The Primary Structure of Caprine PP3: Amino Acid Sequence, Phosphorylation, and Glycosylation of Component PP3 from the Proteose-Peptone Fraction of Caprine Milk

I.M.B. LISTER, L. K. RASMUSSEN, L. B. JOHNSEN, L. MØLLER, T. E. PETERSEN, and E. S. SØRENSEN[1]
Protein Chemistry Laboratory, Department of Molecular and Structural Biology, University of Aarhus, DK-8000 Aarhus, Denmark

ABSTRACT

Proteose-peptone component 3 is a phosphorylated glycoprotein that was isolated from the proteose-peptone fraction of caprine milk. By mass spectrometric analysis, amino acid sequencing, and polymerase chain reaction analysis, the primary structure has been determined and has been shown to contain 136 amino acids. Phosphorylations were identified at Ser_{30} and Ser_{41}. A partial glycosylation was present at Thr_{16}, and a N-linked glycosylation was present at Asn_{78}. Galactosamine was the amino sugar detected at Thr_{16}. Glucosamine and galactosamine were the amino sugars found in the carbohydrate group linked to Asn_{78}. The caprine amino acid sequence exhibits 88% identity with the bovine proteose-peptone component 3 sequence. However, when compared with the bovine sequence, the caprine sequence contains an insertion of a serine residue at position 25.
(**Key words**: proteose-peptone component 3, caprine milk, primary structure, amino acid sequence)

Abbreviation key: **PCR** = polymerase chain reaction, **PP3** = component 3 from the proteose-peptone fraction, **PTH** = phenylthiohydantoin.

INTRODUCTION

Bovine component 3 from the proteose-peptone fraction (**PP3**) is a phosphorylated glycoprotein that is found in the proteose-peptone fraction of milk (22); PP3 has been reviewed by Giradet and Linden (9). The primary structure of PP3 has been determined (23) and comprises a polypeptide backbone of 135 amino acid residues containing 5 phosphorylated serines, 2 threonine-linked O-glycosylations, and 1 N-glycosylation. Studies of the N-linked carbohydrate moiety have revealed the presence of several biantennary N-acetyllactosamine-type carbohydrates (8). Bovine PP3 has been cloned, and the cDNA sequence has been determined (13). Immunological studies with antibodies against highly purified bovine PP3 have shown that PP3 is part of the milk fat globule membrane and forms multimeric aggregates in bovine milk (24). Several functions have been suggested and investigated for bovine PP3 and PP3-enriched fractions, including emulsification (19), inhibition of lipolysis (2, 10, 20), and mitogenesis (15).

In contrast to the numerous studies that have been carried out on bovine PP3, information on the homologous proteins in milks of other mammals is very limited. A camelid whey protein (4) and a murine glycosylation-dependent adhesion molecule-1 GlyCAM-1 (5, 6), present in the milk of the species concerned, have been found to have 54 and 36% identity, respectively, with bovine PP3. A comparative study of the electrophoretic mobility and carbohydrate content of the proteose-peptone fractions of ovine, caprine, and bovine milks has been performed (18), and a protein, GP3, which is similar in some respects to bovine PP3, has been identified in a study (1) of glycoproteins in the proteose-peptone fraction of ovine milk.

Recently, we have identified and purified PP3 from caprine and ovine milks (24). Here we present the entire primary structure of caprine PP3, comprising a polypeptide backbone of 136 amino acid residues, including 2 phosphoserines, 1 N-linked glycosylation, and 1 O-linked glycosylation.

MATERIALS AND METHODS

Materials

Sephacryl S-200 and Mono Q HR 5/5 were obtained from Pharmacia (Uppsala, Sweden). Vydac C_4 and Vydac C_{18} were from The Separations Group

Received November 26, 1997.
Accepted March 27, 1998.
[1]Correspondence should be addressed to E. S. Sørensen, Protein Chemistry Laboratory, University of Aarhus, Science Park, Gustav Wieds Vej 10, DK-8000 Aarhus, Denmark.

