Phosphorus-containing Amino Acids and Peptides from Acid Hydrolysates of Casein and Pepsin

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The importance of the phosphorus-containing amino acids and peptides has been further shown by findings on the function of the phosphoproteins in living cells [1,2] and by experiments with di-isopropyl fluorophosphate [3]. This paper contains results obtained with two phosphoproteins, casein and pepsin.

Experimental and results. For preparation of phosphopeptides technical casein was used. Otherwise casein prepared according to Hammarsten (Grave, Stockholm) or pepsin twice crystallized from ethanol (Worthington Biochem. Sales Co., Freehold, N. J.) was employed. Techniques outlined in earlier papers *cf.* [2] have been applied.

When casein hydrolysed at 100° C for various periods was fractionated on two successive Dowex 50 columns, different proportions of the total phosphorus could be recovered in the phosphoserine peak:

5 hrs	10 hrs	20 hrs
14.8 %	16.4 %	21.4 %

For the preparation of phosphopeptides large amounts of casein were hydrolysed for 20 hours and fractionated through several columns. The two final steps were elution from a Dowex 50 column with 0.01 *N* hydrochloric acid, and elution from a Dowex 1 or 2 column generally with 0.5 *N* formic acid. The separation was in both cases traced with an automatic conductivity recorder [4]. The results are summarized in Table 1. All fractions gave one spot on paper chromatograms coloured with ninhydrin or phosphate reagent. End-group determinations were performed as described by Sanger and Thompson [5]. Some peptides gave no DNP-amino acid after hydrolysis. It may be that the amino group is blocked in some way, although it is possible to prepare the DNP-derivative of free phosphoserine. Phosphoserylglutamic acid from phosphopeptone was described as long ago as 1933 [6]. It is well established that the acid hydrolysis used gives a higher degree of degradation and a simpler mixture than enzymatic digestion [7].

Pepsin was analysed by the same technique. Fig. 1 shows a conductivity curve of the hydrolysate run through a Dowex 50 column. The high peak could be shown to

Table 1.

Dowex 50 column: Effluent volume / Resin bed volume	Final chromatographic system (resin and formic acid concentration)	Amino acids after hydrolysis (2 *N* HCl, 120°, 20 hrs)	N-terminal amino acid
0.95	Dowex 1, 0.25 *N*	Glu, Ser, Val	Glu
		Glu, Ser	Glu
		Val, Ser, Glu	—
1.25	Dowex 2, 0.5 *N*	Ser, Glu	—
		Ser	
1.7	Dowex 2, 0.5 *N*	Ser, Ala	—
		Ala, Ser	Ala
1.9	Dowex 2, 0.5 *N*	Val, Ser	Val
		Thr	
2.5	Dowex 2, 0.5 *N*	Leu, Ser	Leu
		Ileu, Ser	
2.9	Dowex 2, 0.5 *N*	Leu, Ileu, Ser	Leu or Ileu

Abbreviations after Brand and Edsall. The peptides are listed in the same order as they emerge from the Dowex 1 or 2 column.

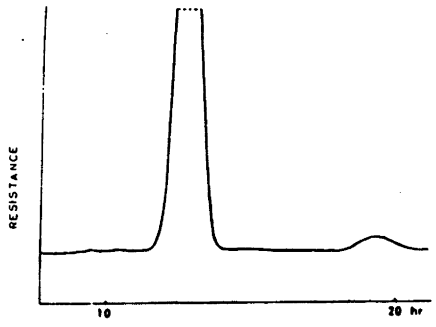

Fig. 1. A conductivity curve from the hydrolysate of 4 g pepsin run through a Dowex 50 column. Elutrient: 0.01 N hydrochloric acid. Column dimensions: 1.03 × 39.5 cm. Flow rate: 6 ml/hr.

be phosphoserine by paper chromatography before and after hydrolysis, and by X-ray diffraction diagram (Fig. 2). It contained 14.8 % of the phosphorus. The lower peak contained after hydrolysis the amino acids serine, threonine and glutamic acid.

There is one phosphorus atom per molecule of pepsin, and Perlmann has shown by experiments with different phosphatases that the phosphorus is probably bound to two different amino acids [8]. The isolation of phosphoserine shows that the phosphorus is bound to serine as in all typical phosphoproteins. A more detailed report of these experiments will be given elsewhere.

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After this investigation was complete the author's attention was drawn to a note reporting the isolation of phosphoserine and threonylphosphoserylglutamic acid from pepsin [9].

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Fig. 2. X-ray powder diagram of phosphoserine from pepsin. Flat film, with a distance of 10.0 cm between specimen and film.

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Viscosities of Dilute High Polymer Solutions

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During the years 1950—51 an apparatus for the very precise determination of relative viscosity [1] was built at this Institute. In the first measurements on high polymer solutions an effect was observed, in which the plot of η_{sp}/c *vs.* c was linear only down to the concentration 0.0001 g/ml, whereas at still lower concentrations the curve tended to bend downwards. A probable explanation was that the concentration, determined by weighing before the viscosity measurement, had changed in the viscometer due to adsorption of solute on its walls. The results of measurements of adsorption by Cutler and Kimball [2] indicated that this explanation is valid. It is of course very difficult to obtain a fairly good estimate of the magnitude of the adsorption effect at these very low concentrations.

It later seemed that the 'bending down' might partly be due to the fact that at these low concentrations the molecules are free from each other, which gives a lower hydrodynamic interaction, so that the viscosity diminishes more than corresponds to the decreasing concentration.

During the past year some measurements on polystyrene in toluene have been done in an attempt to verify this. In these experiments part of each solution was used in the viscometer, and after a measurement its concentration was checked turbi-