Primary Structure of the Calcium Ion-Transporting Adenosine Triphosphatase from Rabbit Skeletal Sarcoplasmic Reticulum

SOME PEPTIC, THERMOLYTIC, TRYPTIC AND STAPHYLOCOCCAL-PROTEINASE PEPTIDES

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The soluble peptides from the peptic digest of the reduced *S*-carboxymethylated 3-carboxypropionylated adenosine triphosphatase protein have been isolated and most of their structures have been determined. About 397 residues of the protein were represented in these peptides. The reduced *S*-carboxymethylated protein was digested with thermolysin, and peptides containing arginine or carboxymethylcysteine were isolated and characterized. Some peptides isolated from tryptic and staphylococcal-proteinase digests of the protein are described. The information contained within the structures of these peptides has been used to reconstruct long stretches of the sequence of the ATPase protein that constitute most of the protein structure external to the lipid bilayer (Allen, Trinnaman and Green (1980) *Biochem. J.* **187**, 591–616). The details of some of the chromatographic steps used in the isolation of the peptides and the properties of the peptides are contained in Supplementary Publication SUP 50104 (45 pages), which has been deposited with the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1978) **169**, 5.

The isolation and determination of the sequences of the soluble tryptic peptides (T1 peptides) from the succinylated (3-carboxypropionylated) ATPase protein are described in an accompanying paper (Allen, 1980a). These T1 peptides have served as the main source of information for the elucidation of the primary structure of that part of the protein external to the lipid bilayer. The isolation and characterization of chymotryptic peptides have also been described [in the preceding paper (Allen, 1980b)]. Further information required for the completion of the sequences of the T1 peptides and for the identification of overlaps between these peptides was obtained from digests of the protein with pepsin, thermolysin, and with staphylococcal proteinase, and from other digests with trypsin, and is presented here.

A large part of the protein is resistant to digestion by proteinases at pH 8, even after denaturation and reduction and carboxymethylation of the cysteine

residues (Allen & Green, 1978; Allen, 1980b). The succinylated protein, which was soluble in water at pH 8, could, however, be cleaved at almost every arginine residue by trypsin (Allen, 1980a). It was anticipated that digestion under acidic conditions with pepsin would lead to more extensive cleavage of that part of the protein embedded in the lipid bilayer in the native protein. The relatively low specificity of pepsin and thermolysin were expected to lead to problems in the purification of peptides from this large (mol.wt. 115000) protein. For the peptic digest, the succinylated protein derivative was used as substrate, since the larger peptides generated would be generally polyanionic and relatively easy to purify. Only peptides containing arginine or carboxymethylcysteine residues were sought from the thermolytic digest.

Several digests of the protein with trypsin were investigated. Two of these digests, which were used mainly for the characterization of sequences around cysteine residues, have already been described (Allen & Green, 1978). Further peptides (labelled T2 and T4), which were isolated from these digests and peptides from a third tryptic digest (labelled T3), are described here.

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Materials and Methods

Most of the materials and methods used in the present work were as described (Allen & Green, 1978; Allen 1980a,b). T.l.c. was most often performed in butanol/acetic acid/water/pyridine (15:3:12:10 by vol.), which is termed 'solvent system 1'.

Peptic digest

The reduced carboxymethylated succinylated lipid-free ATPase protein was prepared as previously described (Allen, 1980a), except that the iodo[14 C]acetic acid used for the carboxymethylation was diluted to a specific radioactivity of 410 c.p.m./nmol. The protein derivative (100 mg; approx. 0.87 μ mol) was suspended in a solution of pepsin (1 mg; from Worthington Biochemical Corp., Freehold, NJ, U.S.A.) in 10.5 ml of 5% (v/v) formic acid. Thiodiglycol (0.01 ml) was added. The mixture was incubated, with shaking, at 35°C for 2 h. Much of the protein remained insoluble. The suspension was homogenized in a glass homogenizer, pepsin (1 mg) was added, and the incubation was continued for a further 3 h at 35°C. The suspension was centrifuged at 10000 g for 10 min. The supernatant was diluted with an equal volume of water and freeze-dried. The precipitate was dissolved in 99% (v/v) formic acid (1.3 ml). A solution of pepsin (1.1 mg) in 12 ml of water was added, with stirring, and digestion was continued at 20°C for 20 h. The mixture was diluted with water (30 ml) and freeze-dried.

The two digests were separately chromatographed on a column (1.8 cm $\times$ 135 cm) of Sephadex G-50 (Superfine grade) in 50 mM-NH₄HCO₃ containing 0.01% thiodiglycol. The second digest was dissolved in 0.5 M-NH₃/8 M-urea (0.6 ml) before chromatography, but the first digest was soluble in 0.1 M-NH₃. Fractions from the column were assayed for radioactivity (by liquid-scintillation counting) and for u.v. absorption at 220 and 280 nm. The elution profiles for the two digests were very similar, except that more material in the second digest was excluded from the Sephadex beads. Combined fractions eluted at the same volumes from the two column runs were therefore mixed, and peptides were isolated from the combined fractions P.A-P.K, as described in the Results section. Details of some isolation procedures are given in Supplementary Publication 50104.

Thermolysin digest

The freeze-dried reduced [14 C]carboxymethylated lipid-free ATPase protein was prepared as described previously (Allen & Green, 1978), except that the specific radioactivity of the iodo[14 C]acetic acid was 405 c.p.m./nmol. The protein (120 mg;

1.05 μ mol) was digested with thermolysin (2.4 mg; from Sigma, Kingston upon Thames, Surrey, U.K.) in 6 ml of 0.1 M-NH₄HCO₃, 1 mM-CaCl₂ for 45 min at 50°C. The suspension was centrifuged for 15 min at 20000 g. The supernatant was applied to a column (1.8 cm $\times$ 140 cm) of Sephadex G-50 (Superfine grade) in 50 mM-NH₄HCO₃/0.01% thiodiglycol. The column was eluted at a flow rate of 15 ml/h. Fractions (3 ml) were collected and assayed for radioactivity by liquid-scintillation counting, and by u.v. absorbance at 280 nm. A second batch (107 mg) of the ATPase protein was similarly digested and the digest was chromatographed under identical conditions.

Peptides containing arginine or carboxymethylcysteine residues were isolated from suitably combined fractions by combinations of ion-exchange chromatography, paper electrophoresis and chromatography, and t.l.c. Arginine-containing peptides were detected on paper or thin-layer cellulose plates with phenanthrene quinone/NaOH (Yamada & Itano, 1966) and carboxymethylcysteine-containing peptides were detected by radioautography or liquid-scintillation counting (Allen & Green, 1978).

