The Amino Acid Sequence of *Neurospora* NADP-Specific Glutamate Dehydrogenase

THE TRYPTIC PEPTIDES

By JOHN C. WOOTTON, JOHN G. TAYLOR, ANTHONY A. JACKSON,
GEOFFREY K. CHAMBERS and JOHN R. S. FINCHAM
Department of Genetics, University of Leeds, Leeds LS2 9JT, U.K.

(Received 23 December 1974)

The NADP-specific glutamate dehydrogenase of *Neurospora crassa* was digested with trypsin, and peptides accounting for 441 out of the 452 residues of the polypeptide chain were isolated and substantially sequenced. Additional experimental detail has been deposited as Supplementary Publication SUP 50052 (11 pages) with the British Library (Lending Division), Boston Spa, Wetherby, W. Yorkshire LS23 7BQ, U.K., from whom copies may be obtained under the terms given in *Biochem. J.* (1975) 145, 5.

The NADP-specific glutamate dehydrogenase (EC 1.4.1.4) of *Neurospora crassa* has been extensively studied in connexion with the effects of gene mutation on enzyme activity (Fincham, 1962), allosteric properties (West *et al.*, 1967), genetic complementation (Fincham & Stadler, 1965) and hybrid enzyme formation (Fincham & Coddington, 1963; Coddington *et al.*, 1966). The enzyme has been shown to be a hexamer composed of identical subunits each of mol.wt. about 49000 (Wootton *et al.*, 1972; Blumenthal & Smith, 1973). The primary structure has been investigated both in our laboratory and by E. L. Smith and his colleagues in Los Angeles, and a provisional complete sequence of 452 amino acid residues has been published (Wootton *et al.*, 1974). Sequences of the peptides obtained by digestion of carboxymethylated or performic acid-oxidized enzyme with trypsin, staphylococcal proteinase (Drapeau *et al.*, 1972), chymotrypsin and pepsin have been determined by our group, and the fragments obtained by CNBr cleavage have been characterized by Blumenthal *et al.* (1975). The results of the analysis of the various proteinase-digestion products are presented in this and the two following papers (Wootton *et al.*, 1975; Holder *et al.*, 1975).

Experimental Methods

Glutamate dehydrogenase purification

The procedures for growth of the fungal mycelium and purification of the enzyme were as described by Ashby *et al.* (1974) with only minor variations. The wild-type organism was usually used for enzyme isolation, but the best preparations of a few peptides were obtained from mutant strains, *am*² (Fincham, 1962) in some cases and *am*¹⁹ R24 (Sundaram & Fincham,

1964) in others. For growth of the auxotrophic mutant *am*₂, the medium was supplemented with 5 mM-mono-sodium L-glutamate. The amino acid replacements that distinguish these mutant glutamate dehydrogenase varieties from the wild-type are now known (G. K. Chambers, A. A. Holder, J. C. Wootton & J. R. S. Fincham, unpublished work), and the tryptic peptides of mutant origin that are described in the present paper are all apparently identical with their wild-type counterparts.

Chemical modification of glutamate dehydrogenase

Purified glutamate dehydrogenase was either carboxymethylated, or oxidized with performic acid, by the methods of Hirs (1967).

Proteinase digestions

Between 100 and 200 mg (2–4 μ mol) of carboxymethylated or performic acid-oxidized glutamate dehydrogenase was dissolved, so far as possible, at a concentration of 5 mg/ml in 0.05 M-NH₄HCO₃, and 1 mg of trypsin (Worthington Biochemical Corp., Freehold, N.J., U.S.A.; three times recrystallized; freeze-dried), dissolved in about 0.2 ml. of 1 M-HCl, was added for each 50 mg of protein to be digested. Digestion was allowed to proceed at 25°C for 6.5 h with stirring, after which the reaction was stopped by the addition of acetic acid to pH 3.5; the digest was dried *in vacuo*. During the digestion particles of undissolved material largely disappeared and a fine precipitate of insoluble digestion products appeared. This precipitate usually represented about 5% by weight of the protein digested. A few large peptides were subdigested with pepsin, chymotrypsin or with staphylococcal proteinase as described in the following papers (Wootton *et al.*, 1975; Holder *et al.*, 1975).

