}Ala)_7$ found in the published sequence. Two explanations may be considered: this variant has experienced a Pro + Ala deletion within this repetitive region, or, perhaps more likely, there has been a Pro → Ala substitution at the first Pro of the published sequence. The latter event would be distinguishable by the presence of seven rather than five consecutive Ala residues in the Ala repeat region.

Table II: Sequence Differences in the LC1 Variants

	1 10
Published sequence:	(TM)Ala-Pro-Lys-Lys-Asp-Val-Lys-Lys-Pro-Ala-Ala-Ala-Ala-Ala
LC1 I	-Lys-Pro-Ala-Ala-Ala-Ala-Ala
LC1 II	-Lys-Pro-Ala-Ala-Ala-Ala-Ala
LC1 III	-Lys-Pro-__-__-Ala-Ala-Ala
	20
Published sequence:	Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala
LC1 I	Ala-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala
LC1 II	Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala
LC1 III	Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala-Pro-Ala
	30 38
Published sequence:	Lys-Pro-Lys-Glu-Pro-Ala-Ile-Asp-Leu-Lys
LC1 I	Lys-Pro-Lys-Glu-Pro-Ala-Ile-Asp-Leu-Lys-
LC1 II	Lys-Pro-Lys-Glu-Pro-Ala-Ile-Asp-Leu-Lys-
LC1 III	Lys-Pro-Lys-Glu-Pro-Ala-Ile-Asp-Leu-Lys-[a]

[a] Assigned from extensive sequencing through a tryptic peptide beginning at Lys-8.

The type II and III variants do not differ in the number of repeated Pro-Ala units but in producing consistently different ratios of thermolysin cleavage products within the repetitive alanine run. Such a result might arise from sequence differences within or prior to the published $(Ala)_5$ run. This possibility, together with the finding for the type I variant, indicated investigation of the variants beginning prior to the alanine run.

The three intact variants and LC3 were digested briefly with trypsin and the digests examined by reverse-phase HPLC (Figure S3). Two peaks (arrowheads), previously identified by sequencing of type II LC1 difference peaks as beginning at Lys-8, were collected, further purified by cation-exchange chromatography (not shown), and sequenced through the $(Ala)_x$ region into the Pro-Ala repeat. The results, together with the data in Table I, established the sequence differences in the variants (Table II).

LC1 type II has the previously published sequence. LC1 type I possesses a Pro → Ala substitution at residue 15, the beginning of the Pro-Ala run. This change, which might most simply have been accomplished by a single cytosine to guanine transversion, turns the $(Ala)_5(Pro\text{-}Ala)_7$ stretch of the published sequence into $(Ala)_7(Pro\text{-}Ala)_6$. The type III variant has an Ala-Ala deletion in the $(Ala)_5$ run, resulting in $(Ala)_3(Pro\text{-}Ala)_7$. Since each variant was not sequenced through its entire length, it is possible that other sequence changes are present.

The differences in migration rate of the three variants on SDS gel electrophoresis which led to their detection may be understood in terms of the above sequence differences. The type I and III variants migrate significantly faster than the type II, while the type III moves just ahead of the type I. The Pro → Ala substitution of the type I thus produces a shortening of the N-terminus of the protein which is almost as effective as the Ala-Ala deletion of the type III. This may be readily understood when the extended rodlike structure of the Pro-Ala run is considered (see below).

SDS gel peptide mapping with *S. aureus* V8 protease distinguished the type II variant from the type I and III forms (Figure S1) as a result apparently of the longer effective length of the $(Ala)_5(Pro\text{-}Ala)_7$ segment of the type II. The absence of differences in the type I and III maps probably is due to the small differences in migration of the starting proteins and the thickness of the digested bands.

The N-terminal peptide of LC1 is remarkable not only for its high content of Ala and Pro residues but also for the concentration of positive charge at its extreme N-terminus (four Lys residues) and the N-terminal trimethylated Ala (Table I).

