APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1999, p. 3540–3546 Vol. 65, No. 8
0099-2240/99/$04.00+0

Characterization of Muscle Sarcoplasmic and Myofibrillar Protein Hydrolysis Caused by *Lactobacillus plantarum*

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Received 16 November 1998/Accepted 1 April 1999

Strains of *Lactobacillus plantarum* originally isolated from sausages were screened for proteinase and aminopeptidase activities toward synthetic substrates; on the basis of that screening, *L. plantarum* CRL 681 was selected for further assays on muscle proteins. The activities of whole cells, cell extracts (CE), and a combination of both on sarcoplasmic and myofibrillar protein extracts were determined by protein, peptide, and free-amino-acid analyses. Proteinase from whole cells initiated the hydrolysis of sarcoplasmic proteins. The addition of CE intensified the proteolysis. Whole cells generated hydrophilic peptides from both sarcoplasmic and myofibrillar proteins. Other peptides of a hydrophobic nature resulted from the combination of whole cells and CE. The action of both enzymatic sources on myofibrillar proteins caused maximal increases in lysine, arginine, and leucine, while the action of those on sarcoplasmic proteins mainly released alanine. In general, pronounced hydrolysis of muscle proteins required enzyme activities from whole cells in addition to those supplied by CE.

The enzymatic systems of lactic acid bacteria responsible for carbohydrate and protein metabolism are those of special relevance during food fermentation (6). In dairy products, the proteolytic system of lactic acid bacteria is involved in casein degradation, thereby providing peptides and free amino acids required for the optimal growth of these bacteria in milk. Initially, a cell wall-associated proteinase generates oligopeptides which can be further hydrolyzed by the coordinated action of a pool of intracellular peptidases showing different specificities (14). These proteolytic events have been thoroughly investigated not only because of their physiological significance but also for their technological connotations in texture and flavor development (33). In meat products, such as dry sausages, proteolysis results from the combined action of enzymes from both endogenous and microbial origins. Therefore, the initial degradation of myosin and actin into peptides is due to cathepsin D, while the later decomposition of peptides into free amino acids is bacterial (19, 31). Thus, a large number of end products, such as peptides and free amino acids, whose concentrations and compositions have been correlated with specific taste descriptors in some cases, are generated (35). It is also known that free amino acids are precursors of other volatile compounds which have an impact in aroma (20, 29). Therefore, studies directed at steering proteolytic phenomena by including well-defined starter cultures or proteolytic enzymes are increasing (5, 8, 9, 35).

The strains of *Lactobacillus plantarum* included in this report were originally isolated from sausages and screened for general fermentation and proteolytic properties for selection as starter cultures (32). *L. plantarum* CRL 681 showed good abilities to grow in a medium based on sarcoplasmic protein extracts and to hydrolyze the proteins (10). Nevertheless, detailed information about the products resulting from the proteolytic activity of this strain has not been reported so far. Research into the components of the proteolytic system of meat lactobacilli is being carried out (21, 23, 25–28), but the information available is still limited compared to that for dairy organisms (14). In fact, there are few reports on the characterization of proteinases and peptidases of *L. plantarum*. In addition, there is scarce information regarding their technological and physiological roles when acting on muscle proteins and peptides. In this respect, recent work on the activities of *Lactobacillus sake* and *Lactobacillus curvatus*, the species most frequently used in meat fermentation, on muscle proteins constitutes a unique exception (11).

This work focuses on the proteinase and peptidase activities of whole cells, cell extracts (CE), and the combination of both from *L. plantarum* CRL 681 on muscle sarcoplasmic and myofibrillar proteins. The compounds generated were analyzed in detail to elucidate the putative mode of action of the enzymatic system of this strain and its impact on proteolytic events during meat curing to provide additional information for evaluating its suitability as a starter culture or enzymatic preparation for sausages.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *L. plantarum* CRL 681, CRL 682, and CRL 685, originally isolated from sausages, were used for proteolytic assays. All strains were routinely grown in MRS broth (E. Merck AG, Darmstadt, Germany) at 30°C for 24 h and then maintained at either 4 or −80°C in 15% (vol/vol) glycerol. For enzymatic assays, cultures were inoculated with strains (1%, vol/vol) that had been subcultured twice and were incubated for 16 h at 30°C.

Preparation of cell suspensions and extracts. Proteinase activity against fluorescein isothiocyanate (FITC)-labeled casein was assayed in whole-cell suspensions. Cells were harvested by centrifugation (10,000 × *g* for 20 min at 4°C), washed twice in 0.085% (wt/vol) NaCl containing 20 mM $CaCl_2$, and resuspended in 50 mM Tris-HCl (pH 6.5) at 2% (wt/vol) the initial volume. The optical density of the cell suspensions was determined at 660 nm, and the corresponding dry weight was deduced from a calibration curve.

