The Amino Acid Sequence of the B-Protein of Bacteriophage ZJ-2

By D. T. SNELL and R. E. OFFORD

*Laboratory of Molecular Biophysics, Department of Zoology, University of Oxford,
South Parks Road, Oxford OX1 3PS, U.K.*

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1. Bacteriophage ZJ-2 is a filamentous male-specific coliphage. Methods are given for its preparation and separation into nucleic acid and the major coat-protein component (B-protein). 2. The determination of the amino acid sequence of the B-protein of bacteriophage ZJ-2 is described. The principal features of this aspect of the work were the special procedures dictated by the insolubility of the protein and the extreme insolubility of its fragments. The sequence is compared with that obtained by other workers for the coat protein of the related bacteriophage fd.

Bacteriophage ZJ-2 (Bradley, 1964) is a member of a group of filamentous male-specific coliphages which also includes bacteriophages f1, M13 and fd (Hofschneider, 1963; Loeb, 1960; Marvin & Hoffman-Berling, 1963). Each of them is about 50 nm in diameter and 7000–8000 nm long, and contains DNA. The DNA is thought to occur as single-stranded circular structures. There is evidence that the protein coat of bacteriophage f1 consists of two components, a single molecule of the so-called A-protein and many copies of a very much smaller polypeptide, the B-protein (Rossomando & Zinder, 1968; Henry & Pratt, 1969). The structure and biology of the filamentous bacteriophages has been well reviewed by Marvin & Hohn (1969).

While this work was in progress, the amino acid sequence of bacteriophage fd B-protein was published (Braunitzer *et al.*, 1967; Asbeck *et al.*, 1969). Bacteriophage ZJ-2 B-protein has been found to differ from that of bacteriophage fd in two respects: (i) the substitution of alanine for threonine at (ZJ-2) position 36 and (ii) the presence of an additional alanine residue at (ZJ-2) position 27. The substitution of alanine for threonine at position 36 is of a type that could have been brought about by a single base change. It cannot be said, of course, whether the difference at position 27 is an insertion in bacteriophage ZJ-2 protein or a deletion in bacteriophage fd protein.

A notable feature of the work presented here is the insolubility of the protein and the extreme insolubility of its fragments. This made it necessary to use some unconventional procedures in the preparation of protein and digests, which are described in the Materials and Methods section.

The sequence work was carried out to provide information for a study of chemical mutagenesis in progress in this laboratory. The amino acid sequence has been reported in preliminary form (Snell & Offord, 1969).

Materials and Methods

Materials

Media. The culture medium employed for preparation of the bacteriophage was Oxoid Nutrient Broth no. 2 obtained from Oxoid Ltd., London E.C.4, U.K. It was made up to 2.5% (w/v) in water and sterilized by autoclaving in accordance with the makers' instructions.

Enzymes. Trypsin and chymotrypsin were supplied by Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. Chymotrypsin-free trypsin, leucine aminopeptidase and carboxypeptidase A were obtained from Worthington Biochemical Corp., Freehold, N.J., U.S.A. (batch nos. of the two last-named ODA and CD17CG respectively). Pepsin was bought from Sigma (London) Chemical Co., London S.W.6, U.K., and thermolysin (batch no. T57C51) from Daiwa Kasei K.K., Osaka, Japan.

Preparation of bacteriophage

Bacteriophage ZJ-2 was cultivated in growing cultures of *Escherichia coli* C3000F⁺. The infection does not bring about any extensive lysis of host cultures (Hoffman-Berling & Maze, 1964; Hofschneider & Preuss, 1963) and plaques formed on lawn cultures of *E. coli* on nutrient-agar plates are characteristically small. Our purification procedure derives from those of D. Kay (personal communication) and of Hobom & Braunitzer (1967).

Bulk cultures were performed at 37°C either in 1-litre batches, in 5-litre conical flasks with aeration by shaking or in 16 litres of medium in a 20-litre bottle with aeration by means of a stream of filtered compressed air. Active cultures of *E. coli* were prepared by adding 50 ml of an overnight culture of *E. coli*/litre of bulk culture medium. When the cell density reached 2×10^8 – 3×10^8 particles/ml the cultures were infected with 0.2 ml of a suspension of

bacteriophage (containing 1.8×10^{11} – 2.1×10^{11} particles/ml)/litre. Incubations were then continued for at least 6 h.