(Hesperia, CA). The HPLC was carried out using a gradient pump (model LKB-Pharmacia 2249; Pharmacia) and a detector (2510 Uvicord SD; Pharmacia) equipped with a 226-nm filter. Problott membranes and reagents used for sequencing were purchased from PE Applied Biosystems (Foster City, CA). Swine anti-rabbit immunoglobulins conjugated to alkaline phosphatase, alkaline phosphatase-conjugated streptavidin, and rabbit anti-bovine PP3 serum raised against highly purified PP3 were purchased at Dako A/S (Glostrup, Denmark). Biotinylated marker proteins for SDS-PAGE were from Bio-Rad Laboratories (Hercules, CA). SeeBlue Prestained Standard was from Novex (San Diego, CA). Coomassie brilliant blue R-250 and thermolysin (E.C. 3.2.24.4) were obtained from Sigma Chemical Co. (St. Louis, MO). Trypsin (EC 3.4.21.4) and *Staphylococcus aureus* V8 protease (EC 3.4.21.19) were obtained from Worthington Biochemical Corporation (Freehold, NJ). α-Cyano-4-hydroxycinnamic acid was from Aldrich Fine Chemicals (Steinham, Germany). All other standard chemicals were of analytical grade. Primers for polymerase chain reaction (**PCR**) analyses were obtained from DNA Technology ApS (Aarhus, Denmark). The Wizard Kit was from Promega Corp. (Madison, WI). Fresh caprine milk was the kind gift of John Hermansen (The National Institute of Animal Science, Foulum, Denmark).

Purification of Caprine PP3

Caprine proteose-peptone was prepared from fresh caprine milk following the procedure described for bovine milk (3). Caprine PP3 was purified from the proteose-peptone by FPLC®-operated Sephacryl S-200 gel filtration and Mono-Q anion exchange, and, as a final step of purification, the protein was applied to a Vydac C_4 reverse-phase column (results not shown). All stages of the purification were monitored by SDS-PAGE and Western blotting with PP3 antibodies. The purity of the isolated protein was verified by amino acid sequencing and SDS-PAGE.

Generation and Isolation of Peptides

Peptides for characterization of the primary structure were generated by enzymatic cleavage with trypsin, *Staphylococcus aureus* V8 protease, and thermolysin. Digestion of trypsin and *Staphylococcus aureus* V8 protease was performed in 0.1 *M* NH_4HCO_3 at pH 8.1. Trypsin was incubated with protein in a 1:50 (wt/wt) ration of enzyme to substrate at 37°C for 3 h. *Staphylococcus aureus* V8 protease was used in a 1:100 (wt/wt) ratio of enzyme to substrate at 37°C for 2 h. Thermolysin subdigests were performed at 55°C in 0.1 *M* pyridine-acetate buffer, pH 6.5, containing 2 m*M* $CaCl_2$; the ratio of enzyme to substrate was 1:50 (wt/wt), and the digestion time was 3 h. Peptides from the different digests were separated by reverse-phase HPLC on a Vydac C_{18} column (4 × 250 mm). The column was operated at 40°C at a flow rate of 0.850 ml/min. The digests were separated in 0.1% trifluoroacetic acid and eluted with a gradient of acetonitrile.

Protein Characterization

Mass spectra were acquired using a BIFLEX matrix-assisted laser desorption and ionization-time of flight (MALDI-TOF) mass spectrometer equipped with a reflector (Bruker-Franzen, Bremen, Germany). Peptide samples (1.5 μl) dissolved in 0.1% trifluoroacetic acid were mixed with 1.5 μl of α-cyano-4-hydroxycinnamic acid (15 g/L), and 1.5 μl were applied to the target. Thirty to 100 calibrated mass spectra were averaged. Theoretical peptide masses were calculated using the GPMAW program (Lighthouse Data, Odense, Denmark). Amino acid sequencing was carried out on a sequencer (model 477A; PE Applied Biosystems) with on-line identification of the phenylthiodantoin (**PTH**) derivatives. For analyses of carbohydrates, phosphates, and amino acids, peptides were hydrolyzed under vacuum at 110°C for 3 and 16 h, respectively, in the presence of 6 *M* HCl and 0.05% phenol. The 3-h hydrolyzation was done to determine the contents of amino sugars and phosphorylated amino aids, and the 16-h hydrolyzation was done to determine the amino acid composition of the peptides concerned (21). Peptides containing phosphoserine were treated with ethanethiol to convert phosphoserine into S-ethyl cysteine as described (17).

