Gly-Ala (166–167), Ser-Leu (279–280), Asn-Gly (408–409) and Gly-Ser (430–431). All these occurred in low yield, and their enzymic basis is in doubt.

Although recovery of peptides was not complete some conclusions may be drawn from the cases where glutamyl bonds showed incomplete cleavage or no cleavage. The single Glu-Glu sequence (319–320) was not split. Of the two Glu-Asp sequences occurring amongst the recovered peptides (out of three in the protein), one (313–314) was found to be unsplit and the other (265–266) gave only a very-low-yield cleavage product (SP18). This suggests that under the conditions used the proteinase does not readily cleave glutamyl bonds which are followed by an acidic residue. Some evidence was obtained indicating that the proteinase does not readily split off the dipeptide product from Glu-X-Glu sequences, but it is not clear if this is because the enzyme is a poor 'exodi-peptidase' or because of the presence of the second acidic group. From the sequence Glu-Gly-Glu-Leu (421–424) the Gly-Glu dipeptide was not recovered but mutually exclusive single splits were observed at each of the glutamyl bonds. The dipeptides Phe-Glu (9–10) and Ala-Glu (321–322) were recovered, but in low yield, and in the latter case an uncleaved product was also found. Another possible dipeptide, Pro-Glu (7–8) was apparently not split off, but Glu-Pro may in any case be resistant to the proteinase. No other cases of incomplete splits of glutamyl bonds were observed. We have no information on Glu-His, Glu-Thr and Glu-Trp sequences which do not occur in the protein. No cleavage of *S*-carboxymethylcysteinyl bonds was observed; Cys-Cys, Cys-Ala, Cys-Lys, Cys-Gly, and Cys-Thr sequences occur in the protein.

Since nearly all the independently assigned glutamate residues, but none of the known glutamine

residues, are found to be sites of staphylococcal proteinase cleavage, we felt it was safe to identify a Glx residue as glutamate on the basis of its *C*-terminal position in staphylococcal proteinase peptide in those few cases where independent evidence is not available. The assignments made in this way (glutamate-10, glutamate-346 and glutamate-349) are indicated in Table 2 of the following paper (Holder *et al.*, 1975).

We thank the Science Research Council for financial support, Margaret Burnley for the amino acid analyses and Dr. G. R. Drapeau for his generous gift of the staphylococcal proteinase.

References

- 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
- Drapeau, G. R., Boily, Y. & Houmard, J. (1972) *J. Biol. Chem.* **247**, 6720–6726
- Fincham, J. R. S. & Stadler, D. R. (1965) *Genet. Res.* **6**, 121–129
- Holder, A. A., Wootton, J. C., Baron, A. J., Chambers, G. K. & Fincham, J. R. S. (1975) *Biochem. J.* **149**, 757–773
- Piszkiewicz, D., Landon, M. & Smith, E. L. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1173–1178
- 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., Taylor, J. G., Jackson, A. A., Chambers, G. K. & Fincham, J. R. S. (1975) *Biochem. J.* **149**, 739–748
- Yamada, S. & Itano, H. A. (1972) *Anal. Biochem.* **48**, 546–556

APPENDIX

The Sequence of the Blocked *N*-Terminal Peptide from *Neurospora* Glutamate Dehydrogenase

By HOWARD R. MORRIS and ANNE DELL

M.R.C. Group, University of Cambridge Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

(Received 23 December 1974)

Mass spectrometry is now becoming a widely used technique in protein structure elucidation. Several major studies encompassing both known and unknown sequences have been carried out on α -lactalbumin (Bacon & Graham, 1972), triose phosphate isomerase (Priddle & Offord, 1974),

dihydrofolate reductase (Morris *et al.*, 1974a) and ribitol dehydrogenase (Morris *et al.*, 1974b).

Apart from such major applications, mass spectrometry is a powerful technique for the study of unusual structural features in proteins or other molecules. In particular, it can be valuable for the

Fig. 1. Mass spectrum of the acetylated blocked N-terminal peptide from *Neurospora* glutamate dehydrogenase

For details see the text.

analysis of repetitive amino acid sequences such as those found in myosin (Bridgen & Morris, 1974) and, more importantly, has played a major role (as has nuclear magnetic resonance; Stenflo *et al.*, 1974) in the identification of a new amino acid in a number of positions in the N-terminal region of prothrombin (Magnusson *et al.*, 1974; H. R. Morris, A. Dell, T. E. Petersen, L. S. Jensen & S. Magnusson, unpublished work).