Aminopeptidase activity was assayed with CE obtained by a modification of the procedure described by Sanz and Toldrá (26). Cells were collected as stated above, washed twice in 20 mM phosphate buffer (pH 7.0), and resuspended in the same buffer (10% the initial volume) containing 0.4 M sucrose and 1 mg of lysozyme (Sigma, St. Louis, Mo.) per ml. After incubation at 30°C for 1 h, the cell wall fraction was removed by centrifugation (15,000 × *g* for 20 min at 4°C). The

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pellet was washed in 20 mM phosphate buffer (pH 7.0), resuspended in the same buffer, and sonicated for 15 min. Cell debris was removed by centrifugation (20,000 × *g* for 20 min at 4°C), and the supernatant constituted the CE.

Assay of proteinase and aminopeptidase activities toward synthetic substrates. Proteinase activity was determined with FITC-labeled casein (type II; Sigma) as a substrate by a modification of the procedure described by Twining (30). The reaction mixture, consisting of 70 μl of 50 mM Tris-HCl (pH 6.5) containing 0.4% (wt/vol) FITC-labeled casein and 20 mM $CaCl_2$ and 100 μl of a whole-cell suspension, was incubated at 37°C for 1 h. The resulting fluorescence was measured with a multiscanning fluorimeter (Fluoroskan II; Labsystems, Helsinki, Finland) at 485 and 538 nm as excitation and emission wavelengths, respectively. One unit of activity was defined as the amount of enzyme hydrolyzing 1 μmol of substrate per h at 37°C. Proteinase activity was expressed as units per milligram of dry weight.

Aminopeptidase activity was measured against several aminoacyl-7-amido-4-methyl coumarin (AMC) derivatives (L-Ala-, L-Lys-, L-Ser-, L-Met-, L-Phe-, L-Val-, L-Arg-, L-Gly-, L-Leu-, L-Tyr-, L-Pro-, and L-Pyr-AMC; Sigma) and L-Glu-1-4-*p*-nitroanilide (pNA) (Fluka Biochemika, Buchs, Switzerland) as described by Sanz and Toldrá (25, 26). Each reaction mixture was incubated at 37°C for 15 min, except for that of the chromogenic substrate, which was incubated for 1 h. One unit of activity was defined as the amount of enzyme hydrolyzing 1 μmol of substrate per h at 37°C. Aminopeptidase activity was expressed as units per milligram of protein. Assays were done in quadruplicate with four samples and controls measured for each experimental point.

Determination of protein concentration. The protein concentration was determined by the bicinchoninic acid method with BCA Protein Assay Reagent (Pierce, Rockford, Ill.). Bovine serum albumin was used as the standard.

Activity on muscle protein extracts. (i) Animals. The samples for protein extraction were removed from the longissimus dorsi muscles obtained from 6-month-old pigs (father, Large White; mother, Large White × Landrace).

(ii) Extraction of muscle proteins. Sarcoplasmic proteins were extracted by the method described by Molina and Toldrá (18) but with 20 mM phosphate buffer (pH 6.5) for homogenization. The final extract was filter sterilized through a 0.22-μm-pore-size membrane (Millipore, Bedford, Mass.). The protein content of the sarcoplasmic extract was 1.80 mg/ml. To prepare the myofibrillar extract, the pellet resulting from the sarcoplasmic protein extraction was resuspended in 100 ml of 0.03 N phosphate buffer (pH 6.5) that had been previously sterilized, and the suspension was homogenized for 4 min in a stomacher model 400 blender. After centrifugation (10,000 × *g* for 20 min at 4°C), the pellet was washed three times in the same buffer to remove muscle proteinases. The resulting pellet was weighed and resuspended in 9 volumes of 0.1 N phosphate buffer with 0.7 M KI (pH 6.5) and 0.02% sodium azide, and the suspension was homogenized for 8 min in a stomacher model 400 blender. After the last centrifugation (10,000 × *g* for 20 min at 4°C), the supernatant was diluted 10 times in the same buffer for enzymatic assays to prevent the possible inhibition of bacterial proteinases by KI. The protein content of the myofibrillar extract was 0.75 mg/ml. For both extracts, sterility was confirmed by determining the absence of bacterial growth on Plate Count Agar (Merck) as described below.

(iii) Enzymatic mixtures. Three independent assays were carried out for each protein extract (sarcoplasmic and myofibrillar) by using as an enzymatic sample either whole-cell suspensions, CE, or a combination (1:1) of both. The reaction mixture consisted of 6 ml of whole-cell suspension or CE aseptically added to 30 ml of protein extract. When whole-cell suspensions and CE were assayed together, both were obtained as previously described but used at half the final volume (3 ml), mixed, and then added to the protein extract. The mixtures were incubated at 37°C in a shaking water bath. Samples were taken initially and after 96 h of incubation for further analyses. In each case, control samples without the addition of any bacterial enzymes were assayed simultaneously.

