JOURNAL OF BACTERIOLOGY, Jan. 1968, p. 181-187
Vol. 95, No. 1

Proteinase Enzyme System of Lactic Streptococci

III. Substrate Specificity of *Streptococcus lactis* Intracellular Proteinase[1]

R. A. COWMAN, S. YOSHIMURA,[2] AND H. E. SWAISGOOD
Department of Food Science, North Carolina State University, Raleigh, North Carolina 27607

Received for publication 16 October 1967

The substrate specificity of an intracellular proteinase from *Streptococcus lactis* was investigated in an effort to understand the role of the enzyme in the cell. Peptides in which the N-terminal residue was glycine were not hydrolyzed by the enzyme (exceptions were glycyl-alanine, glycyl-aspartic acid, and glycyl-asparagine), but the peptide was hydrolyzed if the N-terminal residue was alanine. The enzyme also showed activity toward peptides containing aspartic acid or asparagine. Hydrolysis of only the peptide bonds of alanyl, aspartyl, or asparaginyl residues was confirmed by the action of the enzyme on oxidized bovine ribonuclease A- and B-chain insulin. The N-terminal residues of the peptide fragments liberated were identified. The enzyme attacked both substrates only at alanyl, aspartyl, and asparaginyl residues, releasing these as free amino acids. In addition to alanine, aspartic acid, and asparagine, certain other amino acids were liberated from ribonuclease A, but these were accounted for by the relation of their position to alanine, aspartic acid, and asparagine residues.

When lactic streptococci are propagated in milk, they become dependent upon their proteinase enzyme systems to degrade milk proteins to utilizable forms. This dependence upon an organic nitrogen source can be attributed, in part, to the organisms' requirement for leucine, isoleucine, methionine, valine, glutamic acid, and histidine (6, 9). Peptidases from *Streptococcus lactis* have been isolated which hydrolyze peptides containing essential amino acids (10). Also, dipeptides containing either L-leucine or L-valine have been observed to satisfy the nutritional requirement of *S. lactis* for these amino acids when cells are grown on a chemically defined medium (8).

We have recently purified the intracellular proteinase from *S. lactis* (Cowman et al., *in preparation*). This proteinase exists in a monomer-dimer equilibrium (2). The form which predominates is temperature-dependent, but both forms possess enzymatic activity. To assist in providing a basis for understanding the role of this enzyme in cellular function, especially during biological aging, the activity of the enzyme toward peptide and protein substrates was examined.

[1] Contribution from the Department of Food Science, North Carolina Agricultural Experiment Station, Raleigh. Published with the approval of the Director of Research as paper no. 2435 of the Journal Series.

[2] Present address: Department of Nutritional Biochemistry, Himeji Woman's College, Shinzaike, Himeji, Hyogo Prefecture, Japan.

MATERIALS AND METHODS

Enzyme. The intracellular proteinase of *S. lactis* was purified by passing the supernatant fraction of sonically disrupted cells through a series of Sephadex gel and diethylaminoethyl (DEAE) Sephadex chromatography columns. During purification, activity of individual tubes of eluate was measured with casein used as substrate. In the final step of purification, elution from Sephadex G-100, the ratio of activity to absorbance remained a constant value throughout the elution profile. Preparations of the purified enzyme also were homogeneous to moving-boundary electrophoresis at *p*H 3.0, 3.5, 4.0, 4.5, 5.5, 6.0, 7.0, 8.0, and 9.0, as determined in different buffers. The enzyme also was observed to be homogeneous to sedimentation ultracentrifugation. An impurity in the preparation of up to 5% may not have been detectable by these procedures, but rechromatography and studies of the association-dissociation equilibria (2) and the constant specific activity of all the eluting protein suggest that the enzyme is homogeneous. The enzyme has recently been obtained in crystalline form. The complete and detailed procedure for purification will be described elsewhere (Cowman et al., *in preparation*).