Fractionation of peptides

The strategy described here is based on an appropriate combination of conventional techniques of column chromatography, analytical paper chromatography and the detection and characterization of peptides by a series of staining reactions. The same strategy has also proved successful in separating the very complex mixtures of peptic and chymotryptic peptides (Holder *et al.*, 1975).

Primary fractionation on Dowex AG 50. Digests were initially fractionated on a column (0.9cm×55cm) of the cation-exchange resin Dowex AG 50 (X4) (Bio-Rad, Richmond, Calif., U.S.A.) or on a column (0.9cm×16cm) of the comparable Beckman resin M72, the column being maintained at 55°C in either case. A three-chamber gradient elution system was used, the volumes of the different buffers being varied from one preparation to another. A typical fractionation, which yielded the peptide profile shown in Fig. 1, used the following system: chamber 1 contains 420ml of 0.05M-pyridine-acetic acid buffer, pH2.8, chamber 2 400ml of 0.5M-pyridine-acetic acid, pH3.7, and chamber 3 250ml of 2.0M-pyridine-acetic acid, pH4.85, the three chambers being of equal diameter. The peptide mixture was dissolved so far as was possible in chamber 1 buffer (buffer 1), the insoluble material was removed by centrifuging, and the solution (about 4ml) was applied to the column, which had been equilibrated with buffer 1. Buffer 1 (20ml) was then pumped through the column from chamber 1. Chambers 1 and 2 were then connected and 300ml of buffer of linearly increasing pyridine concentration (0.05–0.22M) was pumped through the column from chamber 1, after which the volume in each chamber was 250ml. The second and third chambers were then connected to give an exponentially increasing pyridine concentration (0.22–2.0M) during the final 750ml of the gradient. A flow rate of 30ml/h was maintained throughout by means of a peristaltic pump. The fraction size was 3.5ml (i.e. about 270 fractions).

Location of peptides. Samples (usually 0.2ml) of alternate fractions were dried *in vacuo*, taken up in 20μl of 0.1M-NH₄HCO₃ or 20μl of 5% (v/v) pyridine–5% (v/v) acetic acid (1:1, v/v), and applied in superimposed 10μl spots 15mm apart on the starting lines of Whatman 3MM paper chromatography sheets (56cm×46cm). The chromatograms were developed in the descending direction for about 16h with butanol–acetic acid–water–pyridine (BAWP; 15:3:10:12, by vol.). After drying, replicate sheets were stained by dipping either in 0.25% ninhydrin in acetone ('plain ninhydrin') or in a cadmium–ninhydrin reagent (Dawson *et al.*, 1969). After at least 6h at room temperature peptide spots were marked; spots that appeared either transiently or permanently

yellow or orange with the cadmium reagent were specially noted. The papers were then bleached by dipping in 1% (v/v) conc. HCl in acetone and further stained for specific amino acids. Usually, cadmium-stained papers were further stained with Ehrlich's reagent (Smith, 1953) for tryptophan, or with 1-nitroso-2-naphthol followed by HNO₃ for tyrosine (Dawson *et al.*, 1969). After plain ninhydrin, papers were stained with alkaline phenanthrene quinone (Yamada & Itano, 1972) to reveal arginine peptides as green fluorescent spots under u.v. illumination, or with Pauly reagent (Bennett, 1967) to show histidine and tyrosine peptides as scarlet and purple respectively. The phenanthrene quinone reagent was found to be very sensitive but not very specific; peptides containing phenylalanine and sometimes those containing tyrosine also showed as fluorescent spots, though their fluorescence tended to be bluer than the typical yellow–green given by arginine. These staining procedures were particularly valuable in giving good discrimination of closely placed peptides on complex chromatograms and were useful as a guide to the best pooling of fractions.

A typical tryptic peptide 'profile', showing only the major peptides, is presented in Fig. 1. On the basis of the pattern on the chromatography sheets developed in BAWP solvent, fractions were pooled so as to separate different peptides, so far as possible, without subdividing single ones. The pools were dried *in vacuo* and, where necessary, refractionated by one of the following procedures.

Separations by paper electrophoresis. In one early fractionation, pools from the primary column were concentrated and applied as bands to Whatman 3MM sheets or strips for high-voltage electrophoresis. 1.25M-Pyridine–acetic acid buffer was used at pH6.5 or formic acid–acetic acid–water at pH2.1; sometimes fractions obtained after electrophoresis at one pH were refractionated at the other pH. After electrophoresis, peptides were located by ninhydrin staining of reference strips and were eluted with either 0.1M-NH₄HCO₃ or 0.1M-NH₃ solution. Yields were often low and much trouble was experienced with minor contaminating peptides. Peptides from subsequent tryptic digests were subfractionated by one or more of the following column procedures, although paper electrophoresis was still resorted to in a few cases.