Tryptic digest

A batch of ATPase lipoprotein was reduced with dithiothreitol (10 mM) in Tris/HCl buffer (50 mM-Tris), pH 8.3, containing sodium dodecyl sulphate (10 g/g of protein) for 1 h at 20°C. The protein was separated from lipids by gel filtration on a column of Sepharose 6B in 0.1% sodium dodecyl sulphate solution as described previously (Allen & Green, 1978). The protein was treated in 1% sodium dodecyl sulphate solution, in 50 mM-Tris/HCl buffer, pH 8.3, with a 10% molar excess over cysteine residues of iodo[14 C]acetate for 1 h at 37°C. The solution was exhaustively dialysed against water adjusted to pH 8 with a trace of NH₃. The protein remained in solution. The incorporation of carboxymethyl groups was only 3.3 per 115000 daltons of protein; equivalent to 12% of the cysteine residues. The protein was therefore used only for the characterization of the smaller tryptic peptides, since the partial modification of the cysteine residues would hinder the isolation of larger peptides, several of which contain cysteine residues (Allen & Green, 1978).

The protein solution [285 mg (2.5 μ mol) in 49 ml of water] was treated with trypsin (3 mg; thrice-crystallized product; Worthington) in a pH-stat (Radiometer, Copenhagen, Denmark) at pH 8.3, 38°C. NaOH was added to maintain constant pH during 2 h. A further portion (2 mg) of trypsin was added and digestion was continued for 1 h. The solution was freeze-dried.

The digest was dissolved in 0.1 M-NH₃ (10 ml) and

chromatographed on a column (1.8 cm $\times$ 143 cm) of Sephadex G-50 (Superfine grade) in 50 mM- NH_4HCO_3 /0.01% thiodiglycol. Fractions (3 ml) were collected and portions (0.05 ml) were subjected to paper electrophoresis at pH 6.5 followed by detection with ninhydrin/Cd reagent (Heilmann *et al.*, 1957). Peptides (labelled T3) were isolated from suitably combined fractions by successive ion-exchange chromatography, paper electrophoresis and chromatography and t.l.c. Some peptides were rechromatographed on a column (1 cm $\times$ 145 cm) of Sephadex G-50 (Superfine grade). Details of the purification methods for each peptide are given in the Results section and the Supplementary Publication SUP 50104.

In addition, some peptides were isolated from other tryptic digests of the protein by similar methods. These digests were used primarily for the characterization of cysteine-containing peptides (Allen & Green, 1978), but other peptides were also purified and sequenced and are labelled T2 or T4.

Staphylococcus aureus proteinase digest

The reduced carboxymethylated succinylated lipid-free ATPase protein was prepared as previously described (Allen, 1980a). The iodo[^{14}C]acetic acid used for the carboxymethylation was diluted to a specific radioactivity of 850 c.p.m./nmol. The protein derivative (130 mg; approx. 1.13 μmol) was dissolved in 16 ml of 0.1 M- NH_4HCO_3 , pH 8.2. *S. aureus* proteinase (3 mg; Miles Laboratories Ltd., Stoke Poges, Slough, Berks., U.K.) was added. The mixture was incubated at 37°C for 5½ h. The digest was freeze-dried.

The digest was dissolved in 50 mM- NH_4HCO_3 (5 ml) with 0.01 ml of NH_3 solution (35% w/v) added. The suspension was centrifuged at 10000 g for 10 min. The supernatant was chromatographed on a column (1.8 cm $\times$ 130 cm) of Sephadex G-50 (Superfine grade) as shown in Fig. 3. Fractions from the column were assayed for radioactivity, by liquid-scintillation counting, and for u.v. absorption at 220 and 280 nm. Peptides were isolated from the combined fractions SP1–SP7, as described in the Results section.

Peptide-structure determinations

Amino acid analyses were performed using a BioCal analyser. Peptides were hydrolysed in evacuated sealed tubes for 20 h at 110°C in 6 M-HCl containing 0.1% phenol. Small peptides were analysed semiquantitatively by the dansyl technique (Allen, 1980a).

Peptides were sequenced by the manual 'dansyl-Edman' method, with identification of dansyl-amino acids on polyamide layers, essentially as described by Hartley (1970). The peptic peptides were sequenced by a more sensitive modification of this

technique, as described previously (Allen, 1980a). [^{14}C]Carboxymethylcysteine residues were identified by liquid-scintillation counting of the extracted phenylthiazolinones. Aspartic acid and asparagine and glutamic acid and glutamine were distinguished wherever possible by using the electrophoretic mobilities of small peptides at pH 6.5 (Offord, 1966), and in some cases the determination of mobility was repeated at each stage of the Edman degradation of peptides. A few peptides were sequenced by the *p*-dimethylaminoazobenzene isothiocyanate method of Chang *et al.* (1978), for identification of aspartic acid, glutamic acid or their amides.

Nomenclature

Peptides labelled P, Th, SP, T2, T3 and T4 were derived respectively from peptic, thermolytic, staphylococcal proteinase and three different tryptic digests. Further symbols are added in accordance with the fractionation methods used. Further details of nomenclature and abbreviations are given in an accompanying paper (Allen, 1980a).

Results

Peptic digest

The protein did not dissolve in 5% (v/v) formic acid. After homogenization and digestion, most (about 60%) of the radioactivity (carboxymethylcysteine residues), but only about one-third of the material absorbing at 280 nm, was solubilized. The insoluble material had a pronounced crystalline sheen, and by optical microscopy was seen to consist of regular rods that aggregated to form clusters. This effect may have been an artefact due to the homogenization. The precipitate gave a viscous almost clear solution upon dispersal in 99% (v/v) formic acid, and only faint turbidity developed upon dilution into the aqueous pepsin solution. A slight precipitate, with a crystalline sheen, formed during the digestion.

The separation of peptides from the supernatant of the first digestion after chromatography on the column of Sephadex G-50 is shown in Fig. 1. The elution profile of the second digest was similar, but with much more u.v.-absorbing material in the void volume fraction P.A.

Peptides in fraction P.A. These were aggregated, and no attempt was made to purify them.

Peptides in fraction P.B. These were partially resolved by ion-exchange chromatography on DEAE-cellulose, but no pure peptides were isolated.

Peptides in fraction P.C. These were chromatographed on a column (11 mm $\times$ 100 mm) of DEAE-cellulose in a gradient of 10 mM–1 M- NH_4HCO_3 , as described in Supplementary Publication SUP 50104. A total of 11 combined fractions were taken, and peptides were purified from these by t.l.c. on silica gel in butan-1-ol/acetic

Fig. 1. Separation of the peptides in the peptic digest of the ATPase protein on a column of Sephadex G-50 (Superfine grade)

The column was 1.7 cm $\times$ 135 cm in size and the buffer was 50 mM-NH₄HCO₃/0.01% thiodiglycol. A 44 ml portion of eluate was collected, followed by fractions of 2.5 ml. —, A_{220}^{1cm} ; ---, A_{280}^{1cm} ; ····, ^{14}C radioactivity (c.p.m./0.05 ml). Fractions were combined as indicated by the horizontal bars.

acid/water-pyridine; 15:3:12:10, by vol. (solvent system 1).