Substrates. The various dipeptides and tripeptides were obtained commercially (Nutritional Biochemicals Corp., Cleveland, Ohio). Bovine ribonuclease A, prepared according to Hirs, was obtained from Calbiochem (Los Angeles, Calif.), and the homoge-

neity of the preparation was determined by Sephadex gel chromatography and ultracentrifugation. The homogeneity of oxidized B-chain insulin (phenylalanine; from Mann Research Laboratories, New York, N.Y.) was established by Sephadex gel chromatography.

Enzymatic hydrolysis of peptides. The dipeptides and tripeptides used were solubilized in 0.05 M sodium phosphate buffer (*p*H 7.0) to give a final concentration of 10 mg/ml. The enzyme assays were performed by incubating 1.0 ml of peptide substrate and 0.1 mg of enzyme at 37 C. A control in which heat-denatured enzyme was used was also analyzed. At intervals, 100-μliter samples of hydrolysis mixture were removed from incubation and spotted on Whatman no. 1 paper strips. The resulting strips were chromatographed by descending chromatography with *n*-buytl alcohol–acetic acid–water (5:1:4) as solvent. After chromatography, the frontal boundary was marked and the strips were air-dried and developed with ninhydrin. Hydrolysis of peptide was qualitatively analyzed by comparing the R_F values of the resulting spots with those for nonhydrolyzed peptide and its constituent amino acids, run simultaneously.

Preparation of oxidized ribonuclease. A 50-mg amount of ribonuclease was oxidized by use of the performic acid procedure outlined by Fraenkel-Conrat et al. (5). Immediately after oxidation, double glass-distilled water was added to stop the reaction, and the solution was lyophilized and stored in a desiccator over P_2O_5. Completeness of the oxidative reaction was confirmed by analytical ultracentrifugation. Both the native and oxidized protein sedimented as a single symmetrical boundary. The oxidized protein had a sedimentation coefficient at 20 C of 0.5*S*, compared with 1.64*S* for the native protein (1).

Preparation of Sephadex columns. Sephadex G-10 and G-25 sufficient to pack a 2 × 50 cm column were dispersed in 0.05 M sodium phosphate buffer (*p*H 7.0), and the gel grains were allowed to hydrate. The slurry was washed repeatedly with buffer until a clear supernatant fluid was obtained. A G-10 and a G-25 column were then packed as described by Flodin (4). The final column of Sephadex was 2 × 40 cm. The void volume of each column was determined by use of Dextran Blue 2,000.

Preparation of dinitrophenyl (DNP) derivatives of amino acids. The DNP derivatives of all 20 amino acids were prepared by the procedure outlined by Fraenkel-Conrat et al. (5). The derivatives were obtained in the crystalline form and were used after three crystallizations. The DNP amino acids were spotted on Whatman no. 3 paper and chromatographed in *n*-butyl alcohol–acetic acid–water (5:1:4); the R_F values were recorded. They again were spotted for the second dimension and chromatographed with methylethylketone–*n*-butyl alcohol–water (2:2:1); R_F values were again recorded. This procedure was repeated, and the average R_F value (±1%) was used to identify the DNP derivatives.

Enzymatic hydrolysis of ribonuclease and insulin. A 20-mg amount of either oxidized ribonuclease or oxidized B-chain insulin was dissolved in 5.0 ml of 0.05 M sodium phosphate buffer (*p*H 7.0). The *p*H of each substrate solution was readjusted to 7.0, if necessary, by use of 0.1 N HCl or 0.1 N NaOH. The substrate solution was tempered to 37 C and transferred to a vial containing 2.0 mg/ml of enzyme of the same temperature. The vial was sealed and incubated at 37 C for 24 hr.

Identification of the peptide fragments of the enzymatically hydrolyzed ribonuclease and insulin proceeded in a stepwise manner as follows.

Step I. Reaction of hydrolysate with 1-fluoro-2,4-dinitro-benzene (FDNB). The hydrolyzed ribonuclease or insulin mixture was treated with a solution of 2.0 ml of 95% ethyl alcohol containing 5% (v/v) FDNB for 2 hr at 37 C. Nonhydrolyzed substrate and enzyme reacted with FDNB giving insoluble DNP derivatives which were removed by centrifugation. The yellow supernatant fluid was decanted and stored at 3 C until used.