After incubation cultures were stored overnight at 4°C. Bacterial cells were then sedimented by centrifugation at 5000 rev./min for 10 min in a 6 × 250 ml rotor of an MSE High-Speed 18 centrifuge. The pH of the supernatant was adjusted to 4.0–4.1 and ethanol was added to 10% (v/v). Under these conditions the bacteriophage underwent isoelectric coagulation; the coagulated particles floated, apparently owing to absorption of air bubbles formed during the addition and mixing of the ethanol. The flotation phenomenon appeared to be diagnostic of the presence of bacteriophage in the culture. On no occasion when the bacteriophage had failed to grow (as established in separate bioassays) did the isoelectric precipitate exhibit buoyancy under these conditions.

The floating layer of solid was decanted off and lightly packed down by means of a brief centrifugation in a bench centrifuge. The supernatant was discarded and the pellet resuspended in 33 mM-sodium phosphate buffer, pH 7.6, to a volume of 15–20 ml/litre of the original bulk culture. The suspension was clarified by centrifugation in the bench centrifuge. Final purification of bacteriophage was achieved by sedimenting bacterial debris at 26000 g for 1 h, followed by sedimenting bacteriophage at 104000 g for 2½ h, both centrifugations being performed in an MSE Superspeed 40 machine. The pellet obtained in the higher-speed centrifugation was resuspended in the phosphate buffer and subjected to further differential centrifugations. Pure bacteriophage forms an almost transparent colourless pellet and could be obtained after as few as three repetitions of the differential centrifugation.

Purified bacteriophage was resuspended in the phosphate buffer at 20–40 mg/ml, under which conditions the suspension was extremely viscous and opalescent. A small crystal of thymol was added to each stock suspension of virus to suppress possible bacterial growth. Bacteriophage was stored under these conditions at 2°C for periods in excess of a year without apparent loss of viability. Yields of purified bacteriophage varied from 60 to 100 mg/litre of culture. Hobom & Braunitzer (1967) describe a more complex isolation procedure for bacteriophage fd. As judged by electrophoresis of whole bacteriophage in agarose and all the analytical work on the protein, there is for bacteriophage ZJ-2 no need for any procedure more complex than that described here.

Separation of protein and nucleic acid

Suspensions of bacteriophage at about 5 mg/ml were shaken for 7–10 min at 0°C with equal volumes of phenol saturated with water. Protein was extracted

into the phenol layer and nucleic acid was extracted into the aqueous phase. The phases were separated by centrifugation and the aqueous layer was removed. The phenol was extracted with water in this manner for a total of five times.

Preparation of solutions of bacteriophage ZJ-2 B-protein

During the early stages of this investigation attempts were made to prepare solutions of bacteriophage ZJ-2 B-protein by a modification of the method described by Knippers & Hoffman-Berling (1966) for bacteriophage fd protein. The phenol solution of protein was mixed with 2 vol. of methanol and dialysed against 8 M-urea. Protein precipitated during the early stages of the dialysis, but redissolved, at least partially, later. The urea solution of protein was diluted to about 0.5 mg/ml and dialysed against 0.5% NH_4HCO_3 . Preparations obtained by this means were extremely cloudy and resulted in poor yields of protein in terms of the starting quantity of bacteriophage. Such preparations were used during the investigations of the tryptic and chymotryptic peptides of bacteriophage ZJ-2 B-protein.

Thermolysin digests were performed on solutions of protein obtained as follows. Phenol was removed from the phenol solution of protein by means of freeze-drying overnight. The residual solid was dissolved in ice-cold 0.1 M-NaOH and rapidly diluted to about 1.5 mg/ml with ice-cold water. The pH was rapidly adjusted to 8.5 by addition of ice-cold 1 M- NaH_2PO_4 solution. Solutions obtained in this way were perfectly clear and showed no tendency to precipitate if kept before use.

Preparation of acylated protein

Solutions of citraconylated (Dixon & Perham, 1968) and succinylated protein were prepared by addition of a 100-fold molar excess of the relevant anhydride to a stirred aqueous solution of protein (pH 8.5) obtained as described above. The pH was maintained between 8 and 9 by additions of 0.1 M-NaOH solution from a Pasteur pipette. Reactions lasted for about 30 min at room temperature.

Desalting of protein solutions

Solutions of both modified and unmodified protein were desalted into aq. 50 mM- NH_3 solution by passage through a column (26.5 cm × 6 cm) of Sephadex G-25. It was found that, if such solutions were freeze-dried, unmodified protein could be brought into solution only by redissolving it in 0.1 M-NaOH solution and repeating the process described

above. Freeze-dried succinylated protein could be redissolved directly in aq. 50mM-NH₃ solution.

