Phosphopeptides in the lactating mammary gland

The presence of free phosphopeptides in milk has been demonstrated by VAN THOAI AND PIN[1,2], who have also obtained evidence for the biosynthesis of such peptides brought about *in vitro* by homogenates of sheep udder. The identification of phosphoserine as a constituent of these biosynthetic peptides focuses attention on the role of this substance as an intermediate in phosphoprotein synthesis. In a study of the occurrence of phosphoserine in mammary tissues, carried out in this laboratory, it has been observed that the lactating mammary gland of rat, while containing no detectable amount of this amino acid, does, however, contain trace amounts of phosphopeptides, the utilization of which might well represent an important pathway for the biosynthesis of casein.

Mammary glands removed from rats, 14 days after parturition, were homogenized in the cold with 5 vol. 2.5% KCl in a Waring blendor. The homogenate was treated immediately with trichloroacetic acid to 2.5% final concentration. All the operations were carried out in the cold to minimize proteolytic activity. The deproteinized sample was filtered, and the clear filtrate repeatedly extracted with ether to remove excess trichloroacetic acid. Any free inorganic phosphate present was removed as magnesium ammonium phosphate. The neutralized solution was then treated with barium acetate followed by alcohol to 50% concentration. After allowing to stand overnight in the cold, the precipitate that separated was centrifuged off, dissolved in water, filtered, and reprecipitated as before. The barium salts of phosphate esters were washed 2–3 times with 50% alcohol, and then decomposed by the quantitative addition of H_2SO_4. The $BaSO_4$ was centrifuged off, and the supernatant concentrated to dryness *in vacuo*.

The solids were analysed by ascending paper chromatography with *n*-butanol: glacial acetic acid: water (4:1:5) as solvent. Spraying with ninhydrin revealed four distinct spots at R_F 0.07, 0.16, 0.27 and 0.32. The method of HANES AND ISHERWOOD[3] was employed to detect phosphate on the paper. With the molybdate reagent, three blue spots were visible, two coinciding with the ninhydrin spots of R_F 0.27 and 0.32, while the third blue spot which had the highest R_F, was found to be neither a peptide nor an amino acid.

For characterization of the individual spots, the chromatogram was run for 48 h to get a clearer separation, according to the technique of RADHAKRISHNAMURTY AND SARMA[4]. With the aid of guide strips, the spots were cut out, eluted with water, hydrolysed with 2*N* HCl in sealed tubes at 110° C for 20 h, and analysed for the constituent amino acids by ascending chromatography using *n*-butanol:glacial acetic acid:water (4:1:5) as solvent. All the four peptides were found to contain the same amino acids, *viz.* isoleucine (and/or leucine, since both have the same R_F in this system), valine, proline, alanine, threonine, serine and glutamic acid. Phosphoserine* was detected on the paper at R_F 0.15 in the hydrolysates of the two faster moving peptides, using the HANES AND ISHERWOOD technique[3].

An incidental observation of interest was the closely similar amino acid pattern given by casein phosphopeptone prepared by the proteolytic degradation of casein. The conditions of the experiments for the isolation of the mammary gland peptides were such as to rule out the possibility of these arising as a result of the proteolytic action of the mammary gland. The close similarity in amino acid composition of these phosphopeptides raises the question of their role in the biosynthesis of casein. While the absence of a phosphorylating enzyme system for serine in the mammary gland[5] is suggestive of the importance of phosphopeptide as intermediate in casein synthesis, the possibility of phosphoserine formation from compounds other than serine, cannot be ruled out[6,7]. A detailed study of the occurrence and biosynthesis of these phosphopeptides in the mammary gland with a view to obtaining information regarding the metabolic roles of these peptides is in progress and will be reported in detail elsewhere.

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