PCR

Cells were isolated by centrifugation of caprine skimmed milk. Cells were disrupted with a solution of 4 *M* guanidinium thiocyanate, 25 m*M* sodium citrate at pH 7, and 0.5% N-laurylsarcosine. Genomic DNA was isolated by phenolation and ethanol precipitation. Primers were designed according to the genomic sequence of bovine PP3 (12). The PCR was then carried out with the following primer pair: forward 5′-CTGGAGGCTCAGCCCACTGA-3′ (sense sequence for residues 11 to 16, LEAQPT) and reverse 5′-AGAGGGAGCTTGGGATTCTG-3′ (anti-sense sequence for residues 62 to 68, QNPKLP). The PCR

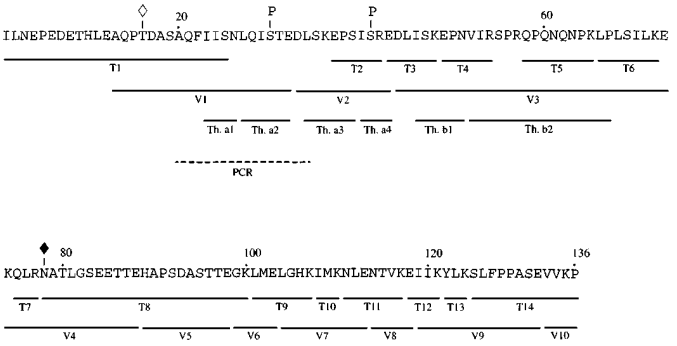

Figure 1. Primary structure of component 3 of the proteose-peptone fraction (PP3). Peptides obtained by enzymatic proteolysis are designated as follows: T = trypsin, V = *Staphylococcus aureus* V8 protease, Th.a = thermolysin subdigest of peptide T1, Th.b = thermolysin subdigest of peptide V3, PCR = sequence obtained by polymerase chain reaction analysis, and P = phosphorylations. Full glycosylation (♦); partial glycosylation (◊).

fragment was purified free of the PCR primers using a Wizard kit and yielded a band on agarose gel electrophoresis of 1.0 kb. The PCR fragment was partially sequenced with the following reverse primer: 5′-CTGGAGATGGAAGGCTCCTT-3′, corresponding to residues 37 to 42, KEPSIS (Figure 1), using a dye terminator kit (PE Applied Biosystems). Sequence analysis was performed on a genetic analyzer (ABIPRISM™ 310; PE Applied Biosystems).

RESULTS AND DISCUSSION

Sequencing Results

Aliquots of highly purified caprine PP3 were subjected to enzymatic digestion using trypsin and *Staphylococcus aureus* V8 protease, and the resulting peptides were separated on a Vydac C_{18} reverse-phase HPLC column. Fractions were sequenced and subjected to mass spectrometric analysis. The majority of the sequence was covered by these two digests (Figure 1). However, peptide V3 showed a mass of 3452.8 Da and could not be sequenced to the end. Accordingly, the peptide was subdigested with thermolysin, and the resulting peptides were used in the construction of the peptide map. Similarly, peptide T1, which represents a large N-terminal fragment of PP3, could not be sequenced to the end. The peptide, which is generated by an incomplete trypsin digestion, was subdigested with thermolysin in order to identify phosphorylation sites in the protein. The results are summarized in Table 1, and a peptide map has been constructed in Figure 1.

Examination of Table 1 shows close similarity between the obtained mass spectrometric data and the calculated mass of the individual peptides, thereby confirming the amino acid sequence presented in Figure 1. Caprine PP3 contains 136 residues as opposed to the 135 of bovine PP3 because of the insertion of a serine residue at position 25. The insertion of Ser_{25} was observed by sequencing of peptides V1 and Th.a1. In order to confirm this insertion, a PCR analysis of the relevant part of the sequence was performed. Sequencing of the obtained PCR fragment gave the sequence: 5′-CTGCTCAGTTCATCA-TCAGCAACCTCCAGATCTCCACTGAGGACCTT- 3′, which was translated into the amino acid sequence AQFIISNLQISTEDL. This analysis verified the insertion of a serine residue at position 25 in the caprine sequence.

TABLE 1. Sequence and mass spectrometric data of peptides used to construct the peptide map (Figure 1).

Residue no.[1]	Peptide[2]	Measured mass	Calculated mass
	Tryptic digest		
1–25	T1	ND[3]	
37–42	T2	688.0	687.8
43–48	T3	703.8	703.8
49–54	T4	727.4	726.8
58–65	T5	953.7	953.0
66–72	T6	783.5	783.0
75–77	T7	416.5	415.5
78–100	T8	ND	
101–107	T9	827.5	827.0
108–110	T10	ND	
111–117	T11	817.4	816.9
118–121	T12	502.1	501.6
122–124	T13	423.2	422.5
125–136	T14	1271.1	1270.5
	Staphylococcus aureus V8 protease digest		
13–32	V1	ND	
33–43	V2	1260.8	1260.4
44–73	V3	3452.8	3453.0
74–88	V4	ND	
89–98	V5	1015.2	1015.0
99–103	V6	577.3	576.7
104–113	V7	ND	
114–118	V8	ND	
119–132	V9	1606.2	1605.9
133–136	V10	442.0	441.6
	Thermolysin digest of peptide T1		
23–26	Th.a1		
27–32	Th.a2		
34–39	Th.a3		
40–43	Th.a4		
	Thermolysin digest of peptide V3		
46–51	Th.b1	687.7	686.8
52–67	Th.b2	1873.4	1872.2

[1]Residue number in the caprine sequence.