Amino acid compositions

Amino acid compositions of peptides and whole protein were determined in analyses performed on a Beckman 120C automatic amino acid analyser. For the determination of the composition of whole protein, samples of freeze-dried unmodified protein were dissolved in 6M-HCl. Each sample was flushed with N₂ and sealed under vacuum. Hydrolyses were performed at 108°C for periods of 24, 47, 72, 96, 168 and 336h. Each protein analysis was done quantitatively by using colour constants obtained in a standard analysis performed on the same column immediately before or after the experimental run. Peptide hydrolyses were performed in 6M-HCl at 108°C for 24h, unless otherwise indicated.

Tryptophan in whole protein was estimated from the spectrum of succinyl-protein in 0.2M-NaOH measured on a Unicam SP.800 spectrophotometer (Goodwin & Morton, 1946). The tryptophan content of certain peptides was determined by hydrolysing the sample of the peptide in sealed tubes in 50μl of 5M-NaOH at 121°C for 5h (Krehl *et al.*, 1946). After the tubes were opened the hydrolysate was diluted into sufficient 0.2M-citric acid to lower the pH to 3 before application of a portion to the amino acid analyser.

Digests

All enzymic digestions except those of pepsin were carried out at pH8–8.5 at 37°C by using an enzyme/substrate ratio of 1:100 (w/w). Digestions normally lasted for 4h.

Fig. 1. Elution profile of the Sephadex G-25 separation of the thermolysin digest of succinylated bacteriophage ZJ-2 B-protein

For details see the Materials and Methods section.

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Peptic digestion was performed overnight in 10% (v/v) formic acid at room temperature. Solutions of protein for peptic digestion were prepared as follows. Salt-free suspensions of bacteriophage were prepared by dialysis. These were freeze-dried and the bacteriophage was dissolved in 98% (v/v) formic acid. The solution was left at room temperature for 1h to denature the virus, diluted to 0.5mg of bacteriophage/ml with formic acid, and water was then added slowly with continuous stirring to give a final concentration of 10% formic acid. It was found necessary to use such very high dilutions since precipitation occurred on addition of water if the bacteriophage concentration exceeded 0.5mg/ml. Even under these circumstances it was not invariably possible to avoid precipitation.

Partial acid digestion was performed under two sets of conditions. Whole protein and peptides known to contain aspartic acid were exposed to 6M-HCl for 3 days at 37°C (Naughton *et al.*, 1960). The 'cores' resulting from digestion of whole protein, and peptides not containing aspartic acid, under these conditions were hydrolysed in 12M-HCl at 100°C

Fig. 2. Sodium dodecyl sulphate-polyacrylamide-gel electrophoresis of bacteriophage ZJ-2 B-protein

●, Marker proteins as identified on the figure; ■, succinyl bacteriophage ZJ-2 protein. Further details are given in the Results section.

Table 1. *Amino acid composition of bacteriophage ZJ-2 B-protein*

Values are molar proportions, calculated relative to Asp (=3). Values for valine, isoleucine and methionine were obtained by plotting residue numbers against the reciprocal of the hydrolysis time ($1/t$) and extrapolating to $1/t = 0$. This is not usually necessary for methionine but follows from the presence of valine adjacent to methionine. Serine and threonine residue numbers were plotted against time and the values reported obtained by extrapolation to $t = 0$. Tryptophan was determined from the spectrum of succinyl B-protein in 0.2M-NaOH measured on a Unicam SP.800 spectrophotometer (Goodwin & Morton, 1946). Details of amino acid analyses are given in the Materials and Methods section.

Residue	Time (h) ...	Molar proportions						Mean/extrapolate	Nearest integer
		24	47	72	96	168	336		
Lys		5.09	4.73	4.79	5.02	4.79	4.70	4.85	5
Asp		3.00	3.00	3.00	3.00	3.00	3.00	3.00	3
Thr		1.89	1.84	1.80	1.71	1.61	1.22	1.96	2
Ser		3.87	3.26	2.92	2.59	2.15	1.24	3.93	4
Glu		2.92	3.02	2.82	2.96	3.07	3.23	3.00	3
Pro		1.13	1.05	1.06	1.17	1.07	0.87	1.06	1
Gly		3.29	3.87	3.57	4.07	3.86	4.43	3.96*	4
Ala		10.08	10.90	11.27	10.74	11.01	10.71	10.93*	11
Val		1.35	2.37	3.02	3.53	3.98	3.73	4.11	4
Met		0.51	0.74	0.86	0.88	0.94	0.90	1.01	1
Ile		2.98	3.41	3.67	3.67	3.71	3.45	3.90	4
Leu		2.06	2.13	2.05	2.09	2.15	1.93	2.07	2
Tyr		1.90	1.96	2.01	1.92	1.92	1.67	1.90	2
Phe		2.95	2.95	3.02	2.92	3.11	2.98	2.99	3
Trp									1
Total									50

* Values for glycine and alanine were the means of the residue numbers for all times of hydrolysis except 24h. The results show that full release of glycine and alanine was delayed.

for 30min. (The 'core' products were not soluble in boiling 6M-HCl.)