Step II. Chromatography of supernatant fluid on Sephadex G-10. A 2-ml amount of the supernatant fluid was applied to a Sephadex G-10 column and eluted with 0.05 M sodium phosphate buffer (*p*H 7.0). The absorbance of the eluate was monitored continuously, by use of a 265 mμ filter, and the profile was recorded. After chromatographic separation, tubes comprising the same fraction were collected and concentrated by evaporation in vacuo to 4.0 ml.

Step III. Chromatography on Sephadex G-25. The fraction (fraction 1) which was eluted in the void volume from Sephadex G-10 was concentrated to 2.0 ml and rechromatographed on Sephadex G-25 with 0.05 M sodium phosphate buffer (*p*H 7.0) as eluting agent. Tubes of eluate comprising a similar component were collected, concentrated, and stored at 3 C.

Step IV. Homogeneity of the separated fractions. Each fraction was chromatographed by two-dimensional paper chromatography with the solvent systems described above. The spots appearing in the individual fractions were compared with a chromatogram of the unfractionated mixture.

Step V. Acid hydrolysis of DNP peptides. When the locations of all peptides were confirmed, the fractions were evaporated to dryness. The dried material was dissolved in constant boiling HCl (5.7 N), placed in vials, evacuated, and sealed. The sealed tubes were heated at 121 C for 20 hr. After hydrolysis, the DNP amino acids were extracted with diethyl ether until the aqueous phase became colorless. The aqueous phase was evaporated to remove HCl and retained for determination of other amino acids present in the peptides.

Step VI. Final identification of DNP amino acids. The ether extracts containing DNP amino acids were spotted on Whatman no. 3 paper and chromatographed two-dimensionally. The R_F values of the yellow spots were determined and compared with the R_F values of the standard DNP amino acids.

RESULTS

Peptide specificity of the enzyme. The hydrolytic activity of the enzyme toward many dipeptides and tripeptides was surveyed. Hydrolysis of each peptide was determined at hourly intervals by examining samples for appearance of constituent amino acids. A representative determination of the hydrolysis of DL-valyl-L-alanine by the enzyme is shown in Table 1. As the time of incubation increased, the spots for valine and alanine became more intense, with a concomitant loss in the intensity for peptide. After 4 hr, the intensity of the peptide spot was appreciably diminished. Many of the peptides tested were not hydrolyzed by the enzyme (Table 2). Most of the nonreactive peptides were glycyl peptides. Leucyl peptides also were not hydrolyzed within 24 hr. When glycyl (N-) was replaced with alanyl (N-), hydrolysis of the peptide occurred. For example, glycyl-DL-phenylalanine was not hydrolyzed but DL-alanyl-DL-phenylalanine was hydrolyzed. The enzyme apparently showed no requirement for the amino or carbonyl side of the alanyl residue since glycyl-alanine, alanyl-glycine, alanyl-valine, and valyl-alanine were all hydrolyzed. However, since this was a qualitative study, the rates of hydrolysis were not determined. In addition to alanine-containing peptides, those which contained either aspartic acid or asparagine also were hydrolyzed within 4 hr of incubation.

Hydrolysis of ribonuclease and insulin. To determine whether only alanyl, aspartyl, and asparaginyl peptide bonds were hydrolyzed, the hydrolytic action of the enzyme on oxidized bovine ribonuclease A and oxidized B-chain insulin was studied. The protein was incubated with the enzyme, and the resulting hydrolytic fragments were converted to their DNP derivatives. The insoluble material was removed by centrifugation and was found to contain only nonhydrolyzed protein and enzyme. The supernatant fraction was first chromatographed on Sephadex G-10 and was eluted with 0.05 M sodium phosphate buffer (*p*H 7.0); the eluate was monitored by absorbance at 265 mμ. The supernatant fluid was eluted as nine different peaks (Fig. 1). Fraction 9 (not shown) was eluted after approximately 1 liter of eluate was collected. Tubes of eluate comprising the same fraction (dotted lines, Fig. 1) were collected and concentrated. After concentration, the separated fractions were stored for 24 hr at 3 C, during which time crystalline material accumulated in fractions 2 and 3. The crystals were removed by centrifugation, redissolved in water, and designated as fractions 2A and 3A. The supernatant fluids were designated as 2B and 3B.

