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Sequence of the *N*-Terminal Half of *Bacillus amyloliquefaciens* α-Amylase

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Bacillus amyloliquefaciens α-amylase (1,4-α-D-glucan glucanohydrolase. EC 3.2.1.1), which is commercially supplied as '*Bacillus subtilis* α-amylase' does not cross-react immunologically with *B. subtilis* α-amylase. This enzyme (from *B. amyloliquefaciens*) was cleaved by treatment with CNBr into seven fragments. Peptide A was selected for sequence determination. It is the longest one, containing 185 amino acids (i.e. approx. 50% of the total molecule) and connects to the hexapeptide of the *N*-terminus. Its primary structure was aligned by use of various proteolytic enzymes. The sequence of amino acids 181–184 is identical with that of amino acids 14–17 of the α-amylase isolated from *B. subtilis* (except that amino acid 183 is asparagine rather than aspartic acid).

Bacillus subtilis produces two types of α-amylase [1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1 (liquefying and saccharifying)] (Fukumoto & Okada, 1963). Although the organism producing the liquefying enzyme has been reclassified as *Bacillus amyloliquefaciens* (Welker & Campbell, 1967), the term '*B. subtilis* α-amylase' has been retained in many publications. Mäntsälä & Zalkin (1979) found that purified *B. subtilis* α-amylase (from strain YY88) has a mol.wt. of 67000 and the terminal sequence: Leu-Thr-Ala-Pro-Ser-Ile-Lys-(Ser)-Gly-(Thr)-Ile-Leu-X-Ala-Trp-Asn-Trp. When tested immunologically, this enzyme cross-reacts with α-amylase from several other strains of *B. subtilis*, but not with that of *B. amyloliquefaciens*. We have initiated structural studies on an enzyme supplied commercially as a product of *B. subtilis* (Sigma A6380). Its mol.wt. is 50000 (Detera & Friedberg, 1979) and its *N*-terminal sequence, which we report here, differs completely from that noted by Mäntsälä & Zalkin (1979).

We also isolated α-amylase secreted into the medium by *B. amyloliquefaciens* N (A.T.C.C. 23845) (mol.wt. 50000). Its *N*-terminal sequence begins like that of the commercial preparation described below and these two enzymes cross-react immunologically. Thus the commercial material should be labelled 'produced by *B. amyloliquefaciens*' rather than as '*B. subtilis* α-amylase'.

Experimental

Materials

A culture of *B. amyloliquefaciens* N was made available by the American Type Culture Collection (A.T.C.C.). Four-times crystallized *B. subtilis* α-amylase was obtained from Sigma. 1-Chloro-4-phenyl-3-L-tosylamidobutan-2-one ('TPCK')-treated trypsin, four-times crystallized α-chymotrypsin and carboxypeptidase Y were obtained from Worthington. *Staphylococcus aureus* V8 proteinase was obtained from Miles Laboratories. Thermolysin and pyroglutamate aminopeptidase were purchased from Boehringer-Mannheim. All sequencing reagents (Sequanal grade) were ordered from Beckman, Pierce, or Jackson and Burdick. Polybrene was supplied by Aldrich, and CNBr by Pierce.

Fragmentation of amylase by CNBr

The cleavage of methioninyl bonds by CNBr was performed as described by Gross & Witkop (1962). α-Amylase (1g) was dissolved in 100ml of 70% (v/v) formic acid. To this solution, CNBr (1.4g) was added. After 20h reaction at room temperature, the solution was diluted with 10vol. of water and freeze-dried.

Trypsin digestion limited to arginyl bonds

The trypsin digestion was performed as described by Heil *et al.* (1974). Citraconylated peptide A (100mg) was treated with trypsin (2mg) in 10ml of 0.5% NH_4HCO_3, pH 8.2, for 4h at 37°C. Termination of the reaction was accomplished either by separation of peptides on a Sephadex G-25 column equilibrated with 0.025 M-NH_3 and freeze-drying of the solution containing these peptides or by addition of acetic acid to a final concentration of 25% (v/v). This solution was kept overnight to remove citra-

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conyl groups and then applied to a Sephadex G-25 column equilibrated with 25% acetic acid.

Digestion with chymotrypsin

Digestion with chymotrypsin was carried out as described above for trypsin.

Cleavage with Staphylococcus aureus V8 proteinase

For enzymic cleavage of glutamyl bonds we utilized the method reported by Hogg & Hermodson (1977). Peptide A (70mg) was dissolved in 6ml of 0.05M-acetic acid. The pH was adjusted to 4.0 with 1M-NaOH, even though the peptide precipitated at this pH. Enzyme (2mg) was added and the suspension was incubated for 20h at 37°C.

Thermolysin hydrolysis

Hydrolysis was performed as described by Heil *et al.* (1974). Succinylated peptide A (130mg) was dissolved in 13ml of 0.5% NH_4HCO_3, pH8.2. Thermolysin (2.6mg) was added and the mixture was incubated at 55°C for 1h.