[2]Peptide number corresponding to Figure 1.

[3]No ions were detected in the mass spectra.

Localization of Phosphoserines

Bovine PP3 contains five phosphoserines. Because of the high similarity between the bovine and caprine sequences, modified residues would be expected to be found in the same positions in caprine PP3. Short-term hydrolysis of amino acids, followed by the composition analysis of amino acids, were carried out on all fractions obtained from the tryptic digest and on fractions obtained from the thermolysin subdigest of peptide T1. From the tryptic digest, peptide T1 was shown to contain phosphoserine, but the peptide was too long to allow localization directly by sequencing. Thermolysin digest of T1 and analysis of the resulting peptides revealed phosphoserine in peptides Th.a2 and Th.a4. Ethanethiol treatment of the peptides, followed by sequence analysis, showed PTH-S-ethyl cysteine at positions corresponding to Ser_{30} and Ser_{41}, thereby showing that these residues are phosphorylated. The presence of Ser_{30} was never observed directly in amino acid sequence analysis, indicating that this residue is fully phosphorylated in caprine PP3. In contrast, sequencing of the underivatized peptides V2 and T2 revealed PTH-Ser in positions corresponding to Ser_{41}, showing that this residue is only partially phosphorylated. The five phosphoserines of bovine PP3 are all located in the recognition sequence of the mammary gland casein kinase sequence; *Ser*-Xaa-Glu/Ser(P) (16). The two phosphoserines of caprine PP3 also lie within such consensus sequences [$Ser(P)_{30}$-Thr_{31}-Glu_{32} and $Ser(P)_{41}$-Arg_{42}-Glu_{43}], and the phosphorylations are likely to be catalyzed by an equivalent caprine enzyme. By comparison with bovine PP3, phosphorylations could have been expected at Ser_{35}, Ser_{39}, and Ser_{47}, which are also located in the mammary gland casein kinase recognition motif, but, during these analyses, no phosphorylations were identified at these serines.

Localization of Amino Sugars

Bovine PP3 contains three glycosylations: a partial O-linked glycosylation at Thr_{16}, an N-linked glycosylation at Asn_{77}, and a full O-linked glycosylation at Thr_{86}. From the great sequence similarity found between bovine and caprine PP3, glycosylated residues at the equivalent positions in caprine PP3 were expected, which was the case for the two glycosylations identified in caprine PP3. Sequence analysis of peptide T1 showed an unidentifiable PTH amino acid at position 16. Amino acid composition analysis following short-term hydrolysis revealed the presence of galactosamine in the peptide, indicating the presence of a glycosylated amino acid at position 16. Amino acid analysis showed that the glycosylated residue was a Thr. Upon sequencing of peptide V1, a Thr was clearly identified at position 16, showing this residue to be partially glycosylated, as was the case with the corresponding residue in bovine PP3 (23). When peptides T8 and V4 were subjected to sequence analysis, no PTH derivatives could be identified in the cycles corresponding to residue 78 in the caprine PP3 sequence. Amino acid analysis of peptide V4 showed that the peptide contained the amino sugar glucosamine, and the composition analysis showed that the peptide contained aspartic acid and asparagine. Based on these analyses, we conclude that residue 78 in caprine PP3 is an N-glycosylated asparagine. The bovine sequence contained an O-glycosylation at Thr_{86}. By sequence analysis of the peptides T8 and V4, a threonine residue was clearly identified at posi-

```
                                     ◊         20         P          P                 60
                                                .         .          .                  .
Caprine PP3           ILNEPEDETHLEAQPTDA-------SAQFIISNLQISTEDLSKEPSISREDLISKEPNVIRSPRQPQNQNPKLPLSILKE
Bovine PP3            :::K::::::::::::::-------::::R-::::N:::::::::::::::::QI::::S::::S::::::::::::
Murine GlyCAM-1       L:PGSK::LQMKT:::::IPAAQSTPTSYTS-EEST:SK::::::::F::E::::DNV::E:TK-:E::EA--------Q
Rat GlyCAM-1          AVPGSK::L::RT:::::IPASQFTPSSH:S-KEST:SK:::::SF:FN:E:V:EDNVGTE:TK-::S:EA--------Q
Camel Whey Protein                                     :NEPKDIMYM:::::::::V::DDV::K:A:RH:::::::LHPVPQ:

                          ◆ 80                 100                 120             136
                            .                   .                   .               .
Caprine PP3           KQLRNATLGSEETTEHAPSDASTTEGKLMELGHKIMKNLENTVKEIIKYLKSLFPPASEVVKP-
Bovine PP3            :H::::::::::::T::::::::::::::::::::R::::::::T::::::::SH:F::::T-
Murine GlyCAM-1       DG::SGSSQL::::RPTT:A:T:S:EN:TKSSQTVEEE:GKIIEGFVTGAEDII:G::RIT:S-
Rat GlyCAM-1          DG::SGSSQQ::::-----S:A:S::::TM:SQAVQ:E:GKVIEGF:SGVEDII:G::GT:R:-
Camel Whey Protein    SSF::TATQ::::K:LT:GA:T:L::::V::T::LI::::::MR:TMDF::::::H:::::::Q
```