(iv) Bacterial counts and pH measurement. Bacterial counts were determined on Plate Count Agar and MRS agar (Merck) after incubation at 30°C for 48 h. The pHs of the reaction mixtures were monitored with a model 2001 pH meter (Crison Instrument S.A., Barcelona, Spain).

(v) Gel electrophoresis. The hydrolysis of muscle proteins was monitored by sodium dodecyl sulfate gel (SDS)-polyacrylamide electrophoresis (PAGE) analysis (15) with 12 and 10% polyacrylamide gels for sarcoplasmic and myofibrillar proteins, respectively. The ratio of acrylamide to bisacrylamide was 200:1. Standard proteins were simultaneously run for protein identification. The standard proteins were myosin (200.0 kDa), β-galactosidase (116.3 kDa), phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), and aprotinin (6.5 kDa) (all from Bio-Rad, Richmond, Calif.). Proteins were visualized by Coomassie brilliant blue R-250 staining after destaining by soaking in several changes of 10% methanol–7.5% acetic acid until a clear background resulted.

(vi) Peptide analyses. The evolution of the peptide contents in protein extracts was analyzed with a model 1050 high-performance liquid chromatograph (Hewlett-Packard; Palo Alto, Calif.) equipped with a multiwavelength UV detector and an automatic injector. Two milliliters of each sample was deproteinized with 5 ml of acetonitrile. The supernatant was concentrated by evaporation to dryness and resuspended in 200 μl of solvent A (0.1% [vol/vol] trifluoroacetic acid in MilliQ water). Samples of 15 μl were applied to a Symmetry C_{18} column (4.6 mm [inner diameter] by 250 mm; Waters Corporation, Milford, Mass.). The mobile phase consisted of solvent A, described above, and solvent B (acetonitrile-water-trifluoroacetic acid [60:40:0.085, vol/vol/vol]). The elution was performed as follows: an isocratic phase in 1% solvent B for 5 min, followed by a linear gradient from 1 to 100% solvent B for 20 min, at a flow rate of 0.9 ml/min at 40°C. Peptides were detected at 214 nm.

(vii) Amino acid and natural dipeptide analyses. The change in free amino acids and natural dipeptide contents in muscle extracts was also monitored. Samples of 500 μl plus 50 μl of an internal standard (0.325 mg of hydroxyproline per ml) were deproteinized with 1,375 μl of acetonitrile. The supernatants (200 μl) were derivatized to their phenylthiocarbamyl derivatives by the method of Bidlingmeyer et al. (4). The derivatized amino acids were analyzed by reverse-phase high pressure liquid chromatography (HPLC) as previously described (1).

TABLE 1. Proteinase and aminopeptidase activities of strains of *L. plantarum* toward synthetic substrates

Substrate	Activity[a] of *L. plantarum*		
	CRL 681	CRL 682	CRL 685
FITC-labeled casein	0.05 ± 0.00	0.01 ± 0.00	0.01 ± 0.01
Ala-AMC	0.49 ± 0.04	5.76 ± 0.80	0.82 ± 0.09
Lys-AMC	3.49 ± 0.64	1.77 ± 0.10	2.31 ± 0.15
Ser-AMC	0.12 ± 0.25	0.05 ± 0.02	0.05 ± 0.01
Met-AMC	0.68 ± 0.17	0.71 ± 0.05	0.94 ± 0.01
Phe-AMC	0.36 ± 0.02	0.30 ± 0.03	0.45 ± 0.01
Val-AMC	0.65 ± 0.02	0.36 ± 0.41	0.71 ± 0.03
Arg-AMC	6.55 ± 0.84	0.79 ± 0.38	5.18 ± 0.20
Gly-PNA	0.10 ± 0.01	0.02 ± 0.00	0.03 ± 0.01
Leu-AMC	2.65 ± 0.96	1.18 ± 0.10	1.93 ± 0.05
Tyr-AMC	0.39 ± 0.17	0.45 ± 0.01	0.26 ± 0.01
Pro-AMC	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
Glu-AMC	0.19 ± 0.95	NH	1.24 ± 0.50

[a] Proteinase activity toward FITC-labeled casein is expressed as units per milligram of dry weight. Aminopeptidase activity toward amino acid-AMC and amino acid-pNA is expressed as units per milligram of protein. The values shown are the means of three experiments ± standard errors of the means. NH, not hydrolyzed.

RESULTS

Screening for proteinase and aminopeptidase activities. The proteinase and aminopeptidase activities of three strains of *L. plantarum* toward synthetic substrates are shown in Table 1. *L. plantarum* CRL 681 showed the highest proteinase activity toward FITC-labeled casein. All tested strains displayed aminopeptidase activity toward every assayed substrate, except for glutamic and pyroglutamic acids (data not shown). The amino acids hydrolyzed at higher rates were L-arginine and L-lysine and, to a lesser extent, L-leucine, except that *L. plantarum* CRL 682 mainly released L-alanine. *L. plantarum* CRL 685 also showed an important glutamyl-hydrolyzing activity. *L. plantarum* CRL 681 showed the highest proteinase and aminopeptidase activities toward the substrates that were preferentially hydrolyzed, so it was selected for further assays on muscle proteins.