Peptide P.C3a. This was identical with peptide P.D2a.

Peptide P.C5a. This had the partial sequence:

Peptide P.C8a [4(39–51)]. This had the structure:

Peptides from fraction P.D. These were similarly chromatographed on a column of DEAE-cellulose, as described in Supplementary Publication SUP 50104. Peptides from the combined fractions P.D1–P.D10 were purified by t.l.c. on silica gel in solvent system 1.

Peptide P.D2a [3(203–212)]. This had the partial sequence:

Peptide P.D5a [3(240–251)]. This had the partial sequence:

The peptide (about 10 nmol) was digested with trypsin (1 μ g) in 0.05 ml of 50 mM-NH₄HCO₃ for 1 h at 30°C. Peptide fragments were separated on a two-dimensional peptide 'map' (pH 6.5 electrophoresis followed by chromatography in solvent system 1) on a cellulose MN300 thin-layer plate. The fragments were characterized by *N*-terminal and semiquantitative analysis by the dansyl method, and their electrophoretic mobilities at pH 6.5 were determined. Peptide P.D5a.T1 had the structure:

Peptide P.D5a.T2 had the structure:

peptide P.D5a.T3 had the structure:

and peptide P.D5a.T4 had the structure:

From this information, the total sequence of peptide P.D5a was deduced as:

Peptide P.D6a [4(39–51)]. This was further purified by thin-layer electrophoresis on silica gel at pH 2 (formic acid/acetic acid/water, 1:4:45, by vol.). The peptide was identical with peptide P.C8a.

Peptide P.D6b. This was further purified by thin-layer electrophoresis on silica gel at pH 2. The analysis was identical with that of peptide P.E9b.

Peptide P.D6c [4(48–51)]. This was further purified by thin-layer electrophoresis on silica gel at pH 2. The analysis and partial sequence:

identified it with peptide P.D6a with an additional N-terminal phenylalanine residue.

Peptide P.D8a [3(110–117)]. This was sequenced as:

Peptide P.D9a was further purified by thin-layer electrophoresis on silica gel at pH 2. The sequence was:

Peptide P.D10a. This was identical with part of a chymotryptic peptide, Ch.B8a (Allen, 1980b).

Peptides in fraction P.E. These were partially resolved by ion-exchange chromatography on a column of DEAE-cellulose, as described in Supplementary Publication SUP 50104. Peptides were purified from each of the 13 combined fractions by t.l.c. on silica gel in solvent system 1.

Peptide P.E4a. This was further purified by thin-layer electrophoresis on silica gel at pH 2. The peptide:

had a mobility at pH 6.5 of -0.30 .

Peptide P.E6g. This was sequenced as:

Peptide P.E7a [3(35–44)]. This was identical with peptide P.D5a. The mobility at pH 6.5 of peptide P.E7a, -0.27 , indicated a net charge of -2 , which was consistent with the results from peptide P.D5a.

Peptide P.E7d [2(35–44)]. This was further purified by thin-layer electrophoresis on silica gel at pH 2. The sequence was:

Peptide P.E7f [3(276–283)]. This was impure by analysis, but the sequence:

was quite unambiguous. The mobility at pH 6.5, -0.38 , indicated a net charge of -2 .

Peptide P.E8h [3(57–71)]. This had the partial sequence:

Peptide P.E9b [2(45–54)]. This was further purified by thin-layer electrophoresis (silica gel, pH 2). It was impure by analysis, but the sequence:

was identical with a known sequence within peptide T1C-3 (Allen, 1980a): Val-Ala-Val-Gly-Asx-Lys-Val-Pro-Ala-Asx. The mobility at pH 6.5 of peptide P.E9b, -0.63 , indicates a net charge of -3 , i.e. two aspartic acid residues.

Peptide P.E10c1 [3(188–202)]. This was further purified by thin-layer electrophoresis (silica gel, pH 2). The partial sequence was:

Peptide P.E10c2 [4(74–79)]. This was further purified by thin-layer electrophoresis (silica gel, pH 2). The sequence was:

Peptide P.E10e [4(20–27)]. This was impure by analysis. The partial sequence was:

The electrophoretic mobility of the peptide at pH 6.5, -0.71 , indicated a net charge of -3 .

Peptide P.E11a [3(110–117)]. This was sequenced with the determination of electrophoretic mobility at pH 6.5 at each step in the Edman degradation:

The peptide was identical with peptide P.D8a.

Peptides in fraction P.F. These were partially resolved by ion-exchange chromatography on a column of DEAE-cellulose as described in Supplementary Publication SUP 50104. A total of 14 combined fractions were taken. Fractions P.F5 and P.F6 were resolved on a preparative peptide 'map' on Whatman 3MM paper as described by Allen (1980b). Other fractions were resolved by t.l.c. on silica gel in solvent system 1.

Peptide P.F2a [3(289–298)]. This was identical with peptide P.G2a except for additional valine and methionine residues.

Peptide P.F2b. This was partially sequenced as:

Peptide P.F5.9. This was sequenced, with determination of the electrophoretic mobilities at pH 6.5 at each step, as:

Peptide P.F5.16 [3(219—223)]. This was sequenced as:

Peptide P.F6.1 [4(28—37)]. The structure was:

Peptide P.F6.9. This was sequenced as:

Peptide P.F6.10 [3(16—22)]. This was not quantitatively analysed. The partial sequence was:

Peptide P.F6.11 [2(106—113)]. The structure was:

Peptide P.F6.13. This peptide, of structure:

had an electrophoretic mobility at pH 6.5 of -0.30 .

Peptide P.F8b2 [3(72—82)]. This was purified by thin-layer electrophoresis (pH 2, silica gel). The structure was:

Peptide P.F13a [3(46—51)]. This was sequenced, with determination of electrophoretic mobilities at pH 6.5 at each step during the degradation, as:

Peptide P.F14a [3(260—265)]. This was sequenced, with determination of the electrophoretic mobilities at pH 6.5 at each step during the degradation, as:

Peptides in fraction P.G. These were partially resolved by ion-exchange chromatography on a column of DEAE-cellulose into ten subfractions, P.G1—P.G10, as described in Supplementary Publication SUP 50104. Peptides in fractions P.G4 and P.G5 were purified on peptide 'maps' (electrophoresis at pH 3.5 followed by chromatography in solvent system 1 on Whatman 3MM paper). Peptides in other fractions were purified by t.l.c. on silica gel in solvent system 1. The following peptides were characterized.