Pyroglutamate aminopeptidase reaction

Removal of pyrrolidinecarboxylic acid (5-oxoproline; pyroglutamic acid), which resulted from cyclization of glutamine, was performed as described by Podell & Abraham (1978), by using pyroglutamate aminopeptidase. The succinylated peptide A was dissolved in 3ml of 'deblocking buffer' [0.1M-sodium phosphate, pH8.0, containing 5mM-dithiothreitol, 10mM-EDTA (sodium salt) and 5% (v/v) glycerol]. Pyroglutamate aminopeptidase (1.5mg) was added and the mixture was flushed with N_2 and incubated at room temperature for 14h. Then the mixture was desalted on a Sephadex G-25 column and the eluant containing the peptide was freeze-dried.

Modification of peptide with citraconic anhydride

Blocking of α-amino groups with citraconic anhydride was performed as described by Atassi & Habeeb (1972). Peptide A (100mg) was dissolved in 10ml of 0.5% NH_4HCO_3, pH8.2, containing 6M-guanidinium chloride. Citraconic anhydride (0.4ml) was added drop by drop while the pH was kept at 8.2 with 5M-NaOH (0.7ml of NaOH was consumed). After 3h reaction, salt was removed on a Sephadex G-25 column equilibrated with 0.025M-NH_3 and the eluant containing the polypeptide was freeze-dried.

Separation of peptides

For the separation of large peptides, column chromatography with Sephadex or Bio-Gel was used. The fractions were analysed by measuring A_{280}.

Small peptides were separated on a column (1cm × 20cm) of cation-exchange-resin (Beckman spherical resin PA 35) by using both pH and concentration gradients at a flow rate of 45ml/h at 50°C.

Volatile pyridine/acetate buffers (Jones, 1970) were used. The initial buffer contained 250ml of pyridine/acetate (0.05M in pyridine), pH2.5, or pyridine/acetate (0.1M in pyridine), pH3.5, and the limiting buffer consisted of 250ml of pyridine/acetate (2M in pyridine), pH5.0. The ninhydrin reaction (Moore, 1968) was used for monitoring the elution of the peptides.

Sequencing

The peptides (0.2–0.4μmol) were sequenced by using the method of Edman & Begg (1967) in a Beckman 890 sequencer. Both the protein/Quadrol (060275) and the peptide/*NN*-dimethyl-*N*-allylamine ('DMAA') (111374) programs were used. Polybrene was used in conjunction with the *NN*-dimethyl-*N*-allylamine program in order to prevent the premature loss of peptides from the sequencer cup (Klapper *et al.*, 1977). When sequencing the entire peptide A fragment (with its terminal glutamine residue removed) sodium dodecyl sulphate was added to the sequencer cup as recommended for insoluble peptides (Bailey *et al.*, 1977). Phenylthiohydantoin derivatives of the amino acids were identified by g.l.c. (Pisano *et al.*, 1972) with a Beckman GC-65-model gas chromatograph. In addition, a t.l.c. procedure was utilized (Kulbe, 1974). Backhydrolysis of the thiazolinone derivatives of arginine and histidine was accomplished with hydroiodic acid (Smithies *et al.*, 1971).

Results and Discussion

When α-amylase was treated with CNBr and separated on a Sephadex column with 0.5M-formic acid as eluting agent, six fragments were isolated (Fig. 1). The largest peak (peptide A) (fractions 84–97), which represents the peptide connected to the hexapeptide at the *N*-terminal end of the intact enzyme, was purified further by application to a Bio-Gel P-60 column. The amino acid composition of peptide A is given in Table 1. The sequence of the *N*-terminal amino acids of the intact enzyme was reported by Friedberg & Thompson (1974) as Val-Asn-Gly-Thr-Leu-Met-Gln-Tyr-Phe-Glu-Trp-Tyr. Since there appears no free amino group at the *N*-terminal end of fragment A as a result of cyclization of the glutamine into pyrrolidonecarboxylic acid in the process of CNBr treatment, this fragment was treated with pyroglutamate aminopeptidase, which removed pyrrolidonecarboxylic acid. Thus the α-amino group of tyrosine, the penultimate amino acid at the *N*-terminus of peptide A was freed, and this made possible further sequencing in the automated

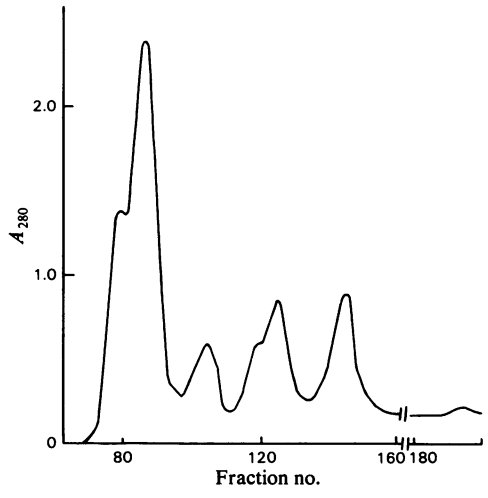


Fig. 1. *Separation of α-amylase peptides obtained after CNBr treatment*

The peptide mixture (100mg) was applied to a column (2.5cm × 190cm) of Sephadex G-50 (fine grade), equilibrated and eluted with 0.5M-formic acid at a flow rate of 20ml/hour. Fractions of 5.3ml/tube were collected. Fractions 84–97 were pooled as peptide A.

Table 1. *Amino acid composition of fragment A*

The amino acid analysis was carried out as described by Moore *et al.* (1958). Abbreviation used: n.d., not determined.