Chromatography on Dowex AG1 (X2). Columns of this anion-exchange resin, of diameter 1.5cm, were packed under gravity in 3% (v/v) pyridine to a length of either 25 or 45cm, depending on the complexity of the pool to be refractionated. The peptide mixture was applied to the column in 2–3ml of the same 3% pyridine and eluted successively with (a) 25ml of 3% pyridine, (b) a 250ml linear gradient from 3% pyridine to 3% pyridine–3% (v/v) acetic acid (1:1, v/v), (c) a 120ml linear gradient from the pyridine–acetic

acid buffer (1:1, v/v) to 3% (v/v) acetic acid, and finally (d) about 30 ml of 3% (v/v) acetic acid followed by similar volumes of 10% (v/v) and sometimes 20% (v/v) acetic acid to recover peptides (especially acidic ones containing tryptophan) that were eluted very slowly from the column.

The column was fed by gravity with a head of about 60 cm; fractions (about 3.5 ml) were collected and the whole procedure took about 10 h. The column was operated at ambient temperature (about 20°C). The system gave excellent resolution but recoveries of some large acidic peptides were poor. Peptides were detected by chromatography in BAWP solvent as described above; tryptophan peptides that were eluted late were sometimes located by E_{280} measurements.

Gel-filtration methods. The most useful gel-filtration agent was Bio-Gel P4, although Bio-Gel P2 and Sephadex G-25 (or very occasionally Sephadex G-50) were also used. In each case the gel was swollen in about 2 litres of 0.05 M-NH₄HCO₃ and packed under gravity into a column (2.5 cm × 90 cm). The peptide mixture was applied in 2–3 ml of 0.05 M-NH₄HCO₃, sometimes with the addition of 0.01% (v/v) thiodiglycol, and eluted with 500 ml of the same buffer over a period of about 20 h. The eluent was fed on to the column by gravity from a head of about 100 cm. Fractions (4–5 ml) were collected and portions analysed by either chromatography in BAWP solvent as described above or, when thiodiglycol was not present, measurements of E_{220} . Tryptophan and tyrosine peptides were located by their E_{280} .

Storage of peptides

Fractions that appeared to contain single peptides were pooled, dried, redissolved in 4 ml of 0.1 M-NH₄HCO₃ containing 0.1% (v/v) thiodiglycol, and stored at –20°C after one-half to one-fifth had been taken for amino acid analysis.

Amino acid analysis

Samples were hydrolysed in sealed evacuated tubes in 6 M-HCl supplemented with phenol at 3 mg/ml to prevent losses of tyrosine. Analyses were performed in either a Beckman Unichrom or a Bio-Cal BC200 analyser. Except where the peptide concentration was relatively high, methionine tended to be lost during the hydrolysis, although there was usually enough left to give a qualitative indication of the presence of the residue. On some occasions the loss was largely avoided by the addition of 4% (v/v) thioglycolic acid to the 6 M-HCl, but this was not usually done. Tryptophan was determined from the absorption spectra of peptides before hydrolysis after any residual pyridine had been removed by butyl acetate following the extraction procedure used the Edman degradation (Hartley, 1970).

Sequence determination

The dansyl-Edman procedure essentially as described by Hartley (1970) was used throughout with the following modifications. (1) Good recovery of Dns-Trp was achieved in most cases by including 4% (v/v) thioglycolic acid in the 6 M-HCl used for hydrolysis of the dansylated peptide. To allow the identification of Dns-Trp, which runs near bis-Dns-Lys in the standard polyamide-layer-chromatography solvents, authentic Dns-Trp was included in the standard mixture of dansyl amino acids. The use of thioglycolic acid also improved the recovery of Dns-Met by preventing the formation of oxidized derivatives. Thioglycolic acid was not used unless *N*-terminal tryptophan or methionine was suspected because of the inconveniently long time required to evaporate it under vacuum. (2) An additional polyamide-layer-chromatography solvent [ethanol-pyridine-acetic acid-water, pH 4.4 (40:27:90:3, by vol.)] was used to separate Dns-His from α - or ϵ -Dns-Lys and Dns-Arg (J. Bridgen, personal communication). (3) Benzene in solvent II was replaced by toluene, and ethyl acetate in solvent III by butyl acetate, with only minor changes in R_F values of the dansyl amino acids.

