WeP-3

PHOSPHORYLATION OF CASEINOMACROPEPTIDE

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Milk contains several bioactive compounds one of which is the caseinomacropeptide (CMP) a polypeptide of 64 amino acids corresponding to α-casein 106-169. CMP is a natural antimicrobial peptide and has a potential as a therapeutic or food preservative agent. CMP naturally exists in several forms due to extensive posttranslational modifications including glycosylation and phosphorylation. We have determined that the most active form of CMP is the non-glycosylated form, however, phosphorylation seems to be important for antimicrobial activity. The aim of this study was to unambiguously locate the phosphorylation sites in the CMP sequence. The method applied was matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) and MALDI post source decay (MALDI-PSD-MS).

106 137 147 151 169
MAIPPKKNQDKTEIPTINTIA**S**GEPTSTPTIE-AVESTVATLE-A**S**PE-VIESPPEINTVQVTSTAV

To obtain peptides suitable for MALDI-PSD-MS analysis, CMP was digested by endoproteainase Glu C (EC 3.4.21.19) for 4 h with a enzyme/substrate ration of 1:100. The major cleavage points are denoted by hyphens in the sequence above.

The sample was dried and dissolved in 30% (aq) acetonitrile and applied to a target prepared with a thin layer of α-cyano 4-hydroxy cinnamic acid matrix. The spectra were acquired in reflector mode with the acceleration and reflector voltage set at 26.5 and 30 kV, respectively. The negative ion mode spectrum of the peptide mixture showed peaks corresponding to the potential phosphopeptides. For example, the peak at m/z 2346.15 has a mass which is 80 Da. Higher than the calculated mass for the sequence (148-169) indicating phosphorylation. The peak 1882.29 corresponds to the calculated molecular weight of the non-phosphorylated peptide 152-169 suggesting that the phosphorylation is located at Ser^{149} and, also, MALDI-PSD-MS analysis showed this to be the case. The fact that no peak could be found at m/z 2266 implies that the peptide (148-169) is fully phosphorylated. The peptide 106-137 was detected in both a phosphorylated and a non-phosphorylated form and MALDI-PSD-MS was used to locate the site to Ser^{127} shown in bold in the above sequence. It is interesting to note that the phosphorylated serine underwent a second fragmentation event and lost the phospho group, hence, the phosphorylated serine shows in the PSD spectra as dehydroalanine.

It has been shown that CMP is phosphorylated completely at Ser^{149}, however, only 10-20% of the peptide is phosphorylated at Ser^{127}. The relevance of this second partial phosphorylation to bioactivity remains to be determined.