TABLE 1. *Hydrolysis of DL-valyl-L-alanine by Streptococcus lactis intracellular enzyme proteinase*

Incubation (hr)	R_F values[a]			Controls	
	Spot 1	Spot 2	Spot 3	Spot	R_F
0	—	—	0.798	DL-Valyl-L-alanine	0.798
1	0.535	0.730	0.790	DL-Valine	0.737
2	0.538	0.735	0.796	L-Alanine	0.530
3	0.531	0.736	0.798		
4	0.530	0.736	0.798		

[a] Solvent, *n*-butylalcohol–acetic acid–water (5:1:4); all R_F values agreed within ±1.0%.

TABLE 2. *Summary of peptides used to determine the specificity of Streptococcus lactis intracellular proteinase*

Peptides not hydrolyzed[a]	Peptides hydrolyzed
Glycyl-DL-phenylalanine	DL-Alanyl-DL-phenylalanine
Glycyl-glycine	Alanyl-alanine
Glycyl-DL-methionine	DL-Alanyl-DL-methionine
Glycyl-valine	Alanyl-valine
DL-Leucyl-glycine	DL-Alanyl-leucine
Glycyl-L-phenylalanine	DL-Valyl-L-alanine
Glycyl-glycyl-DL-phenylalanine	Glycyl-alanine
DL-leucyl-glycyl-DL-phenylalanine	Alanyl-glycine
Glycyl-serine	DL-Alanyl-DL-norleucine
Glycyl-L-tyrosine	Glycyl-L-asparagine
Glycyl-glycyl-glycine	Glycyl-L-aspartic
Glycyl-DL-norvaline	
Glycyl-tryptophan	
Leucyl-DL-isoleucine	

[a] No hydrolysis observed within 24 hr at 37 C.

Fraction 1 was eluted in the void volume from Sephadex G-10, and after concentration was rechromatographed on Sephadex G-25 with 0.05 M sodium phosphate (*p*H 7.0) as the eluting buffer. As a result, this fraction was further separated into four subfractions, 1A, B, C, and D (Fig. 2). Fraction 1A was eluted in the void volume, indicating a molecular weight >5,000. This fraction remained at the origin during two-dimensional paper chromatography, and probably was either nonhydrolyzed protein or enzyme.

The homogeneity of the individual fractions was determined by two-dimensional paper chromatography. The spots observed in each fraction were compared with spots obtained from a chromatograph of the unfractionated supernatant mixture. The unfractionated mixture contained

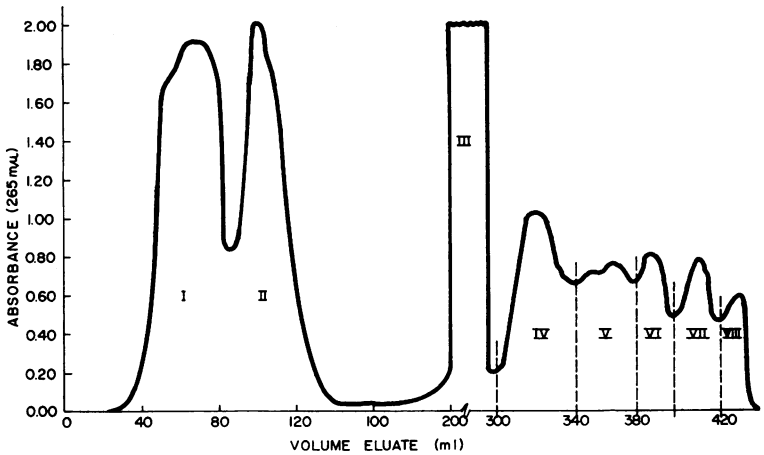

FIG. 1. *Elution profile of DNP peptide fragments from Sephadex G-10 of the enzymatic digest of ribonuclease (oxidized).*