Cyanogen bromide digests of succinyl-protein were performed in solution in 70% (v/v) trifluoroacetic acid. Cyanogen bromide was added in at least threefold excess (by wt.) over the protein to be digested, and the reaction allowed to proceed at room temperature for 1-3 days.

Separation and purification of peptides

Both trypsin and chymotrypsin gave large amounts of insoluble 'core' products during digestion. These were removed by centrifugation and the supernatants either decreased in volume by rotary evaporation or dried completely on the freeze-drier. In neither case could the products of digestion be obtained in clear solution at sufficient concentration to be applied to paper for purification by high-voltage electrophoresis. However, the addition of solid urea to saturation brought about satisfactory solution. The urea did not interfere with the subsequent electrophoresis.

Peptic digestion gave no 'core' product, but the freeze-dried product of digestion was incompletely soluble in aq. 1M-NH₃ solution. The insoluble portion was removed by centrifugation and the components that were soluble were purified by means of high-voltage electrophoresis on paper.

The freeze-dried products of partial acid hydrolysis were soluble in aq. 0.1M-NH₃ solution, and were separated by means of paper electrophoresis. The cyanogen bromide digest of succinyl-protein, when soluble, was treated similarly.

Thermolysin digests were carried out on citraconyl- and succinyl-protein as well as on unmodified protein. The freeze-dried products of digestion of acylated proteins were fully soluble in aq. 1M-NH₃ solution.

Thermolysin peptides of citraconyl-protein were purified exclusively by means of separations carried out on paper. The corresponding peptides derived from succinyl-protein were first fractionated by passage through a column (26.5 cm × 6.0 cm diam.) of Sephadex G-25 in aq. 50mM-NH₃ solution (100ml/h). Sample volumes had to be up to 70ml, containing

up to 100mg of digest. Six fractions were obtained by pooling the contents of the appropriate fraction-collector tubes (see Fig. 1). Each fraction was

freeze-dried and further investigated by means of paper electrophoresis and chromatography.

Methods for these procedures, and for location of

Table 2. *Purification and mobilities of tryptic peptides*

Peptide	Mobility (rel. to Asp* or Lys)			Net charge pH6.5	R_F BAWP†	Purification steps‡			Cd-ninhydrin colour
	pH6.5 (Asp)	pH3.5 (Asp)	pH3.5 (Lys)			1	2	3	
T1	0.92	—	—	+1	0.15	6.5	BAWP‡	—	Red
T2	0.52	—	0.67	+1	0.64	6.5	BAWP	3.5	Red
T2A1	—	—	—	+1	—	3.5	—	—	Red
T2A2	—	—	—	0	—	3.5	—	—	Red
T3	0.46	—	0.62	+1	0.29	6.5	BAWP	3.5	Red
T3A1	—	—	—	+1	—	3.5	—	—	Red
T3A2	—	—	—	0	—	3.5	—	—	Red
T4	0	—	—	0	0.23	6.5	BAWP	3.5	Red
T5	-0.68	0.43	—	-2	—	6.5	3.5	—	Red
T5A1a	0.72	—	—	+1	—	6.5	3.5	—	Red
T5A1b	0.61	—	—	+1	—	6.5	3.5	—	Red
T5A2	0	—	0.42	0	—	6.5	3.5	—	Faint red
T5A3a	-0.55	—	0.29	-1	—	6.5	3.5	—	Red
T5A3b	-0.71	—	0.22	-1	—	6.5	3.5	—	Red
T5A3c	-0.71	—	0.04	-1	—	6.5	3.5	—	Yellow
T5A4	-0.78	—	0.09	-1	—	6.5	3.5	—	Red
T5A5	-1.21	-1.46	—	-2	—	6.5	3.5	—	Red

* Asp = -1 in this and subsequent tables.

† BAWP, Butanol-acetic acid-water-pyridine (15:3:12:10, by vol.; Waley & Watson, 1953).

‡ Electrophoresis in pyridine-acetic acid-water (25:1:225, by vol., pH 6.5, or 1:10:89, by vol., pH 3.5); chromatography in solvent BAWP.