Amino acid	Composition (residues/molecule) From amino acid analysis	From sequence data
Asp	26	28
Thr	10	10
Ser	10	9
Glu	23	23
Pro	6	6
Gly	16	15
Ala	13	13
Val	11	12
Met		1*
Ile	6	6
Leu	10	9
Tyr	11	11
Phe	9	8
His	6	5
Lys	12	11
Arg	10	10
Trp	n.d.	8
Total	179	185

* Present as homoserine.

sequencer. The 20-amino acid-sequence was identified as Tyr-Phe-Glu-Trp-Tyr-Thr-Pro-Asn-Asp-Gly-Gln-His-Trp-Lys-Arg-Ile-Gln-X-Asp-Ala.

Trypsin-digested citraconylated peptide A was

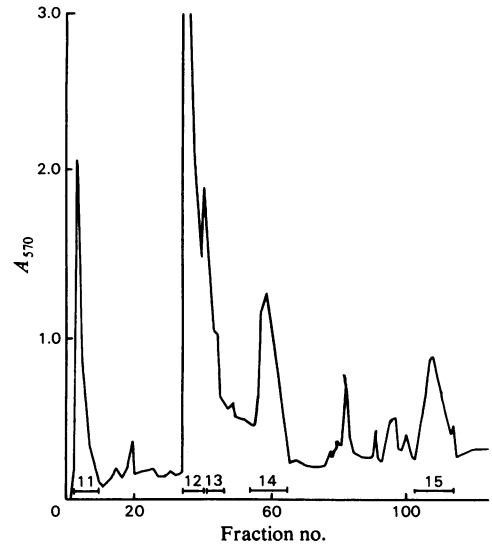


Fig. 2. *Separation of peptide ACT 1 on a cation-exchange-resin column*

Peptide ACT 1 (10mg) was applied to a column (1cm × 20cm) of Beckman PA-35 resin equilibrated with pyridine/acetate (0.05M in pyridine), pH2.5. At fraction no. 20, a gradient starting with 250ml of the same buffer and with 250ml of pyridine/acetate (2M in pyridine), pH5.0, as limiting buffer was begun. Fractions of 2.5ml/tube were collected at an elution flow rate of 45ml/h.

fractionated on a Sephadex column into fractions ACT 1, ACT 2 and ACT 3. After removal of the citraconyl group, each of these three fractions was applied to a cation-exchange-resin column. Fraction ACT 1 yielded five peptide peaks (ACT 11, 12, 13, 14 and 15) as shown in Fig. 2. Fractions ACT 14 and 15 were analysed without further purification. Fraction ACT 14 contained 17 amino acids. Its primary structure was Asn-Gln-Glu-Thr-Ser-Glu-Gln-Tyr-Gln-Ile-Lys-Ala-Trp-Thr-Asp-Phe-Arg (Table 2). Fraction ACT 15, which contained 18 amino acids, was sequenced as Thr-Lys-Tyr-Gly-Thr-Lys-Ser-Glu-Leu-Gln-Asp-Ala-Ile-Gly-Ser-Leu-His-Arg (Table 2). Fraction ACT 13 was rechromatographed on a cation-exchange-resin column and two major peaks resulted. Amino acid analysis of these peptides gave identical compositions for both peptides. They contained, however, no arginine but homoserine. Thus they appeared to represent the *C*-terminus of fragment (peptide) A (Table 2). The separation of two such peptides on the ion-exchange-resin column could be due to the equilibrium state between homoserine and homoserine lactone. To quantify the terminal homoserine residue, the acid hydrolysate of one of these was exposed to 2M-NH_3 at 37°C for 1h as described by Tang &

Table 2. *Amino acid composition of citraconylated Trypsin-digested peptides ACT 13, ACT 14 and ACT 15*
The amino acid analysis was carried out as described by Moore *et al.* (1958). Values in parentheses indicate the number of residues obtained by sequencing. Abbreviation used: n.d., not determined.

Amino acid	Peptide ...	Composition (residues/molecule) ACT 13	ACT 14	ACT 15	Total
Asp		2.2 (2)	2.7 (2)	1.0 (1)	(5)
Thr		—	1.9 (2)	1.3 (2)	(4)
Ser		1.0 (1)	1.0 (1)	2.0 (2)	(4)
Glu		1.9 (2)	5.2 (5)	2.0 (2)	(9)
Pro		—	—	—	—
Gly		1.8 (2)	0.7 (0)	1.7 (2)	(4)
Ala		0.8 (1)	1.4 (1)	1.1 (1)	(3)
Val		0.6 (1)	—	—	(1)
Ile		—	0.8 (1)	0.6 (1)	(2)
Leu		0.6 (1)	—	1.4 (2)	(3)
Tyr		1.1 (1)	1.1 (1)	0.9 (1)	(3)
Phe		—	1.0 (1)	—	(1)
Lys		1.0 (1)	0.9 (1)	2.0 (2)	(4)
His		—	—	1.2 (1)	(1)
Arg		—	1.0 (1)	1.0 (1)	(2)
Trp		n.d. (2)	n.d. (1)	n.d. (0)	(3)
Hse		(1)	—	—	(1)
Total		11.0 (15)	19.2 (17)	16.3 (18)	(50)

Table 3. *Amino acid composition of citraconylated trypsin-digested peptides ACT 12A and ACT 12B*
For further details see the legend to Table 2.