25 well-separated components, all of which were accounted for in fractions 1B to 9 (Fig. 3). Five components were found in fraction 9, four in fraction 2A, and three in fraction 2B, whereas fractions 3B, 7, and 8 each had two components. Fractions 1B, C, D, 3A, 4, 5, and 6 each contained a single component. A certain degree of overlapping of components occurred, particularly in fractions 4 to 8. Inspection of the ribonuclease sequence indicated that 20 peptide fragments should be obtained if only peptide bonds involving alanyl, aspartyl, and asparaginyl residues were hydrolyzed.

After the peptides in each fraction were determined, the fractions were acid-hydrolyzed and the DNP amino acids were extracted with ether (except DNP cysteic acid, which was found in the aqueous phase) and identified by two-dimensional paper chromatography. Eleven different DNP amino acids were identified in the individual fractions (1B to 8; *see* Table 3). Of these, cysteic acid occurred in four fractions, and serine and lysine were found in three fractions each. The positions of these amino acids in the ribonuclease sequence was examined to determine, first, their proximity to alanine, aspartic acid, or asparagine residues, and, second, the possibility of their being an N-terminal residue. The sites of hydrolysis of ribonuclease by the enzyme were determined from these comparisons. For example, serine will be used to illustrate the identification of the peptide fragments (Table 4). Serine as its DNP derivative was found in fractions 3B, 4, and 8. Fractions 3B and 8 contained one additional peptide. Serine occurs in 15 positions in ribonuclease but, if only peptide bonds of alanyl, asparatyl, or asparaginyl residues were hydrolyzed, it could be N-terminal only in positions 15, 21, and 123. Serine would then occur in a

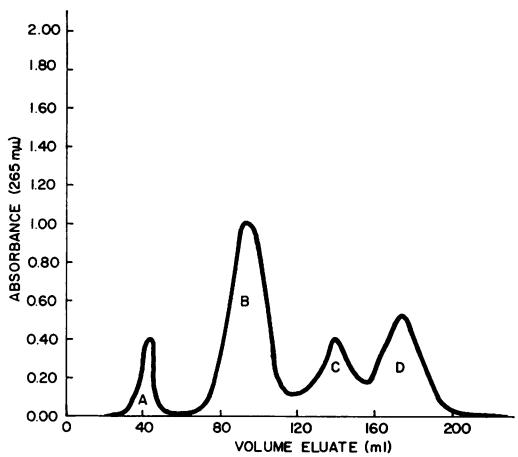

FIG. 2. *Elution profile of fraction I (Sephadex G-10) from Sephadex G-25.*

tetra-, tri-, and dipeptide, provided that the next sequential alanyl, aspartyl, asparaginyl bond was broken. In fraction 3B, the aqueous phase revealed that threonine and serine were present. The ether extract of fraction 3B contained, in addition to DNP serine, DNP leucine. The sequence beginning at position 15 is Ser Ser Thr Ser. The only peptide that would yield a DNP leucine occurs as a tripeptide in positions 35 to 37 (Leu Thr Lys.). The tetrapeptide beginning at position 15 is the only peptide that would give DNP serine (N-terminal) and threonine and serine. Fraction 4 contained a single peptide with N-terminal serine, and the aqueous phase contained only serine. This corresponded to the tripeptide beginning at position 21 (Ser Ser Ser). Fraction 8 contained an N-terminal serine peptide with valine. The other DNP amino acids were identified with a particular segment of the ribonuclease molecule in the same manner.