Peptide P.G2a [3(289—296)]. This had the structure:

Peptide P.G2c [2(66—72)]. This peptide, of structure:

was further purified by thin-layer electrophoresis (silica gel, pH 2) followed by t.l.c. (cellulose MN400; butanol/acetic acid/water, 3:1:1 by vol). The mobilities at pH 6.5 were determined at each step during the degradation of the peptide.

Peptide P.G2h. This peptide, of structure:

was purified further by thin-layer electrophoresis on silica gel at pH 2.

Peptide P.G3b1 [3(162—171)]. This peptide, of structure:

was further purified by thin-layer electrophoresis on silica gel at pH 2, followed by t.l.c. on cellulose MN400 in butanol/acetic acid/water (3:1:1, by vol). The yield was low, and quantitative analysis was not performed. The partial sequence was:

Peptide P.G3b2 [3(134—139)]. This neutral peptide, of structure:

was co-purified with peptide P.G3b1 until the final t.l.c. The yield was low and quantitative analysis was not performed.

Peptide P.G3d [3(284—288)]. This peptide, of structure:

was further purified by thin-layer electrophoresis on silica gel at pH 2.

Peptide P.G3f1. This had the structure:

Peptide P.G4.3 [3(140—147)]. This peptide, of structure:

was not quantitatively analysed.

Peptide P.G4.15 [2(7—10)]. This had the structure:

Peptide P.G5.1 [4(28—38)]. This had the structure:

Peptide P.G5.2 [3(289—293)]. This had the structure:

Electrophoretic mobilities at pH 6.5 were determined at each step during the degradation of the peptide.

Peptide P.G.5.12 [3(83–85)]. This had the structure:

Peptide P.G.5.16a This peptide of structure:

was purified further by t.l.c. on silica gel in solvent system 1.

Peptide P.G6a [4(98–102)]. This peptide, of structure:

was further purified by thin-layer electrophoresis on silica gel at pH 2. Electrophoretic mobilities at pH 6.5 were determined at each step during the degradation of the peptide.

Peptide P.G6c2 This peptide, of structure:

was further purified by thin-layer electrophoresis on silica gel at pH 2. Electrophoretic mobilities at pH 6.5 were determined at each step during the degradation of the peptide.

Peptide P.G6d2. This peptide, of structure:

was further purified by thin-layer electrophoresis on silica gel at pH 2. Electrophoretic mobilities at pH 6.5 were determined at each step during the degradation of the peptide.

Peptide P.G7a [3(52–56)]. This had the structure:

Peptide P.G7b [1(11–13)]. This peptide of structure:

was purified further by thin-layer electrophoresis on silica gel at pH 2.

Peptide P.G8c [4(56–60)]. This had the structure:

The electrophoretic mobilities at pH 6.5 were determined at each step during the degradation of the peptide.

Peptide P.G9a. This had the structure:

The electrophoretic mobility at pH 6.5 was –1.16; mobilities were also determined at each step during the degradation of the peptide.

Peptide P.G9b [3(46–51)]. This had the structure:

and was identical with peptide P.F13a.

Peptide P.G.10a [3(260–265)]. This had the structure:

and was identical with peptide P.F14a.

Peptides in fraction P.H. These peptides were resolved by preparative peptide ‘mapping’ (Allen, 1980b). Peptides neutral at pH 6.5 were excised and subjected to electrophoresis at pH 3.5 before chromatography in system 1. The following peptides were characterized.

Peptide P.Hn8 [4(15–19)]. This was the structure:

and was neutral.

Peptide P.Hn13 [2(31–34)]. This had the structure:

and was semiquantitatively analysed by the dansyl method.

Peptide P.Ha3 [3(254–259)]. This had the structure:

and was purified by t.l.c. on silica gel in solvent system 1. The electrophoretic mobilities at pH 6.5 were determined at each step during the degradation of the peptide.

Peptide P.Ha4 [4(98–102)]. This had the structure:

The electrophoretic mobilities at pH 6.5 were determined at each step in the degradation of the peptide.

Peptide P.Ha8b [3(83–85)]. This had the structure:

and was identical with peptide P.G5.12.

Peptide P.Hb1 [5(1–4)]. This had the structure:

Peptide P.Hb5 [2(55–58)]. This peptide, of structure:

was not completely pure.

Peptides from fraction P.J. These were partially resolved by gel filtration on a column (1.7 cm × 52 cm) of Sephadex G-25 (Fine grade) followed by paper electrophoresis at pH 3.5 and t.l.c. on silica gel in solvent system 1:

Peptide P.J2n1 [3(199–202)]. This had the structure:

Peptide *P.J2n2* [4(52–55)]. This had the structure:

Peptide *P.J2b1*. This had the structure:

Fraction P.J contained more than $1\mu\text{mol}$ of tryptophan (by u.v.-absorbance measurements), but the only Ehrlich-positive material identified was indistinguishable from free tryptophan.

Thermolysin digest

The elution of the soluble thermolysin peptides from the column of Sephadex G-50 is shown in Fig. 2. Fractions were combined as indicated. Fraction Th.A, at the void volume, was a complex mixture of large and aggregated peptides from which no pure peptides were isolated. Peptides in fraction Th.B were subjected to ion-exchange chromatography on a column of DEAE-cellulose, but no pure peptides were obtained.

Peptides in fraction Th.C were partially resolved, by ion-exchange chromatography on a column ($12\text{mm} \times 120\text{mm}$) of DEAE-cellulose in a linear gradient of NH_4HCO_3 , into five arginine- or carboxymethylcysteine-containing fractions (see Supplementary Publication SUP 50104) from which peptides were purified as described in Supplementary Publication SUP 50104, and as outlined below.

Fig. 2. Separation of the peptides in the thermolytic digest of the ATPase protein on a column of Sephadex G-50 (Superfine grade)

The column was $1.8\text{cm} \times 140\text{cm}$ in size and the eluent was $50\text{mM-NH}_4\text{HCO}_3/0.01\%$ thiodiglycol. The fraction size was 3 ml. —○—, $A_{280}^{1\text{cm}}$; ●—, $A_{280}^{2\text{mm}}$; ----, ^{14}C radioactivity (c.p.m./0.05 ml). Fractions were combined as indicated by the horizontal bars.

Fraction Th.C α was resolved by chromatography on a column ($8\text{mm} \times 60\text{mm}$) of sulfoethyl-Sephadex C-25 in a gradient of pyridine/acetic acid/formic acid buffers. Two arginine-positive peaks were obtained.

