Crystalline Phosphoserine from Casein Hydrolysate

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In connection with recent investigations of phosphopeptone in this institute [1] we have tried to test by chromatographic methods the homogeneity of these preparations. In the hydrolysate of electrodialyzed phosphopeptone we could identify two hydroxyamino acids, serine in large amounts and threonine in small amounts, and a priori the possibility could not be excluded that both were esterified with phosphoric acid.

The fact that the phosphoric acid in casein is esterified on the alcoholic group of serine is ascribed to Lipmann, who isolated an amorphous silver salt of serine phosphoric acid from casein, following hydrolysis by boiling with 2.5 *N* hydrochloric acid [2]. In his paper [3] Lipmann gives the following analytical figures for the silver salt:

Calc.	C	7.12	H	0.99	N	2.76	P	6.12	Ag 63.8
Found	»	7.56	»	1.24	»	2.79	»	5.65	» 61.2

Phosphothreonine is about as resistant toward acid hydrolysis as phosphoserine [4] and in our view the possibility that small amounts of phosphothreonine might be present in such a preparation could not be excluded. If present it would possibly be proven by means of paper chromatographic analysis.

EXPERIMENTAL AND RESULTS

The preparative method of Lipmann [3] was followed with a few modifications. 50 g samples of casein were hydrolyzed, neutralized, and precipitated as described by Lipmann. The barium precipitate was thoroughly extracted three times with CO_2-free water and the filtrate concentrated *in vacuo* to a small volume. The reaction was adjusted to pH 4.6 by addition of glacial acetic acid, and the phosphorus-containing compounds precipitated by addition of a saturated solution of lead acetate until no more precipitation occurred. The clear supernatant liquid was removed by centrifugation and the precipitate suspended in water and decomposed by means of hydrogen sulphide. The lead sulphide was centrifuged off and the solution concentrated *in vacuo* to a small volume and precipitated with ethanol and ether in the usual manner. The precipitate was electrodialyzed in an apparatus similar to that described by Theorell and Åkesson [5]. When the anode fraction was concentrated first *in vacuo*, and subsequently over silica gel in a desiccator, a crystalline precipitate was obtained. (Fig. 1.) The precipitate was recrystallized from water, dried to constant weight in a desiccator at 25° C over silica gel, and analyzed.

$C_3H_8O_6NP$

Calc.	C	19.46	H	4.32	N	7.57	P	16.76	
Found	»	19.36	»	4.26	»	7.68	»	15.80	

Total nitrogen was determined by the micro-Kjeldahl method using $KMnO_4$ as a catalytic agent. The usual micro-Kjeldahl procedure with hydrogen peroxide as a catalyst gave low values. On heating the substance in desiccator at 105° C for 3 hours, low nitrogen values were obtained by the Dumas-method. Lipmann [3] reported that his silver salt of phosphoserine lost a large amount of nitrogen when it was dried at 100° C. The quotient of Van Slyke nitrogen to total nitrogen was 1.0.

Phosphorus was determined according to Teorell [6]. A combustion time of 4 days was found to be necessary. On heating, the crystals gave a m. p. of 167° (decomposition). The optical rotation a_D^{23} was + 7.2° (water; *l*, 1; c. 4.17). With two dimensional chromatograms a single spot corre-

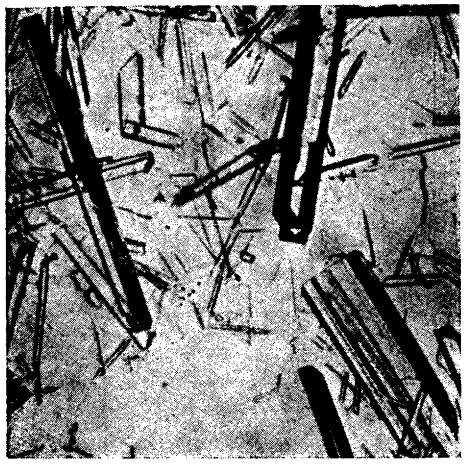

Fig. 1.

sponding to the position of a synthetic specimen of phosphoserine was observed. Serine was completely set free by hydrolysis with 2 *N* HCl at 120° for 24 hours. When large samples of the hydrolysate (1 mg) were analyzed chromatographically, a very faint alanine spot was observed in addition to the large serine spot. At present it is not possible to decide whether the alanine was present as part of a phosphoserine peptide in the crystalline preparation, or formed from phosphoserine during hydrolysis. The phosphopeptides left in the residue after crystallization of phosphoserine are being investigated with the interest focused primarily on the question of whether or not phosphothreonine is present in the mixture.

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On Homospecific Liver Pyrophosphatases

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Recent investigations[1] have demonstrated the existence of four isodynamic pyrophosphatases in rat liver. Swanson [2], however, found only two pyrophosphatases in rat liver. It therefore seems of interest to report some further studies on these enzymes in rat liver and in liver of other species. At the same time a correction regarding the magnesium ion concentration in the previous experiments [1] is given.

The livers of freshly killed animals were used throughout. The assay of pyrophosphatase activity was performed at 37° C as previously described [1]. At pH values below 6 the "third method" was used, *i. e.* the inorganic phosphate liberated was isolated as hydroxyl apatite before estimation. At higher pH values the phosphorus was determined directly on an aliquot of the protein-free filtrate obtained according to the "second method". The pyrophosphate concentration in the enzym digestion mixture was kept at 10^{-3} *M* in all experiments.

For the acid range up to pH 5.8 acetate buffers were used, from pH 6 to 7.6 cacodylate buffers (Plumel [3]) succesfully replaced maleic acid and collidine buffers. Over pH 7.6 ammonium chloride-ammonia buffers were used.

The activating reagent was prepared from a stock solution of molar $MgSO_4$ by dilution with 0.05 *M* buffer. When a new stock solution was prepared (autumn 1950) the enzyme values were lower than before. A check-up study showed that the Mg ion concentration giving maximum activation was about 0.01 *M* instead of 0.2 *M*. As the earlier studies were clearly made with optimal activation con-