A Unique Amino Acid Sequence around a Reactive Serine Residue in Peptidases from French Beans and Yeast

By D. C. SHAW and J. R. E. WELLS

Department of Biochemistry, John Curtin School of Medical Research,
Australian National University, Canberra, A.C.T., Australia, and
Department of Biochemistry, University of Adelaide, Adelaide, South Australia

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Further studies of the properties of a peptidase isolated from French beans by Wells (1965) have shown that the activity against benzyloxycarbonyl-glycyl-L-leucine and benzyloxycarbonyl-L-phenylalanyl-L-leucine is inhibited by DFP.* Recently an enzyme, with similar properties, isolated from brewer's yeast has been reported by Félix & Brouillet (1966) also to be inhibited by DFP. To determine whether this inhibition is due to covalent reaction of DFP or caused by impurities in the batches of reagent (cf. Gould & Liener, 1965), [³²P]DFP has been used in the present work. This communication reports that, for both these enzymes, reaction occurs at a unique serine residue and that the sequence of amino acids about this reactive serine is different from those found in the 'serine' enzymes previously examined (Sanger, 1963).

A partial purification and characterization of a peptidase from French beans (*Phaseolus vulgaris*) has been described (Wells, 1965). The trivial name 'phaseolain' is proposed for this enzyme. Recently it has been shown that phaseolain is difficult to separate from another peptidase, which does not hydrolyse benzyloxycarbonylglycyl-L-leucine or benzyloxycarbonyl-L-phenylalanyl-L-leucine, but which does use casein as a substrate. Preparations of phaseolain at various stages of purification were treated with [³²P]DFP. Application of radiochemical techniques (Naughton, Sanger, Hartley & Shaw, 1960) to the study of the phosphoryl-peptides from a partial acid hydrolysate of the labelled proteins indicated that in the crude preparations two reactive serine residues, with different amino acid sequences, were involved. Separation of the labelled proteins by electrophoresis on polyacrylamide gel and their subsequent elution and hydrolysis showed that phaseolain contained a new serine sequence. The other protein labelled in the experiment contained the sequence Thr-Ser-Met-Ala, which occurs in DFP-treated subtilopeptidase A (EC 3.4.4.16) (Sanger & Shaw, 1960) and DFP-treated mould proteinase (*Aspergillus oryzae*) (Shaw, 1962): the similarity of sequence, however, does not extend beyond the

* Abbreviations: DFP, di-isopropyl phosphorofluoridate; SerP, O-phosphoserine.

alanine residue. On purification of the phaseolain preparation the amount of the phosphoryl peptides arising from the new sequence increased relative to those from the Thr-Ser-Met-Ala sequence.

The phosphoryl-peptides obtained by partial acid hydrolysis after [³²P]DFP treatment of a preparation of peptidase (benzyloxycarbonyl-L-phenylalanyl-L-leucinase activity eluted from DEAE-cellulose) from baker's yeast represented only the new sequence. The peptides in the hydrolysate are interrelated and extend over the sequence A-B-SerP-X-Y. The residue B has been identified as glutamic acid by direct comparison of the dipeptide B-SerP with Glu-SerP. Electrophoretic comparison of the dipeptide SerP-X with SerP-Gly, SerP-Thr, SerP-Met, SerP-Ala and SerP-Ile indicates that the residue X is most probably valine. At pH 3.5, the molecular-weight differences between dipeptides containing SerP led to a clear separation of SerP-Gly, SerP-Ala and SerP-Ile; SerP-X moves to a position, intermediate between SerP-Ala and SerP-Ile, that would be expected for SerP-Val. It therefore appears that the reactive serine residue in this enzyme occurs in the sequence Glu-Ser-Val. Work is in progress to confirm and extend this sequence but certainly the peptides observed are interrelated and represent a new sequence about a reactive serine residue.

The same sequence is the site of reaction with DFP in phaseolain as the three main peptide bands representing this sequence are evident in the preparations examined. It is likely that the enzyme from brewer's yeast (Félix & Brouillet, 1966) that is inhibited by DFP would have the same sequence about a reactive serine residue as the enzyme in our preparation from baker's yeast.

Félix, F. & Brouillet, N. (1966). *Biochim. biophys. Acta*, **122**, 127.

Gould, N. R. & Liener, I. E. (1965). *Biochemistry*, **4**, 90.

Naughton, M. A., Sanger, F., Hartley, B. S. & Shaw, D. C. (1960). *Biochem. J.* **77**, 149.

Sanger, F. (1963). *Proc. chem. Soc., Lond.*, p. 76.

Sanger, F. & Shaw, D. C. (1960). *Nature, Lond.*, **187**, 872.

Shaw, D. C. (1962). Ph.D. Dissertation: University of Cambridge.

Wells, J. R. E. (1965). *Biochem. J.* **97**, 228.