Table 3. *Compositions of tryptic peptides and their fragments*

Peptide	Molar proportions										N-Terminus
	Lys	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Leu	Phe	
T1	1										
T2	1.02								0.91	1.00	Leu
T2A1	1.09									1.00	Phe
T2A2									1.00	1.25	Leu
T3	0.88		1.19	0.96						1.00	Phe
T3A1	0.95			1.00							Ser
T3A2			1.00							1.18	Phe
T4				1.00				1.00			Ala
T5	1.00	1.93			0.95	1.00	1.05	1.80			Ala
T5A1a	1.09							1.00			Ala
T5A1b	1.00					1.05		0.98			Pro
T5A2						1.00		0.97			Pro
T5A3a					1.00		1.08	0.94			—
T5A3b					1.00			0.94			—
T5A3c		1.00					1.10				—
T5A4					1.00		1.16				Glu
T5A5		2.00									—

peptides on paper, were as referred to by Gonzalez & Offord (1971).

Determination of *N*-terminal residues

The 5-dimethylaminonaphthalene-1-sulphonyl (dansyl) chloride method was used to determine the

N-terminal amino acids of peptides (Gray & Hartley, 1963*a,b*). The labelled amino acid was identified in the hydrolysate of a dansylated peptide by means of chromatography on polyamide t.l.c. sheets (Woods & Wang, 1967) obtained from the Cheng-Chin Trading Co. Ltd., Taipei, Taiwan.

The dansyl derivative of the protein was prepared as follows: 0.01 μ mol of protein (0.05 mg) was dissolved in 0.5 ml of 8M-urea, which had been purged of cyanate by passage through a mixed-bed ion-exchange resin. NaHCO₃ (25 mg) was dissolved in the solution and 0.5 ml of a solution of dansyl chloride in acetone (20 mg/ml) was added. The mixture was incubated at 37°C for 3 h. Dansyl-protein was precipitated by addition of a 20% solution of trichloroacetic acid in water to a final concentration of 5%. The precipitate was washed twice with water before being hydrolysed.

The dansyl-Edman technique (Gray, 1967) was used to determine the sequences of several peptides.

Results

Molecular weight of the protein

The molecular weight of the succinyl-protein prepared by us has been determined in a series of measurements of electrophoretic mobilities of small proteins in sodium dodecyl sulphate–polyacrylamide gels (Dunker & Rueckert, 1969) by David Wood of the Microbiology Unit, Department of Biochemistry, South Parks Road, Oxford. Marker proteins were: soya-bean trypsin inhibitor, lysozyme (hen), *Pseudomonas* cytochrome *b*₅₅₁ and insulin. Succinylated bacteriophage ZJ-2 protein was found to have a mobility with respect to Bromophenol Blue identical with that of insulin, indicating a molecular weight of approx. 5500 (see also Fig. 2). Thus, in the present case, the internal inconsistency that might have been expected from the work of Williams &

Fig. 3. First-dimensional separation (pH 6.5) and peptide 'map' [pH 6.5, then butanol–acetic acid–water–pyridine (BAWP)] of the tryptic digest of bacteriophage ZJ-2 B-protein

Details are given in the legend to Table 2.

Table 4. Purification and mobilities of chymotryptic peptides

Peptide	Mobility (rel. to Asp or Lys)			Net charge pH 6.5	Purification steps*			Cd-ninhydrin colour
	pH 6.5 (Asp)	pH 3.5 (Asp)	pH 3.5 (Lys)		1	2	3	
C1	1.02	—	1.10	+2	6.5	3.5	—	Red
C2	0.36	—	—	+1	6.5	3.5	—	Yellow
C2T1	0.55	—	—	+1	6.5	—	—	Yellow
C2T2	0	—	—	0	6.5	—	—	Red
C3	−0.53	0.43	—	−2	6.5	3.5	—	Red
C3T1	0	—	—	0	6.5	—	—	Red
C3T2	−0.65	—	—	−2	6.5	—	—	Red
C4	−0.53	0.12	—	−2	6.5	3.5	1.9	Red

* Electrophoresis at pH 6.5 or pH 3.5 (for details see Table 2).