The identity of the dansylated *N*-terminal residue was usually clear after each step and where several spots were seen on the thin layer, one usually appeared more significant than the others, making due allowance for the generally weaker labelling of serine and the conversion of lysine into the ϵ -phenylthiocarbonyl derivative. In a few instances proline was missed because of destruction during over-long hydrolysis of the dansylated peptide, and methionine and tryptophan also tended to be lost when thioglycolic acid had not been included in the hydrolysis. It was often possible to obtain clear sequences even from contaminated peptides, and on several occasions an ambiguous amino acid analysis was clarified by sequence determination and later confirmed by overlapping sequences from other kinds of digests (Wootton *et al.*, 1975; Holder *et al.*, 1975). We have sometimes found quantities of peptide of 100 nmol or less to be adequate for the unambiguous determination of sequences of 20–25 residues.

On several occasions degradation was blocked where the *N*-terminal residue subsequently turned out to be glutamine; this is presumably due to the formation of the pyrrolidone ring. In such cases the methanolysis procedure of Kawasaki & Itano (1972) was effective in removing the block, revealing glutamate at the *N*-terminus.

Determination of amides

The electrophoretic mobilities of peptides at pH 6.5 were determined relative to aspartic acid, and net charges were calculated on the basis of the observed mobility and the presumed molecular weight in each

case (Offord, 1966). In most, but not all, cases this method gave an unambiguous estimate of net charge. Some amide assignments were obtained by amino acid analysis after limited or total digestion with carboxypeptidase A or A+B (Ambler, 1972) or with aminopeptidase M (Light, 1972). In a few cases cadmium-ninhydrin staining was used, *N*-terminal asparagine giving a yellow-to-orange stain and *N*-terminal aspartic acid giving red; assignments made on this basis were, in all cases, confirmed by charge determinations, either on the same peptide or on an overlapping peptic peptide (Table 1 of Holder *et al.*, 1975).

Results

Fig. 1 shows the typical pattern of the major tryptic peptides revealed by chromatography in BAWP solvent after primary fractionation on the cation-exchange resin. Preparative paper electrophoresis was largely abandoned early in the investigation because of poor yields, but the column procedures themselves gave variable and generally rather low recoveries. This may be attributed partly to peptide degeneracy and losses attendant on pooling selected fractions from complex mixtures, but it also seems likely that some peptides were not eluted efficiently from the ion-exchange columns.

Details of the peptide fractionations, with the yield, R_F value, amino acid analysis and (where determined) electrophoretic mobility of each peptide, are given in Tables 1 and 2 of Supplementary Publication SUP 50052. The sequences of the peptides, so far as they were determined, are shown in Table 1 of the present paper. Peptides and amino acid residues are numbered according to their positions in the complete sequence of the chain given in Holder *et al.* (1975). The 'standard' tryptic peptides expected to result from the complete hydrolysis of arginyl and lysyl bonds are numbered T1–T38 from the *N*-terminus to the *C*-terminus of the chain. Peptides resulting from incomplete hydrolysis are numbered according to the standard peptides which they include, whereas peptides due to chymotryptic or other cleavages within a standard peptide are given the number of that peptide with the suffix A or B. Amide and acidic residues are indicated in Table 2 of Supplementary Publication 50052 where they could be determined on the tryptic peptides themselves; otherwise the symbols Asx and Glx are used.

Most of the tryptic peptides gave clear sequences which are in good agreement with the amino acid analyses, and most of these are used as definitive detailed sequences of the parts of the chain covered. Some peptides which could not be completely sequenced directly were subdigested with pepsin, chymotrypsin or staphylococcal proteinase. The fragments produced, identified in Table 1 by the T number followed by P, C or SP, either gave some