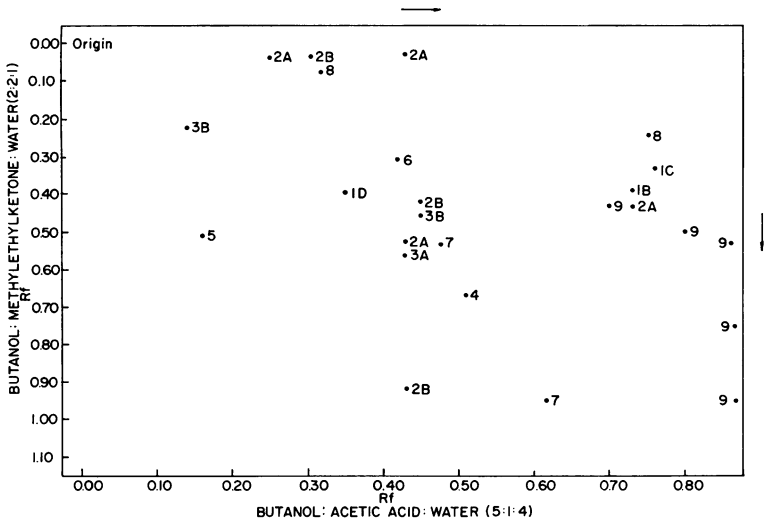

FIG. 3. *Two-dimensional chromatogram of 1-fluoro-2,4-dinitro-benzene-treated enzymatic digest of ribonuclease.*

TABLE 3. *DNP-amino acids identified after acid hydrolysis of the separated peptide fragments of ribonuclease digest*

Amino acid	Fraction of occurrence
Cysteic	1B, 1C, 5, 8
Proline	1D
Valine	2A, 7
Leucine	3B
Threonine	2B
Lysine	2B, 3A, 6
Tyrosine	2A, 6
Glycine	7
Glutamine	2A
Arginine	2A
Serine	3B, 4, 8

TABLE 4. *Identification of the sites of enzyme hydrolysis of ribonuclease with serine used as an illustration*

DNP-amino acid observed	Fraction observed	Size of peptide to next N-terminal DNP-amino acid found	Amino acids in aqueous phase[a]
Serine[b]	3B	4 residues (15–18)	Serine, threonine
Serine	4	3 residues (21–23)	Serine
Serine	8	2 residues (123–124)	Valine

[a] Aqueous phase: after neutralization of ether-insoluble phase.

[b] Serine occurs in the following positions: 15, 16, 18, 21, 22, 23, 32, 50, 59, 75, and 77.

Fraction 9, which was eluted from Sephadex G-10 much later, was found to contain only DNP derivatives of free amino acids; i.e., after subjecting fraction 9 to conditions of acid hydrolysis and ether extraction, no amino acids could be detected in the aqueous portion. The DNP derivatives of five amino acids—alanine, aspartic acid, asparagine (identified in a nonhydrolyzed sample), valine, and cysteic acid—were found in this fraction. From these data, the intracellular proteinase is interpreted as hydrolyzing peptide bonds involving principally alanyl, aspartyl, and asparaginyl residues to yield peptides of various sizes (Fig. 4).

Oxidized B-chain insulin also was subjected to hydrolysis by the enzyme. Only four fractions were eluted from Sephadex G-10 (Fig. 5). Fraction 4 was eluted after 1 liter of eluate was collected. The isolated peptide fractions were homogeneous as determined by two-dimensional paper chromatography and high-voltage electrophoresis. After acid hydrolysis, analysis of fractions 1, 2, and 3 showed that leucine, glutamine, and phenylalanine, respectively, were N-terminal residues (Table 5). Fraction 4 contained only DNP alanine and DNP asparagine (identified in nonhydrolyzed portion). The DNP derivatives of these amino acids would have been obtained only if both peptide bonds at alanyl and asparaginyl residues were hydrolyzed (Fig. 6).