Table 5. *Compositions of chymotryptic peptides and their fragments*

Peptide	Molar proportions											<i>N</i> -Terminus
	Lys	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Leu	Phe	Tyr	
C2	1.03		0.85	1.79				1.00				Thr
C2T1	1.00		0.76	1.12								—
C2T2				1.00				0.94				—
C3	0.95	2.08			1.11	1.04	1.18	4.00		0.94		Ala
C3T1								2.15		1.00		—
C3T2	0.83	1.84			1.00	1.11	1.08	1.92				—
C4		1.00	1.01	1.84	2.48			1.97	0.94		0.79	Asp

Table 6. *Purification and mobilities of peptic peptides*

Peptide	Mobility (rel. to Asp or Lys)		Net charge pH 6.5	R_F BAWP*	Purification steps†			Cd-ninhydrin colour
	pH 6.5 (Asp)	pH 3.5 (Lys)			1	2	3	
P1	0.70	0.85	+3		6.5	3.5	—	Red
P2	0.45	—	+1	0.61	6.5	BAWP	3.5	Red (slow)
P3a	0	—	0		6.5	3.5	—	Red (slow)
P3b	0	—	0		6.5	3.5	—	Red (slow)

* BAWP, Butanol-acetic acid-water-pyridine (15:3:12:10, by vol.).

† Electrophoresis at pH 6.5 or pH 3.5 (for details see Table 2); chromatography in solvent BAWP.

Table 7. *Compositions of peptic peptides*

Peptides P3a and P3b were difficult to obtain in high yield. The high glycine values are attributed to some form of contamination.

Peptide	Molar proportions												<i>N</i> -Terminus
	Lys	Trp	Thr	Ser	Gly	Ala	Val	Met	Ile	Leu	Phe	Tyr	
P1	3.00		0.82	1.59		1.18					2.00		—
P2	1.20								1.00	0.90			—
P3a		+			1.33	2.00	0.73	0.81	1.01			0.84	Ile
P3b		+			1.54	2.00		0.75	0.85			0.91	Ile

Gratzer (1971) with small, highly charged, proteins is not observed.

Amino acid composition of the protein

The results of the amino acid analyses from which the composition of the protein was calculated are shown in Table 1. The molecular weight of the protein, calculated as the sum of the constituent residue weights, is 5206 atomic mass units.

The *N*-terminal residue was identified as alanine.

Tryptic peptides

Table 2 shows the routes by means of which the tryptic peptides were purified. Table 3 shows their compositions.

The 'core' peptide as prepared was always contaminated with other peptides and undigested protein. It resisted all attempts to purify it. It is assumed to have represented a single peptide by virtue of our having found all but one of the lysine residues in the molecule in soluble tryptic peptides.

The subfragments of peptides T2, T3 and T4 were

obtained by partial hydrolysis with hydrochloric acid. Fig. 3 shows the peptide 'map' of the tryptic digest obtained by electrophoresis at pH 6.5 followed by paper chromatography.

Chymotryptic peptides

Tables 4 and 5 describe the purification and composition of the chymotryptic peptides investigated in this study. The fragments of peptides C2 and C3 were produced by trypsin.

The electrophoretic mobility of peptide C4 with respect to aspartic acid (= -1) at pH 6.5 was -0.53. This, taken with its calculated molecular weight, indicates that the peptide carried two negative

charges at pH 6.5 (Offord, 1966). This in turn implies that one of the two residues of glutamic acid in C4 is, in fact, glutamine. Which of them it is may be shown by consideration of the mobilities at pH 6.5 of peptides ThCt2a7a (0) and ThCt4b2 (-0.40) (see Fig. 4). In addition, the pH 6.5 mobility of ThCt4c7 is -0.64.

Peptic peptides

The purifications and compositions of peptic peptides are shown in Tables 6 and 7. Tryptophan was not determined quantitatively in peptides P3a and P3b. Its presence was inferred from a positive reaction of the peptide with Ehrlich reagent.

Table 8. *Purification and mobilities of peptides from partial acid hydrolysis*

Asterisks in peptide designation are used to distinguish peptides obtained in the hydrolysate of the 'core' produced during the first hydrolysis of whole protein (see the Results section). CFF, Cyanol FF (Gurr).