clear partial sequences (as for peptides T1 and T6) or allowed the complete sequence of the parent peptide to be deduced (as for peptides T23, T30, T32 and T36). In most cases agreement between amino acid analyses and deduced sequences was considered acceptable if deviations were not greater than ± 0.2 equivalent. In some cases rather larger deviations (± 0.3 or 0.4 , or rarely ± 0.5 equivalent) were considered acceptable provided only one or a few residues were affected for one of the following possible reasons: hydrolysis losses of serine and threonine; incomplete hydrolysis of valine, isoleucine and occasionally phenylalanine; losses of carboxymethylcysteine, methionine and tyrosine due to the formation of derivatives during hydrolysis; extra partial equivalents of common background amino acids in cases where small samples of peptide were hydrolysed and analysed; specific cases of uncertain baseline due to an analyser fault; the effect of general analysis errors in cases of residues present in three or more equivalents. Among those peptides which were relied on for definitive detailed sequences, a few (T15A, T17A, T23SP1, T30C2, T32C1, T35) gave amino acid analyses which on the above criteria showed unacceptable levels of contamination. Nevertheless, clear sequences which can be taken as reliable were obtained from these peptides. Unacceptably contaminated analyses were also obtained from other peptides (T2A, T2B, T6, T6C1, T22, T28, T32, T32C2,

Fig. 1. Pattern of peptides obtained by fractionating a tryptic digest of carboxymethylated glutamate dehydrogenase on Dowex AG50 (X4) resin as described in the text and chromatographing samples of alternate fractions with butanol-acetic acid-water-pyridine (descending)

The diagram is a composite of several such profiles and not all the peptides were seen on every occasion. Numerous faint and inconsistent spots are omitted. Peptide numbering is as in Table 1. Spots with dotted outlines stain weakly or not at all with ninhydrin; Y, yellow or orange with cadmium-ninhydrin reagent rather than red; diagonal, horizontal and vertical cross-hatching indicates staining reaction for tyrosine, tryptophan and histidine respectively.

Table 1. *Amino acid sequences of tryptic peptides*

—, Round of the Edman degradation with the end group identified by the dansyl procedure; ····, round of the Edman degradation with the end group not definitely identified; <—, residue identified by analysis after limited carboxypeptidase digestion. Residues in parentheses were not sequenced in tryptic peptides but are known from the composition, and from other kinds of peptides. 1E, 2E etc. indicate results obtained after 1, 2 etc. rounds of Edman degradation. Cd-Y, Cd-R indicates yellow, red colour on staining chromatograms with the cadmium-ninhydrin reagent. CP-A, CP-B and AP-M indicate carboxypeptidases A and B and amino-peptidase M respectively. Peptide T25 was sequenced after opening the N-terminal pyrrolidone ring by methanolysis.

		Charge at pH6.5	Amide assignments
T1P1	10 Glx-Glx-Ala-Tyr-Lys 14 —————		
T2	15 Glu-Leu-Ala-Tyr-Thr-Leu-Glu-Asn-Ser-Ser-Leu-Phe-Gln-Lys 28 —————	-1 0 (5E) +1 (6E)	15, 21 by AP-M 21, 22 by charge 27 by CP-A + B 22 by Cd-Y (6E)
T2A	Glx-Leu-Ala-Tyr —————		
T2B		+1	
T3	29 His-Pro-Glu-Tyr-Arg 33 —————	0	31 by charge
T4	34 Thr-Ala-Leu-Thr-Val-Ala-Ser-Ile-Pro-Glu-Arg 44 —————	0	43 by charge
T5	45 Val-Ile-Gln-Phe-Arg 49 —————	+1	47 by charge
T6	50 Val-Val-Trp-Glx-Asx-Asx-Gly-Asx, Val, Glx, Val-Asn-Arg 63 —————	-3	62 by CP-A + B
T6C1		+1	62 by charge and Cd-Y (1E)
T7	64 Gly-Tyr-Arg 66 —————		
T8	67 Val-Gln-Phe-Asn-Ser-Ala-Leu-Gly-Pro-Tyr-Lys 77 —————	+1	68, 70 by charge
T9	78 Gly-Gly-Leu-Arg 81 —————		
T10	82 Leu-His-Pro-Ser-Val-Asn-Leu-Ser-Ile-Leu-Lys 92 —————	+1	87 by charge

Vol. 149

Table 1 (continued)