Peptide *Th.C α 1* [2(45–57)]. This was purified from one peak by paper electrophoresis at pH 2 followed by t.l.c. (cellulose MN400; solvent system 1) of the arginine-positive peptide band. The partial sequence was:

Peptide *Th.C α 2* [2(79–91)]. This was purified from the second peak by paper electrophoresis at pH 6.5 followed by paper electrophoresis at pH 2.

Peptides in fraction Th.C γ were chromatographed on a column ($1\text{cm} \times 143\text{cm}$) of Sephadex G-50 (Superfine grade) in $50\text{mM-NH}_4\text{HCO}_3/0.01\%$ thiodiglycol. The material in the single broad peak was resolved by t.l.c. (silica gel; solvent system 1).

Peptide *Th.Gyl* [1(3–12)]. This had the sequence:

Peptide *Th.C γ 2* [2(37–44)]. This had the sequence:

The presence of two isoleucine–valine sequences led to low values of these two amino acids in the analysis, owing to slow hydrolysis.

Peptides in fraction Th.C δ were similarly chromatographed on a column of Sephadex G-50, followed by t.l.c.

Peptide *Th.C δ 1b* [3(43–53)]. This had the sequence:

Peptide *Th.C δ 1c* [3(42–53)]

The Ile–Ile bonds in these two peptides led to low values for isoleucine in the analyses.

Peptide *Th.C δ 1d* [4(1–15)]. This had the sequence:

Peptides in fraction Th.D. These were separated by paper electrophoresis at pH 6.5 into nine fractions, each of which was subjected to paper electrophoresis at pH 2. Peptides were chromatographed on paper (solvent system 1) and final

purification if necessary was by t.l.c. (silica gel; solvent system 1).

Peptide Th.Dn1 [4(25–34)]. This peptide, of sequence:

was neutral, indicating the presence of two glutamic acid and one glutamine residues.

Peptide Th.Dn2 [3(275–281)]. This had the sequence:

and was neutral.

Peptide Th.Dn3 [3(274–281)]. This had the sequence:

and was neutral.

Peptide Th.Da1 [3(22–30)]. This had the partial sequence:

Peptide Th.Da2 [4(108–114)]. This peptide, of structure:

was identical with peptide Th.Eal.

Peptide Th.Db1 [2(1–12)]. This had the structure:

Peptide Th.Db2 [3(205–211)]. This had the structure:

Peptide Th.Db3 [3(7–13)]. This had the structure:

Peptide Th.Db4 [1(24–33)]. This had the partial sequence:

Peptide Th.Db5 [3(203–211)]. This had the structure:

Peptides in fraction Th.E. These were resolved successively by paper electrophoresis at pH 6.5, paper chromatography (solvent system 1) and paper electrophoresis at pH 2. Further purification if necessary was by t.l.c. (silica gel; solvent system 1). The following peptides were characterized:

Peptide Th.En1 [3(38–41)]. This had the structure:

and was identical with peptide Th.En3.

Peptide Th.En2 [2(65–70)]. This had the structure:

Peptide Th.En3 [3(38–41)]. This had the structure:

Peptide Th.Ea1 [4(108–114)]. This had the structure:

and was an acidic peptide.

Peptide Th.Ea2 [3(196–202)]. This had the structure:

and the mobility at pH 6.5 indicates a net charge of –1.

Peptide Th.Ea3. This had the structure:

Peptide Th.Ea4 [3(288–293)]. This had the structure:

Peptide Th.Eb1 [2(24–28)]. This had the structure:

Peptides in fraction Th.F. These were separated by the same methods as those for fraction Th.E, and the following peptides were characterized.

Peptide Th.Fn1. This had the structure:

Peptide Th.Fn2 [2(101–105)]. This had the structure:

and was a neutral peptide.

Peptide Th.Fn3 [3(234–236)]. This had the structure:

Peptide Th.Fb1 [3(77–81)]. This had the structure:

and was a basic peptide.

Peptide Th.Fb2. This had the structure:

and was a basic peptide.

Peptide Th.Fb3. This had the structure:

Peptide Th.Fb4 [2(24–28)]: This peptide, of structure:

had a mobility at pH 6.5 that corresponds to a net charge of +1.

Peptide Th.Fb5 [2(29–31)]. This had the structure:

and was a basic peptide.

Peptide Th.Fb6 [3(149–152)]. This had the structure:

and was a basic peptide.

Peptide Th.Fb7 [3(162–168)]. This had the structure:

and its mobility at pH 6.5 corresponds to a net charge +2.

Peptide Th.Fb8 [3(294–296)]. This had the structure:

Peptides in fraction Th.G. These were similarly separated, but no pure peptides were isolated.

Tryptic digest T3

The uptake of NaOH during the digestion in the pH-stat was about 50% of that expected for cleavage at all lysyl and arginyl peptide bonds, assuming an average pK_a of 7.8 for the peptide $\alpha\text{-NH}_2$ groups (Steinhardt & Beychok, 1964). The tryptic peptides were eluted from the Sephadex G-50 column between fractions 38 (void volume) and 115 (total column volume). Only the smaller peptides were studied. Fractions 80–89 were combined as fraction T3-D, and fractions 90–115 were combined as fraction T3-E.

Details of the isolation of peptides and evidence for the determination of their sequences are given in Supplementary Publication SUP 50104. The following peptide structures were determined.

Peptide T3-D4a1 [3(243–258)]. This had the structure: Asp-Thr-Pro-Pro-Lys-Arg-Glu-Glu-Met-Val-Leu-Asp-Asp-Ser-Ser-Arg

Peptide T3-D4c1 [2(67–81)] and *peptide T3-D4d*.

These had the structure Val-Asp-Gln-Ser-Ile-Leu-Thr-Gly-Glu-Ser-Val-Ser-Val-Ile-Lys.

Peptide T3-D4c2 [2(3–12)]. This had the structure Asn-Ala-Gln-Asn-Ala-Ile-Glu-Ala-Leu-Lys. One digest of this peptide with thermolysin yielded peptides with mobilities suggesting that one of the amide residues was acidic, as discussed in Supplementary Publication SUP 50104.

Peptide T3-D4f. This had the sequence Ile-Ser-Leu-Pro-Val-Ile-Gly-Leu-Asx-Glx-Ile-Leu-Lys.

Peptides T3-D5d and T3-D5e [3(112–126)]. These had the sequence Val-Gly-Glu-Ala-Thr-Glu-Thr-Ala-Leu-Thr-Thr-Leu-Val-Glu-Lys.

Peptide T3-D6i [3(259–280)]. This had the partial sequence Phe-Met-Glx-Tyr-Glx-Thr-Asx-Leu-Thr-Ala-Val-Gly-Val-Val-Gly-.

Peptide T3-D6a3 [2(3–20)]. This had the partial sequence Asx-Ala-Glx-Asx-Ala-Ile-Glx-Ala-Leu(-)-Glx-Tyr-Glx-.