The benign variability in this sequence documented here is suggestive of a flexible arm function for the LC1 N-terminus, supporting evidence to this effect from other sources. LC1 has long been known to migrate anomalously slowly on SDS gel electrophoresis (Frank & Weeds, 1974), consistent with a region of extended conformation in the molecule. NMR measurements have recently revealed that the Ala/Pro-rich segment of its N-terminus has a well-defined rodlike conformation (Bhandari et al., 1986) which, when LC1 is present in the myosin subfragment 1 complex, exhibits a high degree of mobility (Prince et al., 1981). When actin is added to the complex, this mobility is arrested, suggesting a direct interaction of the N-terminus with actin. Studies of the interaction between actin and the cleaved LC1 N-terminal peptide additionally support the proposal that the positively charged tip of the N-terminus interacts directly with actin during muscle contraction (Henry et al., 1985).

The results of the present study indicate that the precise length of the N-terminal arm is not crucial in this interaction. We anticipate that a benign variability in the N-terminus of LC1 will be found in other species.

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SUPPLEMENTARY MATERIAL AVAILABLE

Three figures showing comparison of the three purified LC1 variants by SDS gel peptide mapping with *S. aureus* V8 protease, verification of the differences in retention time of the major difference peptides of Figure 2, and short-term tryptic peptide mapping of intact LC1 variants (4 pages). Ordering information is given on any current masthead page.

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Characteristics of the Isolated Apical Plasmalemma and Intracellular Tubulovesicles of the Gastric Acid Secreting Cells: Demonstration of Secretagogue-Induced Membrane Mobilization†

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ABSTRACT: Separation of the gradient-purified gastric microsome into two membrane subfractions of distinct enzymatic and phospholipid composition has been achieved by mild SDS (0.033% w/v) treatment followed by sucrose gradient centrifugation of the pig and rabbit gastric microsomes. While the high-density membranes had all of the (H^+,K^+)-ATPase and K^+-pNPPase activities and revealed a single major 100-kDa band on SDS-PAGE, the low-density membranes contained all of the 5′-nucleotidase and nearly all of the Mg^{2+}-ATPase. In the present study, the low-density subfraction has been characterized to be derived from the apical membranes and the high-density one from the intracellular tubulovesicular membranes of the parietal cells. Such characterization was based primarily on sole dependency of the apical plasma membranes on the endogenous activator for (H^+,K^+)-ATPase activity, differential sensitivity of the activator (AF)-dependent and -independent (H^+,K^+)-ATPase on micromolar vanadate and Ca^{2+}, specific vitamin B_{12} binding ability of the apical plasmalemma, phospholipid and protein profiles of the two membrane subfractions, and other parameters. The AF, mentioned previously, has recently been implicated as a cytosolic regulator of the gastric (H^+,K^+)-ATPase [Bandopadhyay et al. (1987) *J. Biol. Chem. 262*, 5664-5670]. Two different forms (i.e., AF-dependent and -independent forms) of the (H^+,K^+)-ATPase are suggested to be present in the tubulovesicles on the basis of differential vanadate sensitivity while the AF-dependent form alone is present in the apical membranes. The data have been discussed in terms of stimulation-induced membrane transformation characteristic of the H^+-secreting epithelia including the acid-secreting cells of the stomach.

Gradient-purified gastric microsomal vesicles have recently been proved to be a unique model system for studies on various aspects of the proton transport process (Ray & Fromm, 1981; Sen et al., 1980; Nandi & Ray, 1982; Ray et al., 1982, 1983; Nandi et al., 1983a-c) including molecular insights into the gastric (H^+,K^+)-ATPase system (Ray & Forte, 1976; Ray & Nandi, 1983; Nandi & Ray, 1987). The purified microsomal vesicles, highly enriched in gastric (H^+,K^+)-transporting ATPase activity, consist of a mixed membrane population derived primarily from the secretory surface (apical plasmalemma) and intracellular tubulovesicular membranes, the latter believed to act as an intracellular reserve for the former. Recycling of the secretory membranes is suggested to occur following cessation of secretion (Sedar & Friedman, 1961; Forte et al., 1977; Zalewsky & Moody, 1977; Ito & Schofield, 1974; Helander & Hirschwitz, 1974).

Previous efforts toward positive identification and biochemical characterization of the secretory plasma membranes and intracellular tubulovesicles were not successful due to their close functional similarities as well as lack of appropriate specific markers which could discriminate between these two membrane types. In a recent report (Nandi et al., 1987), we have demonstrated that treatment of purified gastric microsomes with low (0.033%) SDS under appropriate conditions followed by equilibrium sucrose density gradient centrifugation generates two membrane subfractions having different chemical and enzymatic profiles. In the present study, the use of a pure preparation of cytosolic activator protein (Bandopa-

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