DISCUSSION

In the present study, several dipeptides and tripeptides were tested for their ability to serve as substrate. Only those peptides which con-

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Lys	Glu	Thr	Ala	Ala	Ala	Lys	Phe	Glu	Arg	Gln	His	Me*	Asp	Ser
	Ser	Thr	Ser	Ala	Ala	Ser	Ser	Ser	Asn	Tyr	Cy*	Asn	Gln	Me*	Me*
31	Lys	Ser	Arg	Asn	Leu	Thr	Lys	Asp	Arg	Cy*	Lys	Pro	Val	Asn	Thr
	Phe	Val	His	Glu	Ser	Leu	Ala	Asp	Val	Gln	Ala	Val	Cy*	Ser	Gln
61	Lys	Asn	Val	Ala	Cy*	Lys	Asn	Gly	Gln	Thr	Asn	Cy*	Tyr	Gln	Ser
	Tyr	Ser	Thr	Me*	Ser	Ile	Thr	Asp	Cy*	Arg	Glu	Thr	Gly	Ser	Ser
91	Lys	Tyr	Pro	Asn	Cy*	Ala	Tyr	Lys	Thr	Thr	Gln	Ala	Asn	Lys	His
	Ile	Ile	Val	Ala	Cy*	Glu	Gly	Asn	Pro	Tyr	Val	Pro	Val	His	Phe
121	Asp	Ala	Ser	Val	***										

FIG. 4. *Amino acid sequence of ribonuclease (oxidized). Cy* = cysteic acid; Me* = methionine sulfone.*

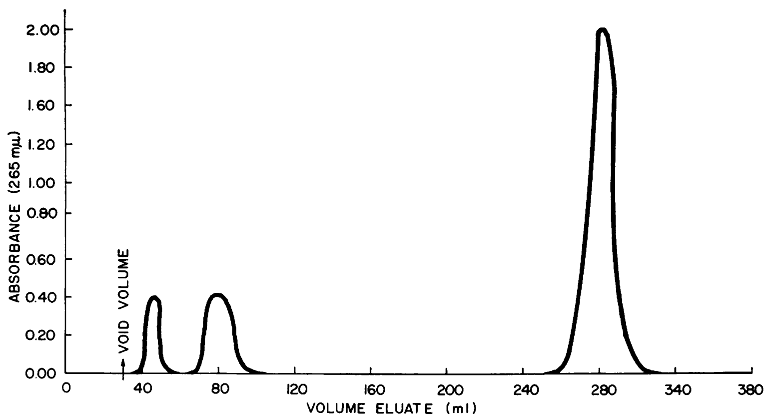

FIG. 5. *Elution profile of DNP-peptide fragments from Sephadex G-10 of the enzymatic digest of oxidized B-chain insulin.*

TABLE 5. *DNP-amino acids identified after acid hydrolysis of peptide fragment of enzymatic digest of oxidized B-chain insulin*

Amino acid	Fraction of occurrence
Phenylalanine	3
Glutamine	2
Leucine	1
Alanine	4
Asparagine	4

tained alanine, aspartic acid, and asparagine were hydrolyzed. In our studies, if a peptide was not hydrolyzed within 4 hr, incubation at 37 C was extended to 24 hr to assure that no hydrolysis of the peptides occurred. Since glycyl-alanine, alanyl-glycine, alanyl-valine, and valyl-alanine were hydrolyzed, it appears that the intracellular proteinase hydrolyzes peptide bonds on both sides of the alanyl residue. This is in contrast to other proteolytic enzymes, such as trypsin and chymotrypsin, which are specific for the amino acid contributing the carbonyl to the peptide bond. Studies are now in progress to ascertain the relative rates of hydrolysis of peptides containing alanine, aspartic acid, or asparagine. Van der Zant and Nelson (10) observed that a cell-free extract of *S. lactis* hydrolyzed glycyl peptides as well as alanyl peptides. In the present study, the intracellular proteinase failed to hydrolyze glycyl peptides except those containing alanine, aspartic acid, or asparagine. The hydrolysis of aspartyl and

		↓	↓	5					10				↓	↓
Phe	Val	Asn	Gln	His	Leu	Cy*	Gly	Ser	His	Leu	Val	Glu	Ala	
					20				25					
Leu	Tyr	Leu	Val	Cy*	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	
30	↓													
Lys	Ala													

FIG. 6. *Amino acid sequence of oxidized B-chain insulin. Cy* = cysteic acid*

asparaginyl peptides in *S. lactis* has not been previously reported.