Peptide T3-D7a [4(19–29)]. This had the sequence Glu-Phe-Asp-Asp-Leu-Pro-Leu-Ala-Glu-Gln-Arg.

Peptide T3-D7b [4(1–13)]. This had the sequence Ile-Gly-Ile-Phe-Gly-Glu-Asn-Glu-Glu-Val-Ala-Asn-Arg.

Peptide T3-E4b3 [3(136–139)]. This had the sequence Asn-Leu-Ser-Lys. Other T3-E peptides had sequences as follows: T3-E4b4 [3(1–3)], Met-Ala-Lys; T3-E4b5, Ser-Pro-Lys; T3-E5n1, Glu-Gly-Gly-Thr-Arg; T3-E5b2, Phe(Ile,Ala,Arg); T3-E5b3 [2(21–23)], Val-Tyr-Arg; T3-E6b3 [4(14–18)], Ala-Tyr-Thr-Gly-Arg; T3-E6b4, Ile-Arg; T3-E7b2 [2(24–27)], Ala-Asp(Arg,Lys); T3-E7b3, a mixture of Asp-Arg-Lys [3(165–167)] and Ala-Asp(Arg,Lys); T3-E7b5, Ala(Arg,Pro,Arg); T3-E9n2 [3(107–111)], Gly-Val-Tyr-Glu-Lys; T3-E9b3 [2(57–61)], Ile-Leu-Ser-Ile-Lys; T3-E9b5 [3(178–180)], Ser(Ser,Arg); T3-E9b6, a mixture of Ile-Lys [2(32–33)] and Leu-Lys; T3-E10b2a [3(220–225)], Ile-Leu-Ser-Val-Ile-Lys; T3-E10b2b [2(91–97)], Ala-Val-Asx-Glx-Asx-Lys-Lys; T3-E10b2c [4(41–46)], Val-Glx(-)-Ser(-)-Lys; T3-E11n3 [3(140–142)], Val-Glu-Arg; T3-E11b1 [2(28–31)], Ser(Gln,Val,Arg); T3-E11b2 [3(205–209)], Val-Gly-Thr-Thr-Arg; T3-E11b3 [2(62–66)], Ser-Thr-Thr-Leu-Arg; T3-E11b5 [4(34)], Arg.

Peptides from other tryptic digests

Two other tryptic digests of the protein have been described (Allen & Green, 1978). These digests were used primarily for the characterization of sequences around the cysteine residues of the protein, but a few peptides lacking cysteine which co-purified with the cysteine-containing peptides until the final stages of resolution were also characterized, and those which gave information useful for the sequence determination of the protein are described here. The details of the purification and sequence determina-

tion of these peptides are given in Supplementary Publication SUP 50104. The following peptide structures were determined.

Peptide T2-Fn4a. This had the sequence Ala-Asx-Glx-Pro-Arg.

Peptide T4-C7a [4(49–74)]. This had the sequence Ile-Val-Glu-Tyr-Leu-Glu-Ser-Tyr-Asp-Glu-Ile-Thr-Ala-Met-Thr-Gly-Asp-Gly-Val-Asp-Asp-Ala-Pro-Ala-Leu-Lys.

Peptide T4-D8b1 [2(102–110)]. This had the sequence Ser-Gly-Thr-Asn-Ile-Ala-Ala-Gly-Lys. The *N*-terminal determination indicated glycine in addition to serine, and the ninhydrin/Cd reagent stained the peptide pink, turning to brown. These unexpected observations have not been explained.

Peptide T4-D8b2 [3(210–217)] This had the sequence Val-Pro-Met-Thr-Gly-Pro-Val-Lys.

S. aureus proteinase digest

The elution of the soluble *S. aureus* proteinase peptides from the column of Sephadex G-50 is shown in Fig. 3. Fraction SP1, at the void volume, was a complex mixture of large and aggregated peptides from which no pure peptides were isolated. Peptides in the other fractions were isolated as described in Supplementary Publication SUP 50104.

The following analyses and partial sequences were determined.

Peptide SP.3C3 [3(195–218)]. This had the sequence (CMCys,Asp₂,Thr₃,Glu,Pro₂,Gly₃,Val₃,

Met,Ile,Tyr,Lys,Arg₃), Gly-Val-Ile-Asx-Arg-CMCys-Asx-Tyr-Val-Arg-Val-Gly-(-)-(-)-Val-(-)-(-)-Gly-Pro-Val-Lys-.

Peptide SP.4A1 [4(31–51)]. This had the sequence (CMCys,Ser₂,Glu₂,Pro,Ala₃,Val₂,Ile,Phe,His,Lys₂,Arg₃), Ala-CMCys-(-)-(-)-Ala-.

Peptide SP.4C2e [3(195–218)] (cf. SP.3C3). This had the sequence Gly-Val-Ile-Asx-Arg-CMCys-Asx-Tyr-Val-(-)-Val-Gly-(-)-Thr-Arg-Val-Pro-(-)-Thr-Gly-Pro-Val-.

Peptide SP.4D1b [4(31–51)] (cf. SP.4A1) This had the sequence Ala-CMCys-(-)-Arg-Ala-CMCys-CMCys-(-)-(-)-Ala-.

Peptide SP.4E2da [4(59–77)]. This had the sequence (Asp₃,Thr₂,Glu,Gly₂,Ala₃,Val,Met,Ile,Leu,Lys₂), Ile-Thr-Ala-(-)-(-)-Gly-(-)-Gly-Val-(-)-Asx-Ala-(-)-Ala-.

Peptide SP.4E3a2 [1(21–)]. This had the sequence Thr-Thr-Gly-Leu-Thr-Pro-(-)-Glx-.

Peptide SP.4F2a [4(95–111)]. This had the sequence (Asp₃,Thr,Ser,Glu₂,Ala₃,Val₃,Met,Ile,Leu,Phe), Met-Val-Leu-(-)-Ala-(-)-Asx-(-)-(-)-Thr-Ile-(-)-Ala-Ala-.

Peptide SP.F2c [4(31–51)] (cf. SP.4A1, SP.4D1b). This had the sequence Ala-CMCys-Arg-Arg-Ala-CMCys-CMCys-Phe-Ala-Arg-Val-Glx-Pro-.

Peptide SP.F2e [3(70–87)]. This had the sequence (Asp₃,Ser,Glu₂,Pro,Gly₂,Val₂,Ile,Leu₂,Phe,Lys₂,Arg), Val-Leu-(-)-Asx-Asx-(-)-Pro-Ile-(-)-(-)-Gly-.