Peptide	Mobility (rel. to compound named)				Net charge pH 6.5	R_F BAWP†	Purification steps‡				Cd-ninhydrin colour
	pH 6.5 (Asp)	pH 3.5 (Lys)	pH 1.9 (Ser)	pH 8.9 (CFF)			1	2	3	4	
A3a1	0	—	—	2.97	0	0.21	6.5	BAWP†	3.5	8.9	Orange
A3a2	0	—	—	2.83	0	0.21	6.5	BAWP	3.5	8.9	Red
A3c1	0	0.47	1.20	—	0	0.48	6.5	BAWP	3.5	1.9	Red
A3c2a	0	0.42	1.35	—	0	0.48	6.5	BAWP	3.5	1.9	Red (slow)
A3c2b	0	0.42	1.08	—	0	0.48	6.5	BAWP	3.5	1.9	Red
A3d1b	0	0.40	1.18	—	0	0.60	6.5	BAWP	3.5	1.9	Orange
A3e	0	0.40	—	—	0	0.68	6.5	BAWP	3.5	—	Red
A*3f	0.68	0.81	—	—	+1	0.61	6.5	BAWP	3.5	—	Red (slow)
A*4f2	0	0.24	—	—	0	0.85	6.5	BAWP	3.5	—	Red (slow)

† BAWP, Butanol-acetic acid-water-pyridine (15:3:12:10, by vol.).

‡ Electrophoresis at pH 8.9, 6.5, 3.5 or 1.9; chromatography in solvent BAWP.

Table 9. *Compositions of peptides from partial acid hydrolysis*

See Table 8 and the Results section for explanation of asterisks used in peptide designations.

Peptide	Molar proportions								N-Terminus
	Lys	Ser	Gly	Ala	Val	Ile	Leu	Tyr	
A3a1		1.00		1.12					Ser
A3a2		1.00		1.00					Ala
A3c1			1.33	1.00		0.93			Ala
A3c2a			1.00			0.85			Ile
A3c2b			1.15	1.82		0.86			Ala
A3d1b		1.00					0.83		Ser
A3e			1.00			0.75		0.28	Tyr
A*3f	1.00					0.93	1.11		—
A*4f2					2.00	0.84			—

Peptides from partial acid hydrolysis

Tables 8 and 9 give details of the purification and compositions of peptides obtained by means of partial acid hydrolysis of the protein. Asterisks are used to distinguish those peptides obtained in the hydrolysate of the 'core' produced during the first hydrolysis of whole protein. Glycine was placed at the C-terminus of A3c2b since, after three Edman-degradation cycles, free glycine remained.

Thermolysin peptides

Peptides prefixed by the letters ThCt were obtained in the digest of citraconylated bacteriophage ZJ-2

B-protein with thermolysin. Peptides prefixed by ThSu were similarly obtained from succinylated bacteriophage ZJ-2 protein. Tables 10 and 11 present the details of purification and composition of thermolysin peptides.

Peptide ThCt2a7bCPA was produced by the action of carboxypeptidase on ThCt2a7b. Peptide ThCt4c7L1 was a product of the action of leucine aminopeptidase on peptide ThCt4c7. Threonine was found to be *N*-terminal in peptide ThCt4b2 after removal of *N*-terminal alanine by the application of one cycle of Edman degradation.

The sequences of peptides ThSu3a7a, ThSu3a9c and ThSu5a2 were determined by means of the Edman degradation. After one cycle of the reaction

Table 10. *Purification and mobilities of thermolysin peptides from succinylated bacteriophage ZJ-2 protein*

Peptide	Mobility (rel. to Asp or Ser)		Net charge pH 6.5	R_F BAWP*	Purification steps†				Cd-ninhydrin colour
	pH 6.5 (Asp)	pH 1.9 (Ser)			1	2	3	4	
ThSu3a7a	0	1.00	0	0.63	G-25	6.5	BAWP	1.9	Red (slow)
ThSu3a9c	0	0.90	0	0.81	G-25	6.5	BAWP	1.9	Red (slow)
ThSu5a1	-0.07	1.00	δ^+	—	G-25	6.5	1.9	—	Red
ThSu5a2	-0.07	0.90	δ^+	—	G-25	6.5	1.9	—	Red

* BAWP, Butanol-acetic acid-water-pyridine (15:3:12:10, by vol.).

† Elution from Sephadex G-25; electrophoresis at pH 6.5 or 1.9; chromatography in solvent BAWP.

Table 11. *Compositions of thermolysin peptides*

Peptide	Molar proportions														<i>N</i> -Terminus
	Lys	Trp	Asp	Thr	Ser	Glu	Gly	Ala	Val	Met	Ile	Leu	Phe	Tyr	
ThCt2a6						0.87		1.20				1.10			Leu
ThCt2a7a					0.93	0.94		1.04				1.00			Leu
ThCt2a7b							1.00				1.01			1.08	Ile
ThCt2a7bCPA							1.00				0.79				Ile
ThCt3a2							1.00				0.91				Ile
ThCt3a4						1.00						0.79			Leu
ThCt3a6								1.00		0.80					Ala
ThCt4a1	2.00												0.94		—
ThCt4b2				0.99		1.22		1.10						0.71	Ala
ThCt4b2A1				1.10		0.88								1.00	—
ThCt4b2A2						1.00								0.67	—
ThCt4b2A3				1.00		1.12									—
ThCt4c3	1.00										1.02				Ile
ThCt4c7			1.00		0.93								1.00		Phe
ThCt4c7L1			1.00		1.40										Asp
ThSu3a7a							1.20		0.79		0.68				Ile
ThSu3a9c									3						Val
ThSu5a1		1.20					0.79								Ala
ThSu5a2		0.79					2.00								Ala