Amino acid	Peptide ...	Composition (residues/molecule) ACT 12A	ACT 12B	Total
Asp		5.7 (7)	7.8 (8)	(15)
Thr		1.3 (2)	1.8 (2)	(4)
Ser		—	2.6 (3)	(3)
Glu		2.2 (3)	6.0 (6)	(9)
Pro		0.5 (1)	1.7 (3)	(4)
Gly		1.6 (2)	5.9 (5)	(7)
Ala		4.8 (5)	4.0 (3)	(8)
Val		5.1 (7)	2.5 (2)	(9)
Ile		—	2.5 (3)	(3)
Leu		0.8 (1)	4.7 (5)	(6)
Tyr		0.4 (1)	3.4 (4)	(5)
Phe		—	1.0 (1)	(1)
Lys		0.6 (1)	1.9 (2)	(3)
His		0.5 (1)	0.9 (1)	(2)
Arg		1.0 (1)	1.0 (1)	(2)
Trp		n.d. (0)	n.d. (1)	(1)
Total		(32)	(50)	(82)

Hartley (1967), dried *in vacuo* and the diluting buffer for amino acid analysis was added just before application to the analyser. (The column in the analyser had been equilibrated with a buffer of pH 3.2 instead of pH 3.25.) Amino acid analysis gave the following composition (residues/peptide): aspartic acid, 2; serine, 1; glutamic acid, 2; glycine, 2; alanine, 1; valine, 1; leucine, 1; tyrosine, 1; lysine, 1; and homoserine (Hse), 0.3. This peptide was analysed in the sequencer as Gly-Glu-Gly-Lys-Ala-Trp-Asp-Trp-Gln-Val. The rest of the peptide could not be identified by this approach. Hence, carboxypeptidase Y digestion of fragment A was attempted by using the procedure suggested by Martin *et al.* (1977). Six amino acids having the sequence Hse-Leu-Tyr-Ser-Asp-Val were released in tandem by treatment with carboxypeptidase Y, when samples were examined 5 or 30 min after the beginning of incubation. Thus information on the primary structure of peptide ACT 13 was complete.

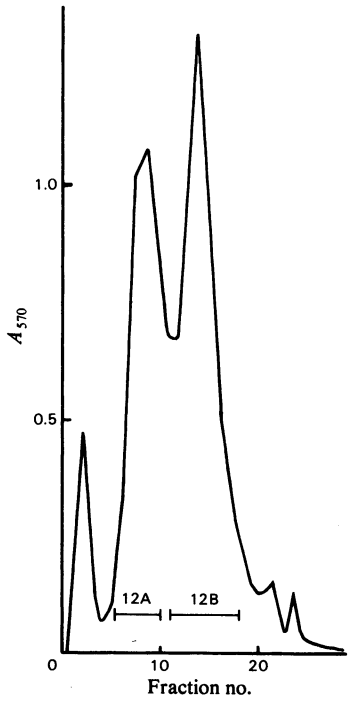


Fig. 3. *Separation of peptide ACT* 12 *on a cation-exchange-resin column*

Peptide ACT 12 was applied to a column equilibrated with pyridine/acetate (0.1 M in pyridine), pH 3.5, and eluted with a gradient composed of 250 ml of the same buffer and 250 ml of pyridine/acetate (2 M in pyridine), pH 5.0.

When fraction ACT 12 was separated on an ion-exchange-resin column, two peptides, ACT 12A and 12B, were obtained (Fig. 3 and Table 3). The sequence of peptide ACT 12A began with Asn-Val-Gln-Val-Tyr- and that of peptide ACT 12B (contaminated with peptide ACT 12A) was analysed as Ile-Gln-Asn-Asp-Ala-Glu. Fraction ACT 12 was exposed again to trypsin digestion (this time without prior citraconylation) to yield peptides ACT 1203, 1214, 1236 and 1283 (Fig. 4). Analysis of peptide ACT 1214 (see Table 4) indicates that this peptide is the *N*-terminal portion of peptide ACT 12A. Peptide ACT 1283 is a tetrapeptide with the

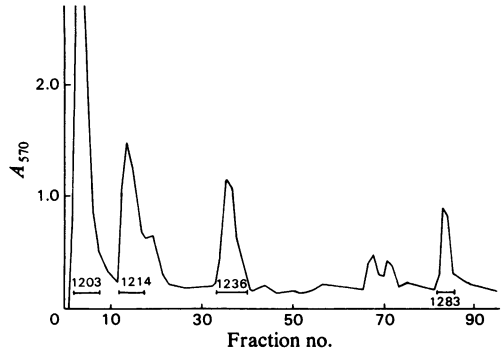


Fig. 4. *Separation of trypsin-digested peptide ACT* 12

Peptide mixture (10 mg) was applied to a cation-exchange-resin column (1 cm × 20 cm). Details were the same as those described in Fig. 2, except that the gradient was started at the beginning of the elution.

Table 4. *Amino acid composition of peptides obtained from peptide ACT* 12 *after digestion with trypsin*

For further details see the legend to Table 2.