		Tyr-Ala-Pro-Gly-Lys → → → → →	
T30C2			
T31	367 Ala-Ala-Asn-Cys-Gly-Gly-Val-Ala-Val-Ser-Gly-Leu-Glx-Met-Ala-Glx(Asx, Ser, Glx, Arg) → → → → →		386
T32	387 Leu(Asx, Trp)Thr-Glx(Ala, Glx)Val-Asx-Glx-Lys → → → → →		
T32A	Ala-Glu-Val-Asp-Glu-Lys → → → → →		
T32C1	Leu-Asn-Trp → → → → →		
T32C2	Thr-Glx-Ala-Glx-Val-Asx-Glx(Lys) → → → → →		
T33	398 399 Leu-Lys → → → → →		
T34	400 Asp-Ile(Met, Lys) → → → → →		
T33/34	Leu-Lys-Asp-Ile-Met-Lys → → → → →		
T35	404 Asn-Ala-Phe-Phe-Asn-Gly-Leu-Asn-Thr-Ala-Lys → → → → →		
T36C1	415 Thr-Tyr-Val-Glx-Ala-Ala-Glx-Gly-Leu-Pro-Ser-Leu-Val-Ala-Gly-Ser-Asx-Ile-Ala-Gly-Phe → → → → →		436
T36C2	Ile-Ala-Gly-Phe → → → → →		
T36C3	437 438 Val-Lys → → → → →		
T37	439 Val-Ala-Glx-Ala(Met, His)Asx(Glx, Gly, Asx, Trp, Ser, Lys) → → → → →		451
T38	452 Asn → → → → →		

369 by AP-M

-2
-1 (2E)

0 (4E) 393, 395, 396 by charge

0 388 by charge
and Cd-Y (1E)

0 400 by charge, Cd-R

+1 400 by charge

+1 All by charge,
404 by Cd-Y0 452 by charge, Cd-Y,
amino acid analyser

T33, T34, T36). From most of these, clear sequence information was obtained (Table 1), but this is not considered to define the detailed sequences and is used either as confirmatory evidence or for alignment with overlapping peptides (Wootton *et al.*, 1975; Holder *et al.*, 1975). Although the analyses shown in Table 2 of Supplementary Publication 50052 are the best obtained, almost all the tryptic peptides have been isolated and analysed several times from separate digests during work on mutationally modified glutamate dehydrogenases. In many of the cases mentioned above as showing unacceptable contamination, the availability of several differently contaminated amino acid analyses of the same peptide has given increased confidence in the compositions deduced.

In some peptides (T11, T12, T13, T14, T21, T22, T26) one or two residues failed to give clear dansyl derivatives and their assignments were originally deduced from the amino acid composition by difference. However, clear assignments at all these positions were obtained from overlapping peptic and chymotryptic peptides (Holder *et al.*, 1975). Peptide T28 does not give the definitive evidence for the sequence of residues 331–353 because incompletely degraded derivatives, lagging one or more steps behind the primary residual peptide, accumulated during the Edman degradation. However, the sequence obtained was adequate for aligning the peptic and chymotryptic peptides (Holder *et al.*, 1975) that define this sequence in detail. This was the only peptide in which lagging end groups caused difficulties in interpretation.

The incompletely sequenced tryptic peptides are T1, T6, T20, T25, T27, T31, T37. In all these cases the missing sequences have been obtained from other types of peptide. The partial sequences obtained from these tryptic peptides are sufficient for the purposes of alignment with the other peptides. Peptide T1, which gave a rather contaminated analysis, proved to have a blocked *N*-terminus, now known to be *N*-acetylserine (appendix by Morris & Dell to Wootton *et al.*, 1975). A peptic subdigestion product, T1P1 (isolated and sequenced by Pierre Baudy in this laboratory), was valuable for the alignment of peptide T1 with the overlapping peptic peptide. A contaminated analysis was also given by peptide T6, but valuable partial sequences were obtained. Peptide T20, a large insoluble peptide, gave *N*-terminal Gly-Leu which was sufficient to allow the alignment of the overlapping chymotryptic peptide. Peptides T25 (partially sequenced after the opening of the *N*-terminal pyrrolidone ring by methanolysis), T27 and T31 gave partial sequences which permitted unambiguous overlapping. For peptide T37 the detailed sequence was obtained from the peptic peptide P45, the chymotryptic peptide C60 (Holder *et al.*, 1975) and the CNBr fragment CN10 (Blumenthal *et al.*, 1975).

Only two tryptic peptides which would have been predicted from the complete sequence were not recovered. One of these, the hypothetical peptide T18, would have the sequence Lys-Ala-Ala-Asn-Arg, and may have been retained on the primary cation-exchange column because of its strongly basic nature. The other, peptide T26, was represented only by a derivative T26B which was six residues shorter than expected because of an unusual Arg-Pro split. The complete T26 peptide would contain two tryptophans, and one each of histidine, lysine and arginine in a total of 13 residues, and presumably it was not eluted from the cation-exchange column.