Figure 2. Alignment of caprine component 3 of the proteose-peptone fraction (PP3), bovine PP3 (21), murine GlyCAM-1 (5), rat GlyCAM-1 (7), and camelid whey protein (4). Identity with the caprine PP3 sequence is denoted by two dots, and gaps in the sequences are indicated by dashes. Numbering and the indicated modifications are according to the caprine sequence. Phosphorylations are denoted P. Full glycosylation (♦); partial glycosylation (◊).

tion 87, corresponding to the bovine Thr_{86}. Therefore, we concluded that caprine PP3 is not glycosylated at Thr_{87}.

Comparison of Caprine PP3 with Homologous Proteins

An alignment of caprine PP3 with the homologous proteins—bovine PP3 (23), murine GlyCAM-1 (5), rat GlyCAM-1 (7), and camelid whey protein (4)—is shown in Figure 2. The alignment shows that caprine and bovine PP3 have identical amino acids at 88% of the positions. Caprine PP3 contains 136 residues, instead of the 135 of bovine PP3, because of the insertion of a serine residue at position 25. Interestingly, this serine insert seems to be unique for caprine PP3.

Comparison of caprine and bovine PP3 shows six substitutions in the potential C-terminal amphipathic α-helix, which has been reported in bovine PP3 (11, 13). In the homologous GlyCAM-1, the helix has been suggested as being used in adhesion of the protein to the cell membrane (14), and a similar role has been suggested for the attachment of PP3 to the milk fat globule membrane (24). In the bovine system, the amphipathic α-helix has also been suggested as a means of PP3 polymer formation (24). The substitution from a bovine threonine to a caprine isoleucine at position 119 would increase the hydrophobicity. Three proline substitutions occur at positions 128, 129, and 136. The former two prolines might affect the helical structure by introducing bends.

ACKNOWLEDGMENTS

The authors thank Roy E. Guldberg for excellent technical assistance. This work is part of the FØTEK programme supported by the Danish Dairy Research Foundation and the Danish Government.