Amino acid	Peptide ...	Composition (residues/molecule) ACT 1214	ACT 1236	ACT 1283	Total
Asp		3.2 (3)	3.4 (3)	—	(6)
Thr		1.0 (1)	—	1.0 (1)	(2)
Ser		1.0 (1)	—	—	(1)
Glu		2.3 (2)	1.3 (1)	—	(3)
Pro		1.1 (2)	—	—	(2)
Gly		1.3 (1)	1.2 (1)	1.2 (1)	(3)
Ala		3.0 (3)	—	—	(3)
Val		1.1 (1)	3.4 (4)	1.0 (1)	(6)
Ile		2.2 (3)	—	—	(3)
Leu		1.8 (2)	1.0 (1)	—	(3)
Tyr		1.0 (1)	1.0 (1)	—	(2)
Phe		—	—	—	(0)
Lys		1.0 (1)	1.0 (1)	—	(2)
His		0.8 (1)	0.9 (1)	—	(2)
Arg		—	—	1.0 (1)	(1)
Trp		n.d. (1)	n.d. (0)	n.d. (0)	(1)
Total		20.8 (23)	13.2 (13)	4.2 (4)	(40)

Table 5. *Amino acid composition of citraconylated trypsin-digested peptides ACT 31, ACT 32, ACT 33, ACT 34 and ACT 35*
For further details see the legend to Table 2.

Amino acid	Peptide ...	Composition (residues/molecule) ACT 31	ACT 32	ACT 33	ACT 34	ACT 35	Total
Asp		1.0 (1)	2.2 (2)	—	2.5 (3)	2.0 (2)	(8)
Thr		—	1.0 (1)	—	—	1.2 (1)	(1)
Ser		—	—	—	0.7 (1)	1.0 (1)	(2)
Glu		1.2 (1)	2.7 (3)	—	1.3 (1)	—	(5)
Pro		—	1.0 (1)	0.7 (1)	—	—	(2)
Gly		—	1.1 (1)	0.8 (1)	1.1 (1)	1.3 (1)	(4)
Ala		1.3 (1)	—	—	0.9 (1)	—	(2)
Val		1.9 (2)	—	—	—	—	(2)
Tyr		—	2.0 (2)	—	0.5 (0)	1.5 (1)	(3)
Phe		—	1.1 (1)	0.8 (1)	1.0 (1)	1.0 (1)	(4)
Lys		—	1.0 (1)	—	—	1.0 (1)	(2)
His		—	1.0 (1)	—	1.1 (1)	0.7 (0)	(2)
Arg		1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	0.6 (0)	(4)
Try		n.d. (1)	n.d. (2)	n.d. (0)	n.d. (1)	n.d. (0)	(4)
Total		6.4 (7)	14.1 (16)	3.3 (4)	10.1 (11)	10.3 (8)	(46)

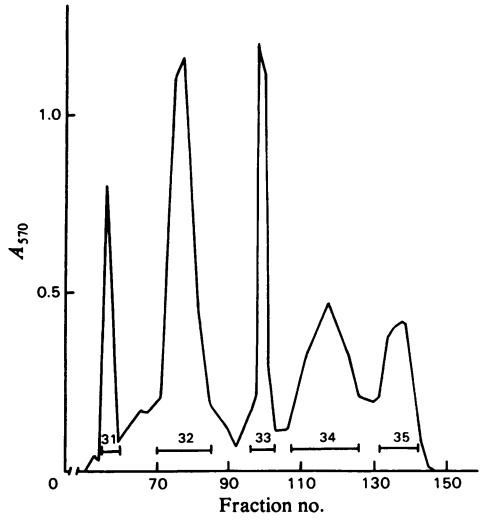


Fig. 5. *Separation of peptide ACT 3 on a cation-exchange-resin column*
The conditions for column development were the same as those described in Fig. 2.

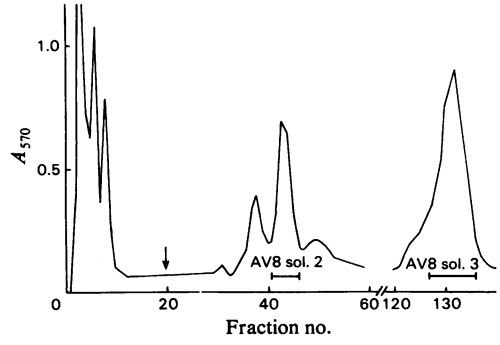


Fig. 6. *Separation of the AV8 sol. fraction on a cation-exchange-resin column*
Approx. 5 mg of peptide mixture was used for separation. Details were the same as those described in Fig. 2.

sequence Gly-Thr-Val-Arg. Peptide ACT 1203 was found to be a mixture of two peptides present in different molar amounts. The peptide representing the larger portion was the arginine-containing peptide that connects to peptide ACT 1236 (see peptide ACT 12A) and contains 19 amino acids. The peptide present in smaller amounts consists of 23 amino acids and has lysine as its *C*-terminal residue.

Fraction ACT 11 was found to be a mixture of peptides ACT 12A and 12B. We cannot explain, however, the erratic behaviour of the ion-exchange resin towards fractions ACT 11 and ACT 12.

When fraction ACT 2 was freeze-dried, the yield was very small. The entire sample was applied to a cationic-exchange-resin column, but no distinct peptide peak was obtained after analysis.

The resolution of fraction ACT 3 by a resin column is shown in Fig. 5. The amino acid compositions of the resulting peptides are given in Table 5. Peptide ACT 31 has the following sequence: Asp-Trp-Val-Gln-Ala-Val-Arg. Peptide ACT 32 has an amino acid composition identical with the first 16 amino acids of peptide A, and its *N*-terminus is blocked. Peptide ACT 33 is a tetrapeptide with the following sequence: Phe-Pro-Gly-Arg. The amino

Table 6. *Amino acid composition of peptides AV8 sol. 2 and 3*

For further details see the legend to Table 2.