Cleavages which could be attributed to chymotryptic-like activity were few; a Phe-Met bond was split in peptide T15, Tyr-Met in peptide T17, and Tyr-Ala in peptide T21. An Asp-Pro cleavage was found in peptide T13. Since the same split was found in a staphylococcal proteinase digest (Wootton *et al.*, 1975), it is possible that it is non-enzymic in origin. Piskiewicz *et al.* (1970) have reported similarly labile Asp-Pro bonds in bovine glutamate dehydrogenase. The reason for the failure of the Lys-Ser bond in peptide T14 to cleave in at least one digest is not known. The general resistance to trypsin of the Lys-Glu bond in peptide T27 is probably connected with the unique concentration of acidic residues in the immediate vicinity.

The sequences reported here, together with those of other kinds of peptides reported in the two following papers (Wootton *et al.*; Holder *et al.*, 1975), and the analysis of the CNBr fragments (Blumenthal, *et al.*, 1975), provide the basis for the complete sequence of 452 residues proposed in Holder *et al.* (1975).

This work was supported by grants from the Medical and Science Research Councils. We thank Mollie Crawford and Margaret Burnley for the amino acid analyses and Andrew Baron for skilled assistance with many other parts of the project.

References

- Ambler, R. P. (1972) *Methods Enzymol.* **25**, 262–272
- Ashby, B., Wootton, J. C. & Fincham, J. R. S. (1974) *Biochem. J.* **142**, 317–329
- Bennett, J. C. (1967) *Methods Enzymol.* **11**, 330–339
- Blumenthal, K. M. & Smith, E. L. (1973) *J. Biol. Chem.* **248**, 6002–6008
- Blumenthal, K. M., Moon, K. & Smith, E. L. (1975) *J. Biol. Chem.* **250**, 3644–3654
- Coddington, A., Sundaram, T. K. & Fincham, J. R. S. (1966) *J. Mol. Biol.* **17**, 503–512
- Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (1969) *Data for Biochemical Research*, 2nd edn., p. 529, Oxford University Press, Oxford
- Drapeau, G. R., Boily, Y. & Houmar, J. (1972) *J. Biol. Chem.* **247**, 6720–6726
- Fincham, J. R. S. (1962) *J. Mol. Biol.* **4**, 257–274

- Fincham, J. R. S. & Coddington, A. (1963) *J. Mol. Biol.* **6**, 361-373
- Fincham, J. R. S. & Stadler, D. R. (1965) *Genet. Res.* **6**, 121-129
- Hartley, B. S. (1970) *Biochem. J.* **119**, 805-822
- Hirs, C. W. H. (1967) *Methods Enzymol.* **11**, 325-329
- Holder, A. A., Wootton, J. C., Baron, A. J., Chambers, G. K. & Fincham, J. R. S. (1975) *Biochem. J.* **149**, 757-773
- Kawasaki, I. & Itano, H. A. (1972) *Anal. Biochem.* **48**, 546-556
- Light, A. (1972) *Methods Enzymol.* **25**, 253-262
- Offord, R. E. (1966) *Nature (London)* **211**, 591-593
- Piskiewicz, D., Landon, M. & Smith, E. L. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1173-1178
- Smith, I. (1953) *Nature (London)* **171**, 43-44
- Sundaram, T. K. & Fincham, J. R. S. (1964) *J. Mol. Biol.* **10**, 423-437
- West, D. J., Tuveson, R. W., Barratt, R. W. & Fincham, J. R. S. (1967) *J. Biol. Chem.* **242**, 2134-2138
- Wootton, J. C., Taylor, J. G. & Fincham, J. R. S. (1972) *Biochem. J.* **127**, 31P-32P
- Wootton, J. C., Chambers, G. K., Holder, A. A., Baron, A. J., Taylor, J. G., Fincham, J. R. S., Blumenthal, K. M., Moon, K. & Smith, E. L. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4361-4365
- Wootton, J. C., Baron, A. J. & Fincham, J. R. S. (1975) *Biochem. J.* **149**, 749-755
- Yamada, S. & Itano, H. A. (1972) *Anal. Biochem.* **48**, 546-556