REFERENCES

1 Alichanidis, E., and A. M. Michaelidou. 1990. Glycoproteins in the heat- and acid-stable fraction of ovine milk. J. Dairy Res. 57:507–515.
2 Anderson, M. 1981. Inhibition and lipolysis in bovine milk by proteose peptone. J. Dairy Res. 48:247–252.
3 Andrews, A. T. 1978. The composition, structure and origin of proteose-peptone component 5 of bovine milk. Eur. J. Biochem. 90:59–65.
4 Beg, O. U., H. von Bahr-Lindström, Z. H. Zaidi, and H. Jörnvall. 1987. Characterisation of a heterogeneous camel milk whey non-casein protein. FEBS Lett. 216:270–274.
5 Dowbenko, D., A. Andalibi, P. E. Young, A. J. Lusis, and L. A. Lasky. 1993. Structure and chromosomal localization of the murine gene encoding GlyCAM 1. J. Biol. Chem. 268: 4525–4529.
6 Dowbenko, D., A. Kikuta, C. Fennie, N. Gillett, and L. A. Lasky. 1993. Glycosylation-dependent Cell Adhesion Molecule 1 (GlyCam-1) mucin is expressed by lactating mammary gland epithelial cells and is present in milk. J. Clin. Invest. 92: 952–960.
7 Dowbenko, D., S. R. Watson, and L. A. Lasky. 1993. Cloning of a rat homologue of mouse GlyCAM 1 reveals conservation of structural domains. J. Biol. Chem. 268:14399–14403.
8 Girardet, J. M., B. Coddeville, Y. Plancke, G. Strecker, S. Campagna, G. Spik, and G. Linden. 1995. Structure of glycopeptides isolated from bovine milk component PP3. Eur. J. Biochem. 234:939–946.
9 Girardet, J. M., and G. Linden. 1996. PP3 component of bovine milk: a phosphorylated whey glycoprotein. J. Dairy Res. 63: 333–350.
10 Girardet, J. M., G. Linden, S. Loye, J. L. Courthaudon, and D. Lorient. 1993. Study of mechanism of lipolysis inhibition by bovine milk proteose-peptone component 3. J. Dairy Sci. 76: 2156–2163.
11 Groenen, M.A.M., R.J.M. Dijkhof, and J. J. van der Poel. 1995. Characterisation of a GlyCAM 1-like gene (glycosylation-dependent cell adhesion molecule 1) which is highly and specifically expressed in the lactating bovine mammary gland. Gene 158:189–195.
12 Johnsen, L. B., T. E. Petersen, and L. Berglund. 1996. The bovine PP3 gene is homologous to the murine GlyCAM 1 gene. Gene 169:297–298.
13 Johnsen, L. B., E. S. Sørensen, T. E. Petersen, and L. Berglund. 1995. Characterization of a bovine mammary gland PP3 cDNA reveals homology with mouse and rat adhesion molecule GlyCAM-1. Biochim. Biophys. Acta 1260:116–118.
14 Lasky, L. A., M. S. Singer, D. Dowbenko, Y. Imai, W. J. Henzel, C. Grimley, C. Fennie, N. Gillett, S. R. Watson, and S. D. Rosen. 1992. An endothelial ligand for L-selectin is a novel mucin-like molecule. Cell 69:927–938.
15 Mati, A., F. Moulti-Mati, J. M. Girardet, E. Fokou, F. Belleville-Nabet, P. Nabet, and G. Linden. 1993. Mitogenic activity of hydrophobic fractions of proteose peptone from cows', ewes' and goats' milk measured with MARK 3 hybridoma culture. J. Dairy Res. 60:442–448.
16 Mercier, J. C. 1981. Phosphorylation of caseins, present evidence for an amino acid triplet code posttranslationally recognized by specific kinases. Biochemie (Paris) 63:1–17.
17 Meyer, H. E., E. Hoffmann-Posorske, H. Korte, and M. G. Heilmeyer, Jr. 1986. Sequence analysis of phosphoserine-containing peptides. Modifications for picomolar sensitivity. FEBS Lett. 204:61–66.
18 Ramos, M., R. M. Sánchez, A. Olano, J. Sanz, and I. Martinez-Castro. 1988. Comparative studies on "acid-stable, heat-stable polypeptides" of ovine, caprine and bovine milks. Z. Lebensm. Unters. Forsch. 186:22–24.
19 Shimizu, M., K. Yaumauchi, and M. Saito. 1989. Emulsifying properties of the proteose peptone fraction obtained from bovine milk. Milchwissenschaft 44:497–500.
20 Sundheim, G., and G. Bengtsson-Olivecrona. 1987. Methods to assess the propensity of milk fat globules toward lipolysis and the ability of skim milk to inhibit lipolysis. J. Dairy Sci. 70: 2040–2045.
21 Sørensen. E. S., P. Højrup, and T. E. Petersen. 1995. Posttranslational modifications of bovine osteopontin: identification of twenty-eight phosphorylation and three O-glycosylation sites. Protein Sci. 4:2040–2049.
22 Sørensen, E. S., and T. E. Petersen. 1993. Purification and characterization of three proteins isolated from the proteose peptone fraction of bovine milk. J. Dairy Res. 60:189–197.
23 Sørensen, E. S., and T. E. Petersen. 1993. Phosphorylation, glycosylation and amino acid sequence of component PP3 from the proteose peptone fraction of bovine milk. J. Dairy Res. 60: 535–542.
24 Sørensen, E. S., L. K. Rasmussen, L. Møller, and T. E. Petersen. 1997. The localization and multimeric nature of component PP3 in bovine milk: Purification and characterization of PP3 from caprine and ovine milk. J. Dairy Sci. 80:3176–3181.