THE EFFECT OF DEPHOSPHORYLATION ON THE CONFORMATION OF PEPTIDES FROM β-CASEIN

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Peptides β(1−28) and β(1−52) incorporating residues 1−28 and 1−52 of β-casein were prepared by proteolysis of the protein using plasmin and chymotrypsin respectively. Analysis of the circular dichroism spectra of the isolated peptides revealed that limited levels of α-helix were formed only by peptide β(1−52) and then only in the presence of >40% trifluoroethanol (TFE). Partial dephosphorylation of the peptides by treatment with alkaline phosphatase resulted in the formation of significant levels of α-helix in both peptides in the presence of TFE. However, no α-helix was detected in either peptide in the absence of TFE.

Casein accounts for 80% of the total protein content of bovine milk and is mainly present in the form of colloidal aggregates known as casein micelles. The caseins are phosphoproteins and their primary structure is characterized by a non-random distribution of charged and hydrophobic groups within the molecule. The N-terminal polar region of β-casein accounts for only 10% of the polypeptide chain, but one third of the total charged groups, with a charge of −12 at pH 6·6 (ref.[1]). The rest of the molecule has virtually no net charge.

Circular dichroism (CD) measurements have shown that there is little α-helix present in β-casein in aqueous solution (5% at 20°C) (ref.[2]). This may be partly due to the presence of α-helix breaking proline residues in the sequence. The induction of secondary structure into poorly structured proteins such as β-casein, has been demonstrated by the addition of a variety of alcohols, and in particular 2,2,2-trifluoroethanol (TFE) (ref.[2]).

In this study the secondary structure of two peptides, β(1−28) and β(1−52), derived from the N-terminal region of β-casein was examined in relation to their primary sequence, and in particular the high concentration of phosphorylated serine residues present in the peptides and consequently in the N-terminal region of β-casein.

METHODS

β-Casein was prepared from bulk milk using DEAE-cellulose anion exchange chromatography[3]. The peptides β(1−28) and β(1−52) were prepared by proteolysis of β-casein with plasmin[4]

and α-chymotrypsin[3-5] respectively and purified by chromatography. Alkaline phosphatase (Sigma) was used to dephosphorylate β-casein, β(1–28) and β(1–52). The extent of dephosphorylation was estimated from the phosphorus content of the supernatant[6]. Polyacrylamide gel electrophoresis (PAGE) was performed as described previously[5].

Far-UV (260–190 nm) CD spectra were measured with a Jasco J600 spectropolarimeter and data recorded on-line using an IBM PC. Samples (approx. 1 mg/ml) were prepared in 10 mM sodium phosphate buffer (pH 7·0), containing appropriate amounts of TFE (0–66% (v/v)). The final peptide concentration was accurately measured by amino acid analysis. The spectra, which are an average of two scans, were recorded at 19·5°C, using silica quartz demountable cells of 0·1 mm pathlength, with data resolution of 5 points per nm. An instrument sensitivity of +/– 20 millidegrees full scale and a time constant of 4 s were routinely used. The data are presented as molar CD (Δε) based on a mean amino acid residue molecular weight of 113. The secondary structure content of the spectra was carried out off-line using the 'CONTIN' programme of Provencher and Glockner[7].

RESULTS AND DISCUSSION

The two peptides β(1–28) and β(1–52) were purified to electrophoretic homogeneity and their structure was confirmed by their amino acid composition, molecular weight and C-terminal analysis[5]. The amino acid sequence of β(1–52) which also incorporates the sequence for β(1–28) is shown in Fig. 1. β(1–28) contains phosphorylated serine residues at positions 15, 17, 18 and 19. β(1–52) has an additional phosphorylated serine at position 35. Approximately 60% dephosphorylation of the peptides and β-casein was achieved by treatment with alkaline phosphatase. Electrophoresis showed that the mobility of the dephosphorylated samples was significantly reduced on the non-denaturing gels, as expected. In addition, the retention times of the dephosphorylated samples on a hydrophobic interaction FPLC column was increased, consistent with the reduction in charge which accompanied the removal of the hydrophilic phosphate groups.