Amino acid	Peptide ...	Composition (residues/molecule) AV8 sol. 2	AV8 sol. 3
Asp		2.5 (3)	—
Thr		—	2.6 (3)
Ser		—	0.7 (1)
Glu		1.9 (2)	3.0 (3)
Pro		n.c.* (1)	—
Gly		0.3 (0)	2.0 (2)
Ala		1.2 (1)	—
Val		1.0 (1)	0.5 (1)
Ile		—	—
Leu		—	—
Tyr		—	0.6 (1)
Phe		—	0.6 (1)
Lys		—	1.9 (2)
His		—	—
Arg		0.7 (1)	1.0 (1)
Total		(9)	(15)

* Not calculated.

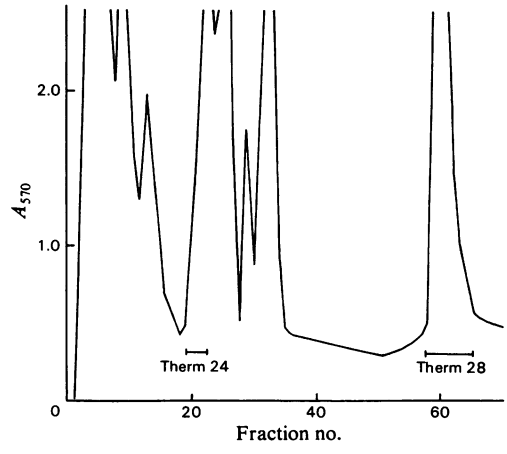


Fig. 7. *Peptide Therm 2 applied to cation-exchange-resin column*

Approx. 10 mg was applied to the column. Details were the same as those described in Fig. 2.

acid composition of peptide ACT 34 was identical with that of peptide T 26, which is a peptide obtained after tryptic digestion of succinylated peptide A and the sequence of which was His-Phe-Asp-Gly-Ala-Asp-Trp-Asp-Glu-Ser-Arg (detailed results not shown). Peptide ACT 35 was the only lysine-containing peptide that was cleaved by tryptic digestion of citraconylated peptide A, suggesting either difficult citraconylation or facile decitraconylation. Its sequence is as follows: Gly-Asn-Thr-Tyr-Ser-Asp-Phe-Lys.

Peptides from treatment with Staphylococcus aureus V8 proteinase

After peptide A was treated with *Staphylococcus aureus* V8 proteinase in 0.05 M-sodium acetate, pH 4.0, the suspension was separated by centrifugation and freeze-dried. When the buffer-insoluble fraction was dissolved in 0.5 M-formic acid and chromatographed on a column (2.5 cm × 190 cm) of Sephadex G-50, five peaks were observed. The sequence of peptide AV8 insol. 55 (fractions 115–125; 5.3 ml/fraction), however, was found to be: Leu-Gln-Asp-Ala-Ile-Gly-Ser-Leu-X-X-X-Asn-Val-Gln-Val-Tyr-Gly. This peptide contains the overlaps for peptides ACT 15 and ACT 1236. Two of the three unidentified amino acids were the histidine and arginine residues of peptide ACT 15. The third unknown amino acid (X) can only be arginine, since the peptide ACT 1236 (i.e., the *N*-terminal portion of peptide ACT 12A) starts with Asn-Val-Gln-Val-Tyr-. Peptide AV8 insol. 75 was analysed without further purification. It represents a mixture of peptide AV8 insol. 55 and a peptide that begins at residue no. 27 of intact α-amylase (Ala-Glu-His-Leu-Ser-Asp- etc.). Peptide AV8 insol. 110 (fractions 170–190) was found to have the following sequence: Gln-Tyr-Gln-Ile-Ala-Trp-Thr-Asp-Phe-Arg-Phe-Pro-Gly-X-Gly-Asn-X-Tyr-X-Asp-Phe-Lys. This peptide made it possible to align sequences of peptides ACT 14, ACT 33 and ACT 35. The remaining peaks were not analysed.