Bacterial counts and pH evolution in meat extract mixtures. When whole-cell suspensions of *L. plantarum* CRL 681 were incorporated into sarcoplasmic and myofibrillar protein extracts, bacterial counts were about 10^9 CFU/ml at the beginning of incubation. When these extracts were analyzed after 96 h of incubation, bacterial counts of about 1.2×10^4 CFU/ml were still detected in sarcoplasmic protein extracts, while counts were undetectable in myofibrillar protein extracts. In lactic acid bacteria, the osmotic stress caused by the presence of high salt concentrations is quite deleterious to cell growth (12); thus, the presence of KI in myofibrillar extracts could explain the low viability detected. As expected, bacterial counts were not detected at either 0 or 96 h of incubation when only CE were incorporated into both protein extracts. The simulta-

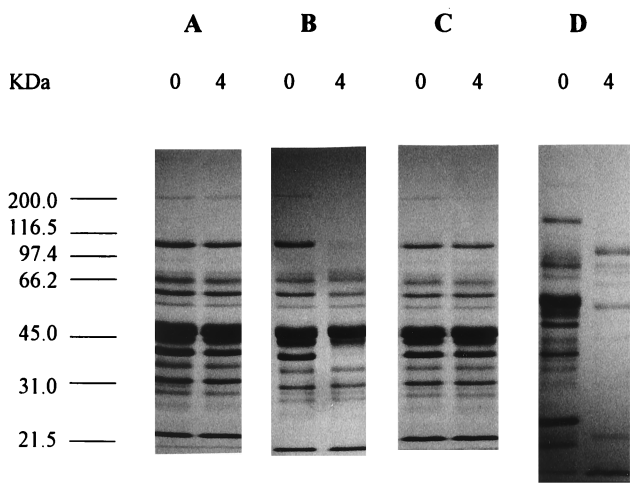

FIG. 1. SDS-PAGE (12% polyacrylamide) of sarcoplasmic protein hydrolysis by *L. plantarum* CRL 681. (A) Control samples at 0 and 96 h (4 days) of incubation. (B) Samples containing whole cells at 0 and 96 h (4 days) of incubation. (C) Samples containing CE at 0 and 96 h (4 days) of incubation. (D) Samples containing both whole cells and CE at 0 and 96 h (4 days) of incubation.

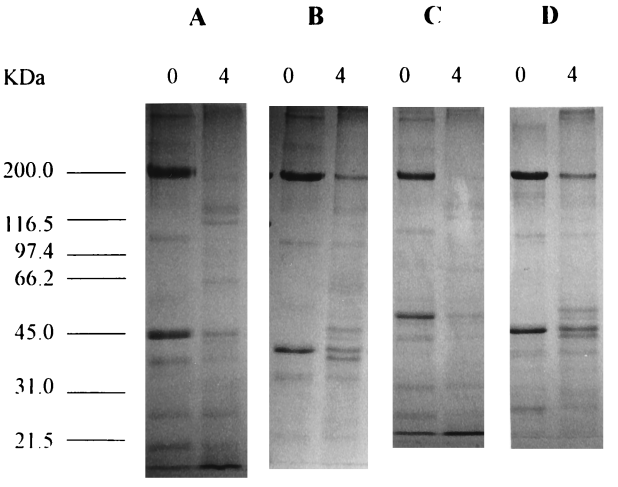

FIG. 2. SDS-PAGE (10% polyacrylamide) of myofibrillar protein hydrolysis by *L. plantarum* CRL 681. (A) Control samples at 0 and 96 h (4 days) of incubation. (B) Samples containing whole cells at 0 and 96 h (4 days) of incubation. (C) Samples containing CE at 0 and 96 h (4 days) of incubation. (D) Samples containing whole cells and CE at 0 and 96 h (4 days) of incubation.

neous addition of whole cells and CE to both protein extracts allowed a higher level of survival of the inoculated bacteria throughout the incubation period. Thus, bacterial levels initially present (about 10^9 CFU/ml) decreased to 10^8 and to 10^2 CFU/ml in sarcoplasmic and myofibrillar extracts, respectively. The pH values remained in the range of 6.5 to 7.0 during the entire incubation period.

Electrophoretic analyses. The protein profiles resulting from the hydrolysis of muscle sarcoplasmic proteins by *L. plantarum* CRL 681 are shown in Fig. 1. Control samples, without the addition of any bacterial enzyme, did not reflect proteolytic changes (Fig. 1A). The activity of whole cells resulted in the degradation of bands of about 97, 45, 37, and 26 kDa, which disappeared or decreased in intensity (Fig. 1B). The addition of CE did not cause major changes (Fig. 1C). However, the simultaneous action of both CE and whole cells greatly intensified the proteolytic changes (Fig. 1D).