Peptide SP.F3a [1(3–11)]. This had the sequence (Thr,Ser₂,Glu₂,Ala₂,His,Lys), Ala-Ala-His-Ser-Lys-Ser-Thr-Glx-Glx.

Peptide SP.4G1h2 [3(67–87)]. This had the sequence (Asp₃,Ser,Glu₃,Pro,Gly₃,Val₂,Ile,Leu₂,Phe,Lys₂,Arg), Gly-Glx-Val-Leu-.

Peptide SP.4H2 [3(88–104)]. This had the Sequence (CMCys₂,Asp₄,Thr,Ser₂,Glu,Ala₂,Ile,Leu₃,Phe), Leu-Ala-(-)-Ile-CMCys-Ala-Leu-CMCys-Asx-Asx-Ser-Ser-Leu-Asx-Phe-(-)-Glx

Peptide SP.5A1γ [3(27–)]. This had the sequence (-)-Thr-Gly-Leu-Thr-(-)-Asx-Glx-. This peptide results from a cleavage after aspartic acid-[3(26)].

Peptide SP.5A2β [4(10–19)]. This had the sequence Val-Ala-Asx-Arg-Ala-Tyr(Thr)Gly-Arg-Glx.

Peptide SP.5A2γ [2(10–13)]. This had the sequence Ala-Leu-(-)-Glx.

Peptide SP.5B1b [3(111–114)]. This had the sequence Lys-Val-Gly-Glx.

Peptide SP.5B1c [3(134–141)]. This had the sequence (Asp,Ser,Glu,Val₂,Leu,Lys,Arg), Val-Arg-Asx-Leu-Ser(Lys)Val-Glx.

Peptide SP.5B1e [3(118–125)]. This had the sequence (Thr₃,Glu,Ala,Val,Leu₂), Thr-Ala-Leu-(-)-(-)-Leu-(-)-Glx.

Peptide SP.5C2 [3(57–67)]. This had the sequence

Fig. 3. Separation of the peptides in the *S. aureus*-proteinase digest of the ATPase protein on a column of Sephadex G-50 (Superfine grade)

The column was 1.8cm × 130cm in size and the buffer was 50mM-NH₄HCO₃/0.01% thiodiglycol. The fraction size was 2.5 ml. —, $A_{1\text{cm}}$; ---, $A_{2\text{cm}}$; ···, A_{220} ; ———, ^{14}C radioactivity (c.p.m./0.01 ml). Fractions were combined as indicated by the horizontal bars.

(Thr₂,Ser₂,Glu,Pro,Gly,Ala,Ile,Tyr,Phe), Phe-Ser-Ile(-)-Gly-Ser-Thr-Tyr-Ala(-)-Glx.

Peptide SP.5D1a1α [2(10–13)]. This had the sequence Ala-Leu-Lys-Glx.

Peptide SP.5D1a3α [3(111–114)]. This had the sequence Lys-Val-Gly-Glx.

Peptide SP.5E2b [4(20–30)]. This had the sequence (Asp₂,Glu₃,Pro,Ala,Leu₂,Phe,Arg), Phe-Asx-Asx-Leu(-)-Leu-Ala-Glx.

Peptide SP.5E2c [3(105–110)]. This had the sequence (Thr,Glu,Gly,Val,Tyr,Lys), Thr(-)-Gly-Val-Tyr-Glx.

Peptide SP.5F1 [4(20–27)]. This had the sequence (Asp₂,Glu,Pro,Ala,Leu₂,Phe), Phe-Asx-Asx-Leu-Pro-Leu-Ala-Glx.

Peptide SP.5F2 [1(12–20)]. This had the sequence CMCys-Leu-Ala-Tyr-Phe-Gly-Val(-)-Glx.

Peptide SP.5G1. This had the sequence (CMCys, Asp,Glu,Gly,Leu), Gly-Leu-Asx-CMCys-Glx.

Peptide SP.6B1a [1(3–11)]. This had the sequence Ala-Ala-His-Ser-Lys-Ser-Thr-Glx-Glx.

Peptide SP.6B1b [3(134–141)]. This had the sequence (Asp,Ser,Glu,Val₂,Leu,Lys,Arg), Val-Arg-Asx-Leu-Ser-Lys-Val-Glx.

Peptide SP.6B1d [3(118–125)]. This had the sequence Thr-Ala-Leu-Thr-Thr(-)-Val-Glx.

Peptide SP.6B2a [3(115–117)]. This had the sequence (Thr,Glu,Ala), Ala-Thr-Glx.

Peptide SP.6B2c [2(6–9)]. This had the sequence (Asp,Glu,Ala,Ile), Asx-Ala-Ile-Glx.

Peptide SP.6B2d [3(57–67)]. This had the sequence (Thr₂,Ser₂,Glu,Pro,Gly,Ala,Ile,Tyr,Phe), Phe-Ser-Ile-Thr-Gly-Ser-Thr-Tyr-Ala(-)-Glx.

Peptide SP.6B3a [1(3–11)]. This had the sequence Ala-Ala-His-Ser(-)-Ser-Thr-Glx-Glx.

Peptide SP.6B3b [3(111–114)]. This had the sequence Lys-Val-Gly-Glx.

Peptide SP.6B3d [3(118–125)]. This had the sequence Thr-Ala-Leu-Thr-Thr-Leu-Val-Glx.

Peptide SP.6C1 [3(57–67)]. This had the sequence (Thr₂,Ser₂,Glu,Pro,Gly,Ala,Ile,Tyr,Phe), Phe-Ser-Ile(-)-Gly(-)-Thr-Tyr-Ala-Pro-Glx.

Peptide SP.6D1b [3(111–114)]. This had the sequence Lys-Val-Gly-Glx.

Peptide SP.6D3a and SP.6D2c [2(10–13)]. This had the sequence Ala(Leu,Lys,Glx).

Peptide SP.6E2 [2(14–17)]. This had the sequence (Glu₂,Pro,Tyr), Tyr-Glx(Pro)Glx.

Peptide SP.6E4a [3(105–110)]. This had the sequence (Thr,Glu,Gly,Val,Tyr,Lys), Thr-Lys-Gly-Val-Tyr-Glx.

Peptide SP.6E5 [4(20–27)]. This had the sequence (Asp₂,Glu,Pro,Ala,Leu₂,Phe), Phe-Asx-Asx-Leu(-)(-)(-)-Glx.

Peptide SP.6F1. This had the sequence (CMCys, Asp,Glu,Gly,Leu), Gly-Leu-Asx-CMCys-Glx.

Peptide SP.7E1. This had the sequence CMCys-Glx-Ile-Glx.

Peptide SP.7E2 [3(158–161)]. This had the sequence Phe-Thr-Leu-Glx.

Peptide SP.7H1. This had the sequence Asx(-)-Pro(-)-Phe-Glx.