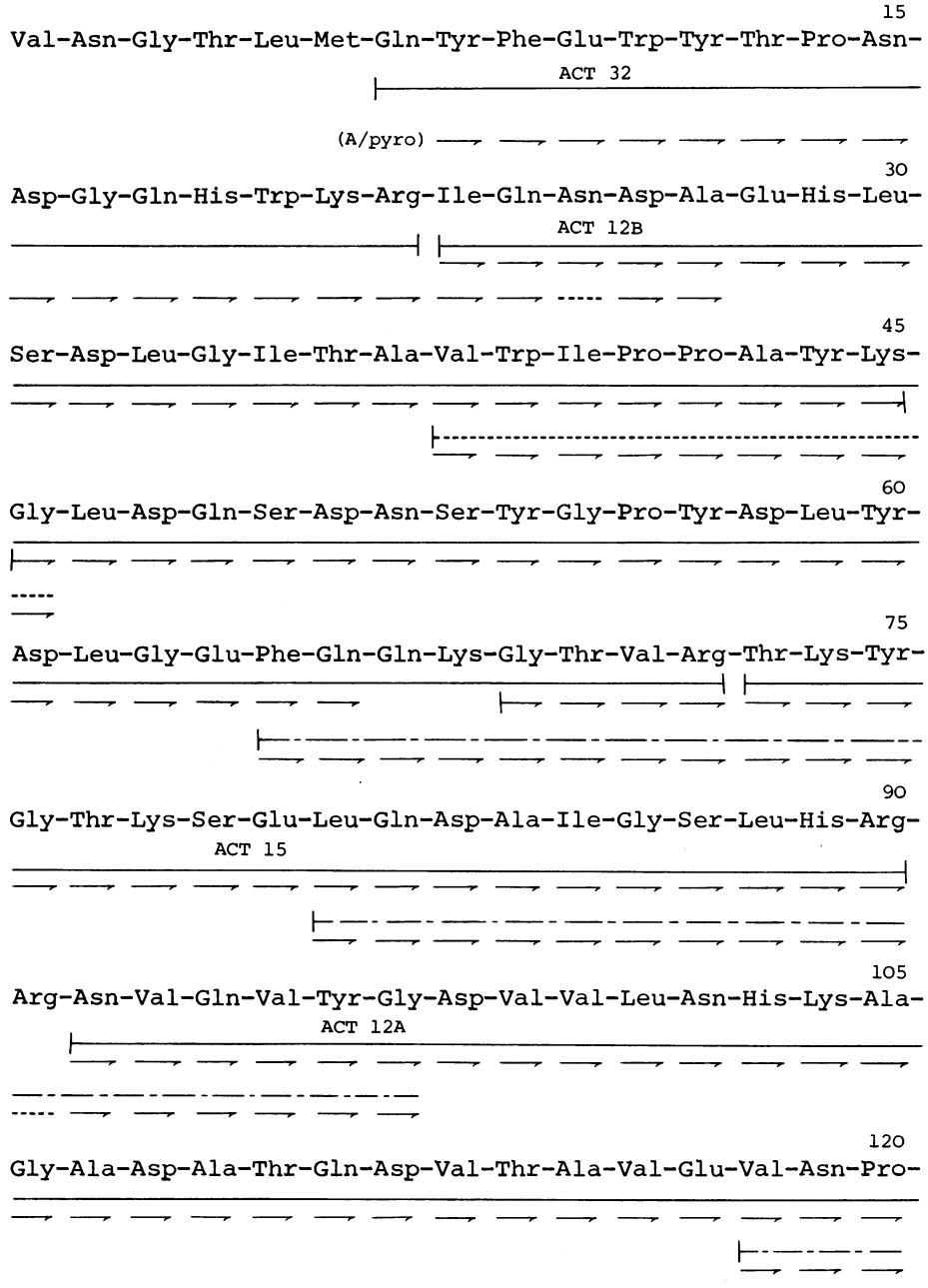
The buffer-soluble fraction was further separated on a cation-exchange column. Only two peaks, AV8 sol. 2 and AV8 sol. 3, were analysed (see Fig. 6 and Table 6). Peptide AV8 sol. 2 contains nine amino acids and has the following sequence: Val-Asn-Pro-Ala-Asn-X-Asn-Gln. Val-Asn-Pro-Ala-Asn-Arg is the *C*-terminal end of peptide ACT 12A. Thus peptide ACT 12A was aligned with peptide ACT 14. Peptide AV8 sol. 3, on the other hand, sequenced as Phe-Gln-Gln-Lys-Gly-Thr-Val-X-X-Lys-Tyr-Gly-Thr-Lys-Ser-Glu, with two missing amino acids. By comparison with the amino acid analysis and the sequence of ACT peptides, these were found to be arginine and threonine. Thus peptide AV8 sol. 3 contains the overlaps for peptides ACT 12B and ACT 15.

Thermolysin peptides

Thermolysin hydrolysis of succinylated peptide A yielded four peaks, Therm 1, 2, 3 and 4, on chromatography on a Sephadex G-25 column. Fraction Therm 2 was resolved further on an ion-exchange column (Fig. 7) into peptides Therm 28 and Therm 24. The former has the following sequence: Phe-Arg-Gly-Glu-Gly-Lys-, enabling us to attach peptide T

33 to peptide ACT 1328. (T 33 is a peptide obtained from succinylated A by digestion with trypsin.) Peptide Therm 24 contains the peptide having the sequence: Phe-Lys-Asp-Trp-. This peptide is needed to connect peptide ACT 35 to peptide ACT 31.

Fig. 8 shows the completed sequence for 191 amino acids beginning at the *N*-terminus of α-amylase isolated from *B. amyloliquefaciens*. Note that the sequence of amino acids 181–184 of this enzyme is identical with that of amino acids 14–17 of amylase isolated from *B. subtilis* (Mäntsälä & Zalkin, 1979) (except that residue no. 183 is asparagine rather than aspartic acid).