The hydrolysis of principally those peptide bonds containing alanyl, aspartyl, and asparaginyl residues by the intracellular proteinase was confirmed by the results when oxidized bovine ribonuclease A and oxidized B-chain insulin were used as substrates. These proteins also were subjected to a 24-hr incubation period with the enzyme to assure complete hydrolysis of all peptide bonds susceptible to enzymatic attack. Neither alanine, aspartic acid, nor asparagine was identified as an N-terminal residue on a peptide in either substrate. These amino acids were found as free amino acids in the proteinase digests of both proteins. No requirement for the amino or carbonyl side of alanine was observed in the peptides, and the data obtained with the protein substrates suggest that both peptide bonds of aspartyl and asparaginyl residues are similarly hydrolyzed.

Free valine and cysteic acid were found in the proteinase digest of ribonuclease but not in the insulin digest. In ribonuclease, valine and cysteic acid occur in the sequence with one of the required amino acids (alanine, aspartic acid, asparagine) on each side. For valine, this occurs at positions 62 to 64 (Asn Val Ala), and for cysteic acid at positions 94 to 96 (Asn Cy^+ Ala). Hydrolysis of these tripeptides by the enzyme would liberate these amino acids. In contrast, the same type of peptide sequence does not occur in insulin, and, therefore, it is not surprising to find that the only free amino acids released upon incubation of this polypeptide are alanine and asparagine. Studies are in progress to determine whether or not, upon shorter periods of hydrolysis, peptide fragments containing terminal alanine, aspartic acid, or asparagine can be isolated.

The data presented provide only tentative evidence that the intracellular proteinase from *S. lactis* is specific for the alanyl, aspartyl, and asparaginyl residues in peptides and proteins. Since alanine, aspartic acid, and asparagine occur frequently in proteins (3, 7), this enzyme would be useful as an additional biochemical reagent when the specificity of the enzyme is confirmed. Studies are in progress to provide quantitative information on the kinetics of hydrolysis on both peptides and proteins.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service Training Grant ES-61 from the Division of Environmental Health Science.

The technical services of Elizabeth M. Keele were appreciated.

LITERATURE CITED

1. ANFINSEN, C. B., R. R. REDFIELD, W. L. CHOATE, J. PAGE, AND W. R. CARROLL. 1954. Studies on the gross structure, cross-linkages and terminal sequences in ribonuclease. J. Biol. Chem. **207:** 201.
2. COWMAN, R. A., AND H. E. SWAISGOOD. 1966. Temperature - dependent association - dissociation of *Streptococcus lactis* intracellular proteinase. Biochem. Biophys. Res. Commun. **23:** 790–793.
3. ECK, R. V., AND M. O. DAYHOFF [ED.]. 1966. Atlas of protein sequence and structure. National Biomedical Research Foundation, Silver Spring, Md.
4. FLODIN, P. 1961. Methodological aspects of gel filtration with special reference to desalting operations. J. Chromatog. **5:**103–115.
5. FRAENKEL-CONRAT, H., J. I. HARRIS, AND A. L. LEVY. 1955. Recent developments in techniques for terminal and sequence studies in peptides and proteins. Methods Biochem. Analy. **2:** 359–425.
6. HUSAIN, I., AND I. J. MCDONALD. 1957. Amino acids and utilization of sodium caseinate by lactic streptococci. Can. J. Microbiol. **3:**387–491.
7. KONING, P. J. DE, AND P. J. VAN ROOLJEN. 1965. Genetic variants of α_{S1}-casein; amino acid composition of the variants B, C, and BC. Biochem. Biophys. Res. Commun. **20:**241–245.
8. MACLEOD, P., AND D. F. GORDON, JR. 1961. Peptides as sources of essential amino acids for lactic streptococci. J. Dairy Sci. **44:**237–242.
9. REITER, R., AND J. ORAM. 1962. Nutritional studies on cheese starters. I. Vitamin and amino acid requirements of single strain starters. J. Dairy Res. **29:**63–77.
10. ZANT, W. C. VAN DER, AND F. E. NELSON. 1954. Characteristics of some endocellular peptidases of *Streptococcus lactis*. J. Dairy Sci. **37:**795–804.