Table 12. *Composition of fragment CNBrSu3*

For details of the origin of fragment CNBrSu3 see the Results section. Values are molar proportions. Hsr, homoserine.

Fig. 4. *Amino acid sequence of bacteriophage ZJ-2 B-protein*

The principal fragments used in obtaining the sequence are shown. End groups or sequences obtained by dansyl-Edman or leucine aminopeptidase procedures (see the Materials and Methods section): —, by carboxypeptidase (see the Materials and Methods section), ←. The sequence of peptide ThCt4b2 was obtained by partial acid hydrolysis; the peptides resulting from this operation are omitted for clarity, but are fully described in Table 11. For peptides ThCt4a1 and A*4f2, there are alternative positions from which the peptides could have come. These pairs of alternatives are shown in parentheses.

peptides ThSu3a7a and ThSu3a9c contained *N*-terminal valine. Peptide ThSu5a2 was converted into a peptide with electrophoretic mobility at pH1.9 identical with that of peptide ThSu5a1, but staining yellow with cadmium-ninhydrin, behaviour to be expected of *N*-terminal tryptophan. After two cycles of the Edman reaction, peptide ThSu3a7a yielded free glycine, peptide ThSu3a9c yielded free valine and peptide ThSu5a2 yielded free alanine.

Treatment with cyanogen bromide

It was in this stage of the work that solubility problems were most severe. A common feature of the protein and digest, the difficulty of redissolving material once it has been brought out of solution, was particularly apparent. However, we were on only one occasion fortunate enough to obtain a digest of succinylated protein from which fragment CNBrSu3 could be obtained by electrophoresis at pH8.9. Its amino acid composition is shown in Table 12 and shows the peptide to be reasonably pure in spite of the difficulties in purification, although the glycine value is high.

Discussion

These results lead to the conclusion that the sequence of bacteriophage ZJ-2 B-protein is that shown in Fig. 4. The assignment of the non-overlapped tetrapeptide (Ala,Ala,Ile,Gly) to positions 35–38 (rather than at the beginning or end of the molecule) is most consistent with the composition of peptide CNBrSu3 and the tryptic peptides that are obtained. It is difficult to see how peptide T5 could have arisen were the *N*-terminal sequence Ala-Ala-Ile-Gly-Ala-Glu-Gly. Equally, it is difficult to explain the presence of peptide T4 in the digest of a protein whose C-terminal sequence was Lys-Ala-Ser-Ala-Ala-Ile-Gly.

Bacteriophages fd and ZJ-2 B-proteins therefore differ only at (ZJ-2) position 36 (alanine→threonine, a single base-change) and at (ZJ-2) position 27 (ZJ-2 has an extra alanine). Bacteriophages fd and ZJ-2 B-proteins are thus almost as close in sequence as might have been supposed from amino acid compositions published by Asbeck *et al.* (1969). The extra alanine residue is not found after a short hydrolysis and was not reported by these workers. Both proteins contain conspicuously acidic *N*-termini and basic C-termini separated by very hydrophobic sequences. However, it appears that the differences that do occur render the bacteriophage ZJ-2 protein digests even more insoluble than corresponding materials from bacteriophage fd. For example, Knippers & Hoffman-Berling (1966) found it possible to dissolve bacteriophage fd protein in

neutral solution at concentrations of approx. 3 mg/ml. Unmodified bacteriophage ZJ-2 protein was soluble only up to 1.5 mg/ml, and that only above pH12.5. Only very dilute solutions were obtainable in 8M-urea–50mM-ammonia.

The substitution of alanine for threonine at position 36 also altered the susceptibility of the protein to thermolysin and to pepsin. The pattern of release of some peptides (in addition to those including residue 36 itself) differs from that found for bacteriophage fd protein.

The resemblance between the two sequences, bacteriophages fd and ZJ-2, supports the idea that these viruses are very closely related. The substitution alanine→threonine at position 36 is easily explained as the result of a single base-change. There is no such simple explanation for the extra alanine residue at position 27.

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