Far-UV CD-spectra obtained from β(1–28) and β(1–52) are shown in Figs 2 and 3. The spectra of the native peptides in aqueous solution were typical of β-casein, consistent with a structure composed exclusively of sheet and random coil. Native β(1–28) showed minor spectral changes upon addition of TFE. The CONTIN analysis of these spectra showed only small increases in the levels of sheet present. The effect of TFE on native β(1–52) was more pronounced with a clear shift in the negative minimum from <200 nm to 207 nm and the formation of a secondary minimum at 222 nm. This change signified the formation of approximately 15% α-helix in the presence of 66% TFE. The effect of dephosphorylation was most pronounced with β(1–28). Although the spectrum obtained in aqueous solution was distinguishable from the spectrum of the native peptide the CONTIN analysis confirmed the absence of helix. However, in the presence of 66% TFE, the spectrum became characteristic of a α-helix containing peptide and the CONTIN analysis confirmed this to constitute >16% of the total structure. Dephosphorylation of

β(1–52) resulted in an intensification of the spectrum in 66% TFE, consistent with an increase in α-helix content.

CONCLUSION

The formation of α-helix in these peptides was inhibited by phosphorylated serine residues. Examination of the sequence in helical wheel notation revealed that the serine phosphates at positions 15, 18 and 19 lie in very close proximity to each other in two successive turns of the α-helix. We postulate that a reduction in phosphate content is a prerequisite for limited α-helix formation in this region of the sequence.

The amino acid sequence of peptide β(1—52). The phosphorylated serine residues are printed bold

NH_2-ARG-GLU-LEU-GLU-GLU-LEU-ASN-VAL-PRO-GLY-
-GLU-ILE-VAL-GLU-**SER**-LEU-**SER**-**SER**-**SER**-GLU-
-GLU-SER-ILE-THR-ARG-ILE-ASN-LYS-LYS-ILE-
-GLU-LYS-PHE-GLN-**SER**-GLU-GLU-GLN-GLN-GLN-
-THR-GLU-ASP-GLU-LEU-GLN-ASP-LYS-ILE-HIS-
PRO-PHE-COOH

FIG. 1

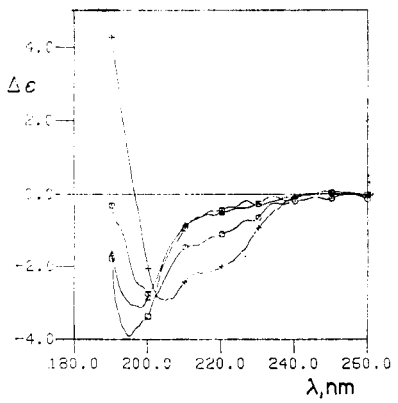

FIG. 2

Far-UV CD spectra of peptide β(1—28). Native peptide in (□) 0% and (○) 66% TFE. Dephosphorylated peptide in (△) 0% and (+) 66% TFE

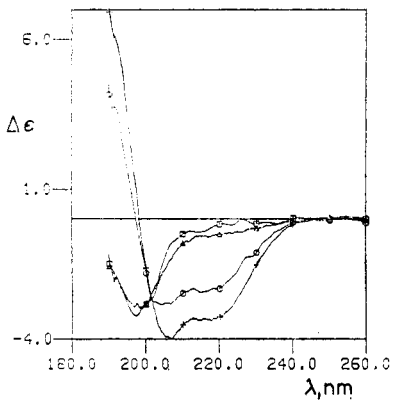

FIG. 3

Far-UV CD spectra of peptide β(1—52). Native peptide in (□) 0% and (○) 66% TFE. Dephosphorylated peptide in (△) 0% and (+) 66% TFE

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