The protein profiles resulting from the hydrolysis of muscle myofibrillar proteins are shown in Fig. 2. In control samples, the activity of endogenous proteinases was responsible for the degradation of protein bands of 200 kDa (myosin), 66 kDa, and 43 kDa (actin) (Fig. 2A). When whole cells were inoculated, myosin and actin were partially hydrolyzed, while other, faint bands of intermediate molecular masses (50 to 35 kDa) appeared (Fig. 2B); the same results were obtained when both whole cells and CE were used (Fig. 2D). The protein pattern obtained when only CE were incorporated was identical to that obtained for control samples (Fig. 2C).

Peptide analyses. Peptide maps resulting from the proteolytic activity of *L. plantrum* CRL 681 on sarcoplasmic and myofibrillar proteins are shown in Fig. 3 and 4. Control samples of sarcoplasmic protein extracts showed minor changes with respect to the initial peptide profile after 96 h of incubation (Fig. 3A and B). When whole cells were inoculated, peaks eluting at 7, 10, and 12 min of retention time appeared while others disappeared (15.5, 17.5, 25, and 30 min) after incubation (Fig. 3C and D). When CE were added, new, tiny peaks were detected in the range of 10 to 25 min but others (8 and 11.5 min) were missing (Fig. 3E and F). The peptide profile obtained from the combination of whole cells and CE was crowded with new peaks, especially in the range of 10 to 15 min (Fig. 3G and H).

Control samples of myofibrillar protein extracts showed slight modifications after 96 h of incubation (Fig. 4A and B). When whole cells were added, many new peaks were detected at 10 to 15 min of retention time, while the peak eluting at 30 min disappeared (Fig. 4C and D). Few modifications were observed when only CE were added (Fig. 4E and F). The strongest changes were observed when whole cells and CE were added together, although the peptide map was similar to that obtained when only whole cells were incorporated. Thus, many small peaks became detectable in the range of 15 to 25 min, and others (10 to 15 min) increased in intensity (Fig. 4G and H).

Amino acid analyses. The generation of free amino acids and natural dipeptides resulting from the activity of *L. plantarum* CRL 681 is shown in Table 2. When whole cells were inoculated into sarcoplasmic protein extracts, the levels of most of the amino acids and dipeptides (carnosine and anserine) decreased and only the concentrations of threonine and taurine increased. The activity of CE mainly released β-alanine, histidine, alanine, and anserine, while the levels of glycine, glutamine, and carnosine decreased. The content of almost all amino acids analyzed increased by the end of incubation when whole cells and CE were used together. The largest increases were detected first for alanine and then for glutamic acid, glycine, histidine, γ-aminobutyric acid, and leucine (Table 2).

In myofibrillar protein extracts, whole cells gave rise to significant amounts of only alanine (Table 2). The effect of the addition of CE to these extracts was not remarkable for amino acid generation, while the combination of CE and whole cells promoted the release of mainly alanine, lysine and, to a lesser extent, glutamic acid and arginine.

DISCUSSION

The use of whole cells, CE, and both enzymatic sources from *L. plantarum* CRL 681 made it possible to study their different contributions to the proteolytic events in meat extracts. The proteinase activity of whole cells of *L. plantarum* CRL 681 was

