Caseino-glycopeptides : Characterization of a methionine residue and of the N-terminal sequence

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When rennin reacts at pH 6.8 on casein, paracasein precipitates in presence of Ca^{++} in the case of whole casein and without Ca^{++} in the case of κ-casein. The supernatant contains mainly a caseino-glycopeptide, soluble in 12% trichloroacetic acid and not dialyzable. The glycopeptides from cow, sheep and goat caseins have previously been purified and analyzed : it was found that they contain : a) no aromatic or sulfur-containing amino acids and no arginine; b) almost all the sugars of the casein complex; no N-terminal amino acid had been characterized (Jollès and Alais, 1959; Nitschmann and Beeby, 1960; Jollès et al., 1961, 1962).

Jollès et al. (1963, 1964) continuing their chemical structure studies concerning the milk clotting process reinvestigated recently the question of the N-terminal sequence of the cow κ- and sheep caseino-glycopeptides. These substances were prepared following the procedure of Alais and Jollès (1961).

Amino acid analyses with a Technicon Autoanalyzer were previously made after total hydrolysis (HCl 6 N; sealed tube) during 24 and 48 hours. New determinations were tried after hydrolysis of 4, 8 and 12 hours. Under these conditions, the cow κ-casein-glycopeptide gives rise to a peak eluted at the same

place as methionine (one residue/mole for a hydrolysis of 8 hours); this peak disappears very rapidly when hydrolysis is continued. Sheep caseino-glycopeptide gives rise after hydrolysis during 4 and 8 hours respectively to 0.86 and 0.93 residue of methionine-sulfoxide/mole and only to traces of methionine. This methionine sulfoxide disappears again very rapidly when the total hydrolysis is continued.

The characterization of an N-terminal sequence was attempted by the methods of Edman (1950) and Sanger (1945) and by digestion experiments with an aminopeptidase.

The cow κ-caseino-glycopeptide was submitted twice in succession to the technique of Edman. The N-terminal amino acid obtained after each step was identified in the Autoanalyzer after regeneration of the PTH-derivative; methionine and alanine were characterized. The sheep caseino-glycopeptide was submitted successively three times to the same procedure : methionine, alanine and isoleucine were obtained and identified in this case by paper chromatography after regeneration of the PTH-derivative.

The DNP-sheep-caseino-glycopeptide was hydrolyzed (HCl 6 N; 5 h.; 110°; sealed tube) and the ethero-soluble DNP-amino acids were characterized by paper chromatography. A small amount of DNP-methionine was identified.

Cow κ-caseino-glycopeptide was digested with aminopeptidase : aliquots were withdrawn after different reaction times and submitted to paper chromatography or to analysis in the Autoanalyzer. When the digestion was made at pH 7.5 in the presence of trimethylamine, methionine and alanine were identified by paper chromatography and methionine, methionine sulfoxide and alanine by column chromatography. By preparative paper chro-

matography, a small amount of the substance with the R_f of methionine was prepared. This substance reacts positively with the iodo-platinic acid reagent of Toennies and Kolb (1951) and gives in the Autoanalyzer a peak which elutes at the place of methionine. When the digestion was made in the presence of a 0.05 M veronal buffer of pH 7.5, methionine sulfoxide and alanine were identified by paper chromatography. A small amount of the substance with the R_f of methionine sulfoxide was prepared : it gives in the Autoanalyzer a peak which elutes at the place of methionine sulfoxide and after performic acid oxidation a peak which elutes at the place of methionine sulfone (yield 30%). Similar results were obtained with the sheep caseino-glycopeptide.

In conclusion, it was established that cow κ- and sheep caseino-glycopeptides contain a very labile methionine residue, which is rapidly destroyed during total hydrolysis and easily oxidized to methionine sulfoxide but not to methionine sulfone. This methionine residue seems to occupy the N-terminal end of these glycopeptides, followed by an alanine residue. Further studies are necessary to establish whether the C-terminal phenylalanine residue of cow para-κ-casein (Jollès et al., 1963; Dennis and Wake, 1965) and the methionine residue characterized in this study are linked together or not.

Finally it is interesting to mention the fact that the caseino-glycopeptide itself seems to be very labile, especially in an acidic medium, in which a part of the molecule can be split off; this part gives in the Autoanalyzer a peak which elutes at the place of cysteic acid. More details on the chemical structure of the caseino-glycopeptides will be given in a forthcoming paper.

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