Another important application is the identification of blocked N-termini in peptides and proteins (de Haas *et al.*, 1969; Frank & Weeds, 1974). This is normally achieved by the use of stable isotopic derivatives, and is based on the known behaviour of all amino acids under conditions of acetylation and permethylation. This is not always a safe assumption, and indeed the new amino acid mentioned above behaved somewhat unexpectedly in both reactions (H. R. Morris, A. Dell, T. E. Petersen, L. S. Jensen & S. Magnusson, unpublished work). However, no such problems were experienced with the study on the N-terminal peptide from *Neurospora* glutamate dehydrogenase described below.

Experimental

The peptide (100–200 nm) was acetylated by the method of Thomas *et al.* (1968) except that the [$^2\text{H}_6$]-acetic anhydride was used instead of acetic anhydride. The acetyl peptide was permethylated (Morris, 1972; Morris *et al.*, 1973) with a reaction time of approx. 2 min. Sample handling, partial fractionation and mass spectrometric parameters were as described previously (Morris *et al.*, 1971).

Results and Discussion

Several spectra were recorded, and the various relative peak heights observed during the temperature gradient assisted in the interpretation. One typical spectrum is shown in Fig. 1. A signal at m/e 158 shows the presence of N-terminal acetylserine (not deuterio-acetyl) thus showing the natural acetyl blocking group. A sequence can then be generated via signals at m/e 314, 441, 538, 621 and 778 giving Ac-Ser-Asn-Leu-Pro-Ser-Glu. Asparagine can give rise to N-C cleavage with hydrogen rearrangement (Morris *et al.*, 1974b) and a sequence arising from this type of fragmentation is also observed via signals at m/e 126, 253, 350, 465 and 622 giving ...Asn-Leu-Pro-Ser-Glu. A further signal at m/e 719 suggests a proline residue following the glutamate, although in this case the amino acid analysis (Ser₂, Glu₂, Pro₂, Leu, Asx) is consulted to confirm the assignment, since m/e 719 can also be due to threonine-32. A number of other signals in the spectrum, for example m/e 282, 409, 433, 590 etc., are due to losses of methanol from the relevant (serine-containing) sequence ions.

Thus far the data suggest a sequence, Ac-Ser-Asn-Leu-Pro-Ser-Glu-Pro-, but the mass spectrum was not sufficiently strong to differentiate the C-terminal residue as glutamine or glutamate. However, in the light of the known specificity of the staphylococcal proteinase used to generate the peptide a sequence Ac-Ser-Asn-Leu-Pro-Ser-Glu-Pro-Glu is likely.

References

- Bacon, J. R. & Graham, G. N. (1972) *Biochem. J.* **127**, 76P–77P
- Bridgen, J. & Morris, H. R. (1974) *Eur. J. Biochem.* **44**, 333–334
- de Haas, G. H., Franek, F. F., Keil, B., Thomas, D. W. & Lederer, E. (1969) *FEBS Lett.* **4**, 25–28
- Frank, G. & Weeds, A. (1974) *Eur. J. Biochem.* **44**, 317–332
- Magnusson, S., Sottrup-Jensen, L., Petersen, T. E., Morris, H. R. & Dell, A. (1974) *FEBS Lett.* **44**, 189–193
- Morris, H. R. (1972) *FEBS Lett.* **22**, 257–260
- Morris, H. R., Williams, D. H. & Ambler, R. P. (1971) *Biochem. J.* **125**, 189–201
- Morris, H. R., Dickinson, R. J. & Williams, D. H. (1973) *Biochem. Biophys. Res. Commun.* **51**, 247–255
- Morris, H. R., Batley, K. E., Harding, N. G. L., Bjur, R. A., Dann, J. G. & King, R. W. (1974a) *Biochem. J.* **137**, 409–411
- Morris, H. R., Williams, D. H., Midwinter, G. G. & Hartley, B. S. (1974b) *Biochem. J.* **141**, 701–713
- Priddle, J. D. & Offord, R. E. (1974) *FEBS Lett.* **39**, 349–352
- Stenflo, J., Fernlund, P., Egan, W. & Roepstorff, P. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2730–2733
- Thomas, D. W., Das, B. C., Gero, S. D. & Lederer, E. (1968) *Biochem. Biophys. Res. Commun.* **33**, 519–521