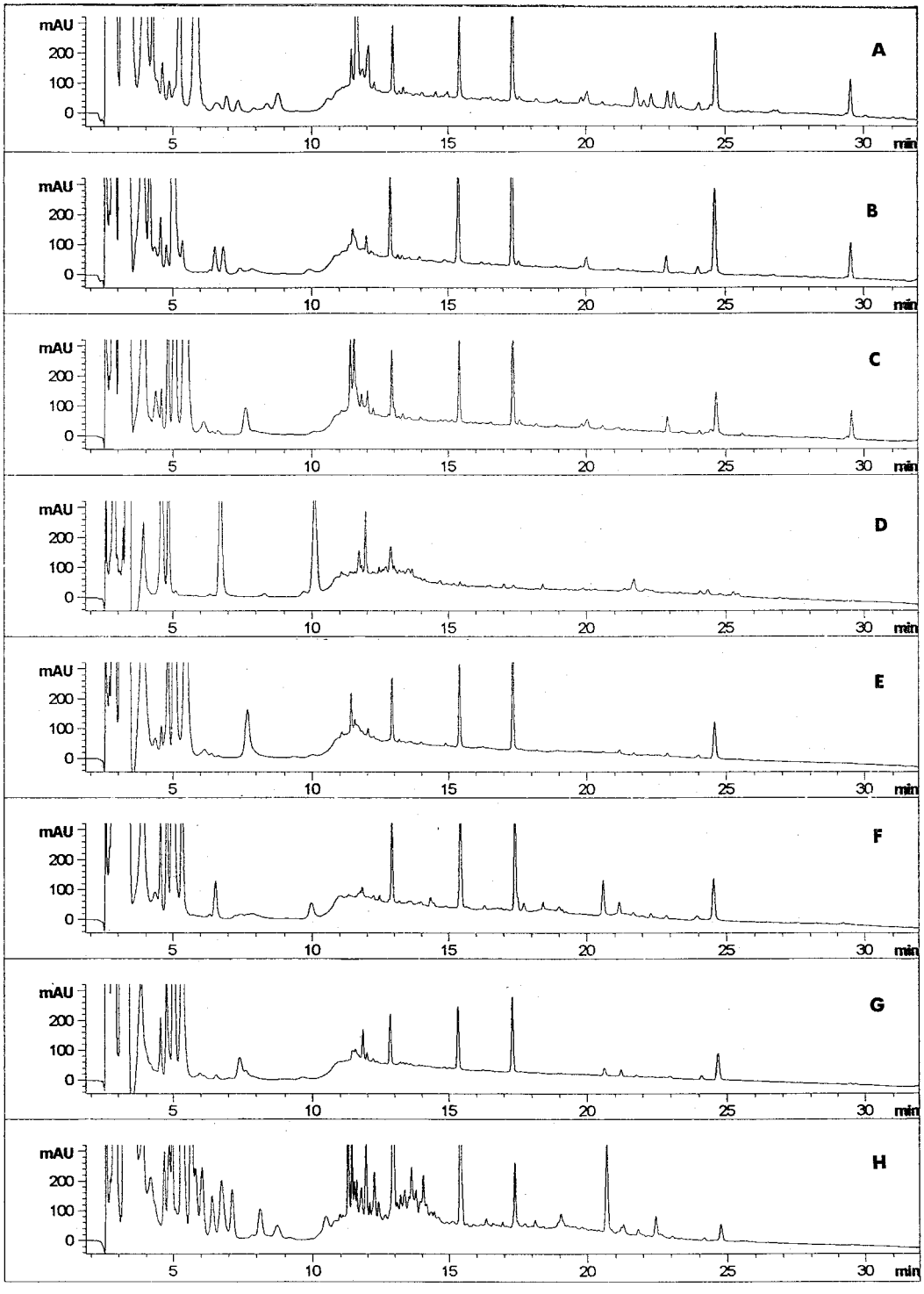

FIG. 3. Reverse-phase HPLC patterns of soluble peptides contained in sarcoplasmic protein extracts treated with *L. plantarum* CRL 681 at 0 (A, C, E, and G) and 96 (B, D, F, and H) h of incubation. Control samples (A and B), samples containing whole cells (C and D), samples containing CE (E and F), and samples containing whole cells plus CE (G and H) were tested. mAU, milli-absorbance units.

able to initiate pronounced hydrolysis of sarcoplasmic proteins. This activity was tentatively associated with the cell wall, since no hydrolytic change was detected in the protein patterns obtained when only CE were added. These results are comparable to those of Parra et al. (24), who did not report any significant increase in proteolysis when CE were added to a model involving goat's milk curds. In fact, most dairy lactic acid bacteria possess a single extracellular proteinase which initiates the hydrolysis of caseins into oligopeptides (14). The cell wall proteinase of several dairy lactobacillus strains has been puri-