Peptide SP.7H2 [2(14–17)]. This had the sequence Tyr-Glx-Pro-Glx.

Peptide SP.7H4 [1(12–20)]. This had the sequence CMCys(-)(-)-Tyr-Phe-Gly-Val(-)-Glx.

Discussion

The total number of unique residues from the ATPase protein identified in the peptic peptides was 397. This number is considerably less than that identified in tryptic (Allen, 1980a) or chymotryptic (Allen, 1980b) peptides. However, the relatively low specificity of pepsin led to cleavage at many alternative sites and the production of many more peptides than with the other two proteinases. A large number of other peptides were almost completely purified, but in yields too low for structural analysis, and these peptides are not reported here. It is noteworthy that all but 36 of these 397 residues were also identified in these other digests of the protein. This indicates that the regions of the polypeptide chain resistant to digestion at pH 8 are also resistant to digestion in 5% (v/v) formic acid.

This conclusion is in agreement with the distribution of carboxymethylcysteine and tryptophan residues between the soluble and the insoluble or aggregated peptide fractions. As in other digests of the protein, most of the carboxymethylcysteine residues were found in the soluble peptides, obtained from the part of the protein outside the lipid bilayer, whereas most of the tryptophan residues were present in aggregated or insoluble peptides. The only tryptophan-containing material identified in the peptic digest was free tryptophan. Small amounts of Ehrlich-positive peptides were observed on peptide 'maps', but the yields were too low to permit characterization.

Since pepsin has a high specificity for hydrophobic and especially aromatic residues, the poor cleavage of the tryptophan-rich relatively hydrophobic part of the protein associated with the lipid bilayer, indicates that it is the physical state (secondary structure or aggregation) of this part of the protein, even after dilution from 99% formic acid, that prevents digestion from taking place. Peptic digestion of a phage protein with solubility properties similar to those of the ATPase was aided by dilution from formic acid (Snell & Offord, 1972).

The peptide bonds cleaved by pepsin in the present work are typical of those for pepsin. The peptides in highest yield originated from cleavage between adjacent hydrophobic residues.

Thermolysin digestion of the protein gave rise, as expected, to a very large number of small peptides. The purification of these peptides presented considerable difficulty, several steps of electrophoresis and chromatography being required in most cases. The yields were generally low, and although about 40 peptides could be detected on analytical peptide 'maps' stained for arginine with phenanthrene quinone/NaOH (not shown), only 27 unique arginine residues could be determined in sequences in the purified peptides. Similarly, less than half of the carboxymethylcysteine residues of the protein were identified in the purified peptides. However, the thermolysin digest was not performed with the intention of isolating most of the polypeptide chain in small peptides, but rather with providing evidence for aligning the tryptic peptides derived by cleavage at arginine residues. Several alignments were made, but, in many cases, unambiguous alignments could not be made. However, in conjunction with results from other digests of the protein, the information from the thermolysin digest was quite important for the reconstruction of extended lengths of the polypeptide chain [the following paper (Allen *et al.*, 1980)]. The tryptic peptides described in the present paper were derived mainly from a batch of protein that was not successfully carboxymethylated. The carboxymethylation reaction in sodium dodecyl sulphate solution, although described in the literature (Cole *et al.*, 1958), appears to be very slow, allowing competing side reactions (mainly oxidation) to occur. The reaction of negatively charged reagents, such as iodoacetate, with thiol groups in proteins complexed with dodecyl sulphate would be expected to be hindered by the negative charge on the mixed protein-detergent micelles, both through inhibition of the approach of the reagent and through suppression of the necessary deprotonation of the thiol group. Neutral reagents, such as *N*-ethylmaleimide (Thorley-Lawson & Green, 1977), react quite well.

Since several of the larger tryptic peptides of the protein contain cysteine, and the isolation of these peptides would be hindered by the probable distribution of cysteine-containing peptides (with the cysteine partially modified and partially oxidized) over ion-exchange columns as multiple peaks, only the shorter peptides were isolated from this digest. The digestion was limited to about half of the lysyl and arginyl bonds, probably by aggregation of the protein.

The main use of these tryptic peptides has been in confirming sequences determined within some of the longer tryptic peptides from the succinylated (3-carboxypropionylated) protein, and in distinguishing between aspartic acid and asparagine and

between glutamic acid and glutamine in several positions in these sequences.

The study of the *S. aureus*-proteinase peptides was made after information from other digests of the protein had been used to reconstruct most of the longer peptide sequences described in the following paper (Allen *et al.*, 1980). The main purpose of the investigation of these staphylococcal-proteinase peptides was therefore to confirm sequences that had already been determined, and especially to confirm the identifications of glutamic acid residues, some of which had not been distinguished from the amides (glutamine) in the previous work. Complete purification of all the peptides was not attempted, but attention was focused on those peptide fractions that appeared likely to yield useful information.

As with other digests discussed in the present paper, almost all of these peptides arose from that part of the polypeptide chain that also yielded soluble tryptic peptides. All of the peptides that were characterized were found to arise through *S. aureus*-proteinase digestion at the C-terminal side of glutamic acid residues, except for peptide SP.5A1 γ , which followed an aspartic acid residue.

The use of the peptide sequences determined in the present work for the alignment of the tryptic peptides from the succinylated protein is discussed in the following paper (Allen *et al.*, 1980).

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References

- Allen, G. (1980a) *Biochem. J.* **187**, 545–563
- Allen, G. (1980b) *Biochem. J.* **187**, 565–575
- Allen, G. & Green, N. M. (1978) *Biochem. J.* **173**, 393–402
- Allen, G., Trinnaman, B. J. & Green, N. M. (1980) *Biochem. J.* **187**, 591–616
- Chang, J. Y., Brauer, D. & Wittmann-Liebold, B. (1978) *FEBS Lett.* **93**, 205–214
- Cole, R. D., Stein, W. H. & Moore, S. (1958) *J. Biol. Chem.* **233**, 1359–1363
- Hartley, B. S. (1970) *Biochem. J.* **119**, 805–822
- Heilmann, J., Barollier, J. & Watzke, E. (1957) *Hoppe-Seyler's Z. Physiol. Chem.* **309**, 219–220
- Offord, R. E. (1966) *Nature (London)* **211**, 591–593
- Snell, D. T. & Offord, R. E. (1972) *Biochem. J.* **127**, 167–178
- Steinhardt, J. & Beychok, S. (1964) in *The Proteins* (Neurath, H., ed.), 2nd edn., vol. 2, pp. 139–304. Academic Press, New York
- Thorley-Lawson, D. A. & Green, N. M. (1977) *Biochem. J.* **167**, 739–748
- Yamada, S. & Itano, H. A. (1966) *Biochim. Biophys. Acta* **130**, 538–540