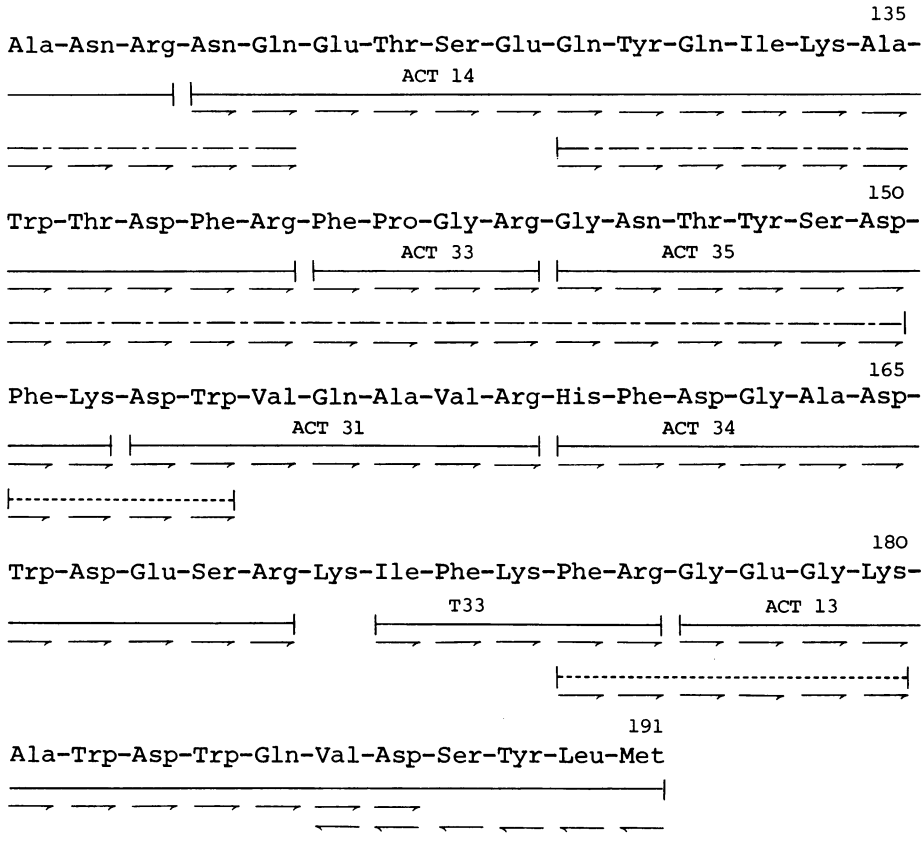


Fig. 8. *Sequence of the N-terminal polypeptide chain of α-amylase*

The sequence of residues 1–12 has been published previously (Friedberg & Thompson, 1974). ——, peptides obtained from citraconylated or succinylated peptide A by trypsin digestion; •–•–, peptides from peptide A obtained by digestion with *S. aureus* V8 proteinase; – – –, peptides from peptide A obtained by digestion with thermolysin; →→, sequence established with the automatic sequencer; ←←, sequence established by digestion with carboxypeptidase; ····, if appearing between two → symbols, indicates unidentified amino acid residue. 'A/pyro' refers to peptide A after treatment with pyroglutamate aminopeptidase.

References

Atassi, M. Z. & Habeeb, A. F. S. A. (1972) *Methods Enzymol.* **25**, 546–553

Bailey, G. S., Gillett, D., Hill, D. F. & Petersen, G. B. (1977) *J. Biol. Chem.* **252**, 2218–2225

Detera, S. D. & Friedberg, F. (1979) *Int. J. Peptide Protein Res.* **14**, 364–372

Edman, P. & Begg, G. (1967) *Eur. J. Biochem.* **1**, 80–91

Friedberg, F. & Thompson, J. (1974) *Acta Chem. Scand.* **28**, 815–816

Fukumoto, J. & Okada, S. (1963) *Ferment. Technol.* **41**, 427–434

Gross, E. & Witkop, B. (1962) *J. Biol. Chem.* **237**, 1856–1860

Heil, A., Mueller, G., Noda, L., Pinder, T., Schirmer, H., Schirmer, I. & von Zabern, I. (1974) *Eur. J. Biochem.* **43**, 131–144

Hogg, R. H. & Hermodson, M. A. (1977) *J. Biol. Chem.* **252**, 5135–5141

Jones, R. T. (1970) *Methods Biochem. Anal.* **18**, 205–258

Klapper, D. G., Wilde, C. E., III & Capra, J. D. (1977) *Anal. Biochem.* **85**, 126–131

Kulbe, K. D. (1974) *Anal. Biochem.* **59**, 564–573

Mäntsälä, P. & Zalkin, H. (1979) *J. Biol. Chem.* **254**, 8540–8547

Martin, B., Svendsen, I. & Ottesen, M. (1977) *Carlsberg Res. Commun.* **42**, 99–102

Moore, S. (1968) *J. Biol. Chem.* **243**, 6281–6283

Moore, S., Spackman, D. H. & Stein, W. H. (1958) *Anal. Chem.* **30**, 1185–1190

Pisano, J. J., Bronzert, T. J. & Brewer, H. B. (1972) *Anal. Biochem.* **45**, 43–59

Podell, N. N. & Abraham, G. N. (1978) *Biochem. Biophys. Res. Commun.* **81**, 176–185

Smithies, O., Gibson, D., Fanning, E. M., Goodfliesh, R. M., Gilman, J. G. & Ballantyne, D. L. (1971) *Biochemistry* **10**, 4912–4921

Tang, J. & Hartley, B. S. (1967) *Biochem. J.* **102**, 593–599

Welker, N. E. & Campbell, L. L. (1967) *J. Bacteriol.* **94**, 1124–1130