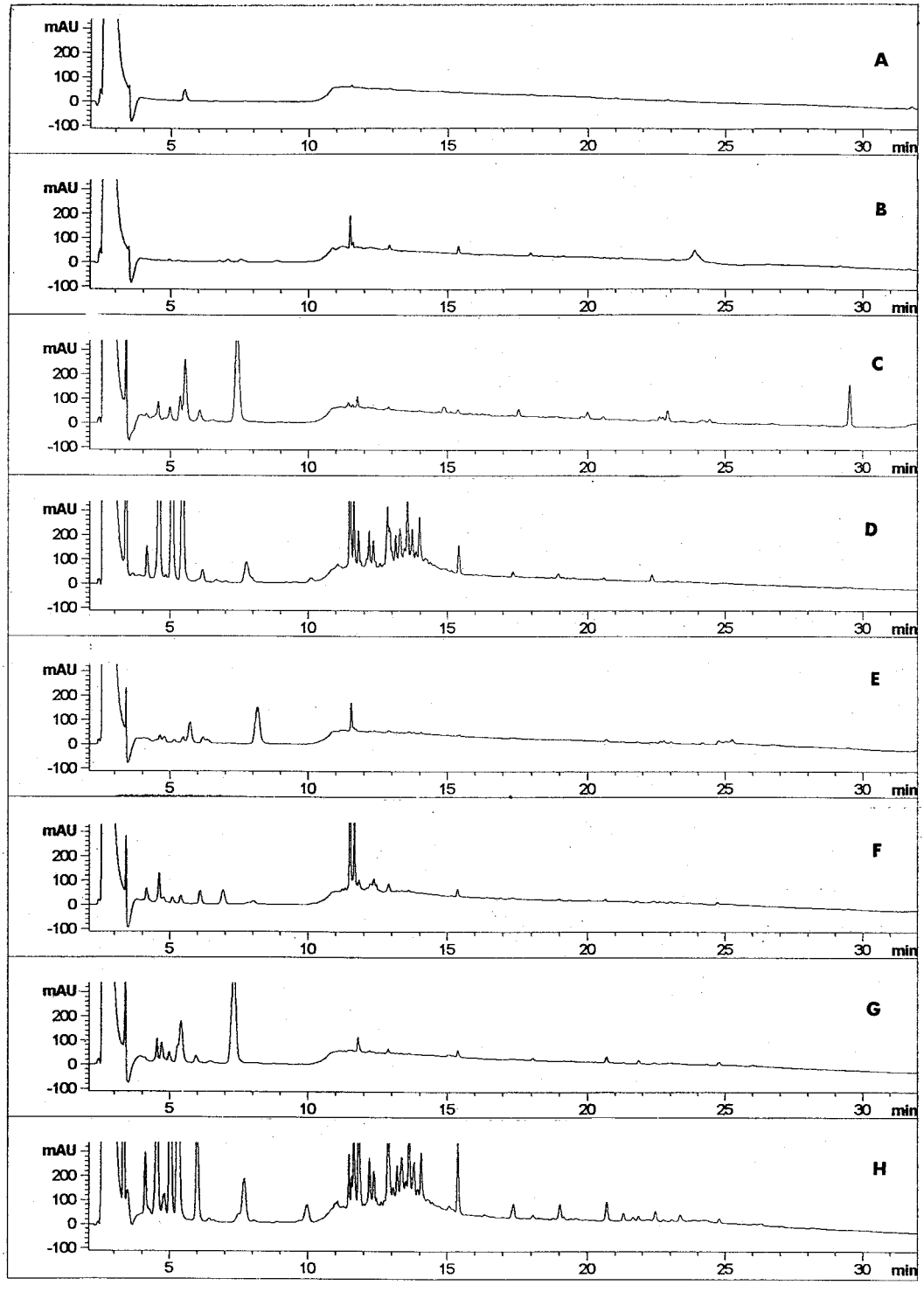

FIG. 4. Reverse-phase HPLC patterns of soluble peptides contained in myofibrillar protein extracts treated with *L. plantarum* CRL 681 at 0 (A, C, E, and G) and 96 (B, D, F, and H) h of incubation. Control samples (A and B), samples containing whole cells (C and D), samples containing CE (E and F), and samples containing whole cells plus CE (G and H) were tested. mAU, milli-absorbance units.

fied and characterized (16, 17, 22), although these reports do not refer to strains of the species *L. plantarum*. The absence of initial hydrolysis of meat proteins by the action of CE alone indicates that muscle proteins are not suitable substrates for this activity. It is likely that hydrolytic changes have to be preceded by the action of cell-bound proteinases, as is the case for casein degradation (13, 14). Nevertheless, it should be mentioned that the incorporation of intact cells also means the addition of an extra source of intracellular enzymes. The initial breakdown of the main myofibrillar proteins, myosin and actin,

TABLE 2. Evolution of free amino acid and natural dipeptide content after incubation at 37°C of sarcoplasmic and myofibrillar extracts containing whole cells, CE, and a combination of both

Amino acid or dipeptide	Amino acid or dipeptide content[a] in the following protein extract with the indicated addition:							
	Sarcoplasmic				Myofibrillar			
	None (control)	Whole cells	CE	Cells + CE	None (control)	Whole cells	CE	Cells + CE
Asp	6.41	−0.14	−0.95	1.33	0.60	0.00	0.19	−2.53
Glu	−22.89	−4.37	4.04	15.18	0.19	−0.17	1.87	4.68
Ser	2.26	−1.60	2.74	1.93	−0.26	1.50	0.37	2.54
Asn	0.38	−1.32	0.74	5.40	−0.12	0.41	0.23	1.30
Gly	2.75	−6.79	−11.50	13.71	−0.08	1.25	0.80	3.39
Gln	−17.97	−61.39	−81.35	−72.02	0.00	0.22	0.29	0.85
β-Ala	−0.04	−2.20	19.43	3.03	0.00	0.00	0.13	0.37
Hys	2.34	−2.46	57.21	15.01	0.00	0.40	0.58	1.76
γ-Aminobutyric acid	1.06	−0.94	1.84	27.42	0.00	0.74	0.04	0.38
Thr	5.39	25.03	6.27	4.04	7.26	3.31	0.16	1.27
Ala	7.07	−14.91	28.62	69.02	57.97	20.36	2.44	33.77
Arg	0.57	−4.26	0.83	6.81	11.83	0.00	0.93	6.59
Pro	2.93	−4.61	−3.24	−4.80	7.42	−0.47	0.08	1.03
Tyr	0.81	−1.53	0.73	3.57	5.76	1.20	0.11	0.33
Val	2.11	−2.71	4.49	10.70	9.44	1.96	0.47	3.92
Met	1.08	−1.36	1.01	1.99	3.29	0.43	0.24	2.49
Ile	1.13	−1.54	1.58	7.23	5.70	−0.92	0.41	2.75
Leu	2.83	−2.75	2.47	20.54	8.78	−0.24	0.52	3.93
Phe	1.46	−1.80	1.43	8.02	6.50	0.00	0.25	1.36
Trp	0.27	−0.66	4.23	1.20	2.08	0.00	0.00	0.25
Orn	0.63	−0.46	3.00	0.27	2.03	0.00	0.00	0.00
Lys	2.90	−3.63	−2.79	9.91	11.17	5.04	1.78	38.30
Anserine	0.31	−10.27	11.60	2.28	63.73	0.00	0.00	1.01
Carnosine	−11.62	−165.09	−61.45	−38.68	−0.06	0.76	−0.46	2.43
Taurine	0.52	5.31	−0.08	6.98	0.18	0.00	0.10	0.42

[a] Expressed as milligrams per 100 ml of extract of net change relative to respective control.

has been mainly attributed to the activity of muscle proteinases (19). The limited sensitivity of the electrophoretic analyses did not reveal the possible activity of enzymes from *L. plantarum* CRL 681 in initiating the hydrolysis of myofibrillar proteins. Nevertheless, the activity of these enzymes will be important only for peptides that can be translocated, which in milk represent only a minor portion of the casein (11, 17), or once cellular lysis occurs.

Whole cells generated hydrophilic peptides from both sarcoplasmic and myofibrillar proteins, while no major changes were detected when only CE were added. In addition to the hydrophilic peptides (10 to 15 min of retention time), others of a hydrophobic nature (15 to 25 min of retention time) were generated by the combination of both enzymatic sources (Fig. 3 and 4). Thus, the peptide changes described required the presence of enzymes accessible as whole cells and CE. The hydrophilic nature of most of the generated peptides indicates the potential contribution of *L. plantarum* CRL 681 to the development of desirable cured-meat taste (2).

The reduction of almost all amino acid levels was extremely large when whole cells were incorporated into sarcoplasmic protein extracts. Amino acids could partially contribute to maintaining viability in these extracts. Indeed, the use of proteolytic products to sustain growth was demonstrated when CE and whole cells were added together as an extra source of enzymes; viability remained almost constant, although other soluble compounds could have contributed to survival in these extracts. Accordingly, the incorporation of a lactobacillus proteinase into raw sausage mixtures also increased the rate of acidification and bacterial growth (5). Conversely, in myofibrillar protein extracts, the initial bacterial numbers drastically declined, although low levels were still maintained, with the combined use of whole cells and CE. In sarcoplasmic extracts, the net balance between decreases and increases in free amino acid contents was positive only when the combination of whole cells and CE was incorporated. The high negative values for glutamine concentration correlated quite well with the increase in glutamic acid concentration as a result of their possible interconversion, which may depend on the balance of ammonium, glutamate, and glutamine concentrations (7). Also, high levels of β-alanine and histidine may come from carnosine degradation. Indeed, the gene encoding the general dipeptidase PepV, described for dairy strains, was named the carnosinase gene, for its ability to hydrolyze this compound (34). The proteolytic activity exhibited for myofibrillar proteins caused maximal increases in lysine, arginine, and leucine levels, according to the specificity of the studied strain for the synthetic substrates (see Table 1). These amino acids also constitute some of the major components of pork myosin (3). In contrast, the increase in alanine content was larger than those of lysine and arginine in sarcoplasmic extracts. This result could be due to differences between the specificities for synthetic and natural peptides (6), a rapid metabolism of basic amino acids, or the effect of the availability of these amino acids in the substrates to be hydrolyzed.

In conclusion, pronounced hydrolysis of muscle proteins requires available enzyme activities as both whole cells and CE, and cell-associated proteinases seem to be dispensable for initiating some hydrolytic changes. Nevertheless, the coexistence of endogenous enzymes makes it rather difficult to establish the proteolytic pathway in these systems. Further investigations to identify the peptides generated and how the muscle and bacterial proteases interact must be carried out, especially in relation to the hydrophilic peptides which may contribute to desirable tastes.

ACKNOWLEDGMENTS

This work was supported by grant ALI98-0890 from Comisión Interministerial de Ciencia y Tecnología (CICYT, Madrid, Spain). Support was also provided by scholarships to S. Fadda from CONICET (Buenos Aires, Argentina) and to Y. Sanz from FPI/MEC.

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