Afslutningsrapport

Struktur af kaseinmiceller

Proteiner i fedtkuglemembranen

Valleproteiner

Proteolytiske enzymer i mælk

Mejeribrugets ForskningsFond

Rapport nr. 1996-10

Juni 1996

mejeriforeningen
danish dairy board

PREFACE

Etableringen af Laboratorium for Proteinkemi under Aarhus Universitet er en direkte følge af FØTEK-lovgivningen og en positiv indstilling fra Danske Mejeriers Fællesorganisation samt en række ministerier og forskningsråd. Det er ikke almindeligt, at man som offentlig ansat forsker får mulighed for at deltage i opbygningen af et helt nyt laboratorium med budgetter i millionklassen. Det har været en spændende og interessant udfordring at starte med et stort tomt rum og ende med et laboratorium, hvor omkring tyve personer har deres daglige arbejde med udførelse af moderne molekylærbiologiske teknikker og tilhørende betjening af kompliceret apparatur.

Denne rapport afviger fra normale afslutningsrapporter under Mejeribrugets ForskningsFond ved hovedsageligt at fungere som en oversigt over de videnskabelige artikler, som vi har offentliggjort indenfor emnet "Mælkeproteiners molekylærbiologi". Herunder er inkluderer enkelte artikler, der er udarbejdet før FØTEK-perioden, men de er medtaget her for af give et samlet billede af det forskningsarbejde, der er udført indenfor området. De forskellige bevillingsgivere er anført under de respektive artikler i oversigten. Denne rapport er udarbejdet dels som en afslutningsrapport for de fire FØTEK I-projekter, dels med henblik på dokumentation i forbindelse med den forestående midtvejsevaluering af FØTEK-programmet.

- - - - - - - - - - - - - - - -

The establishment of the Protein Chemistry Laboratory at the University of Aarhus was a consequence of the Government's research and development programme for food technology (the FØTEK programme) and a positive point of view from the Danish Dairy Board, ministries, and research councils. It is unusual as a publicly employed scientist to get the possibility to participate in the establishment of a new laboratory with budgets counted in millions. It has been an exciting and interesting challenge to start with one big empty room and end up with a laboratory, in which about twenty people can work daily with modern molecular biological techniques and operate advanced, complicated equipment.

This report deviates somewhat from ordinary final reports for projects under the Danish Dairy Research Foundation (MFF) by mainly being an overview of these articles, which have been published on the subject "Molecular Biology of Milk Proteins". The overview does, however, include a few articles that were published before the FØTEK programme. They have been included to give a complete picture of the research that has been carried out on the subject. The different sponsors are named in the articles in the overview. The report has been prepared partly as a closing report for the four local FØTEK projects, partly as full documentation in connection with the current midterm evaluation of the FØTEK research programme.

Aarhus, January 6, 1997

Torben Ellebæk Petersen

BACKGROUND

With the Danish Government's initiative to promote research and development in food technology, named the FØTEK programme, it became attractive to apply for funds to finance research within food science.

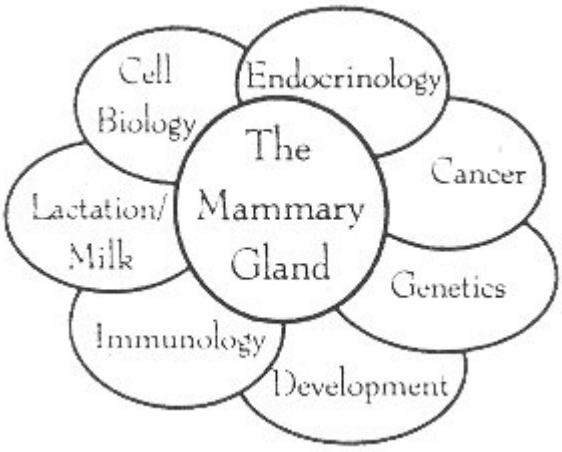
In collaboration with the Danish Dairy Board and the University of Aarhus an application was submitted to establish a laboratory dedicated to study the molecular biology of milk proteins. In the beginning of 1992 the funds were granted for four FØTEK 1 projects. As the space available in the building of Molecular Biology was insufficient to hold the projects, a floor in the Science Park Aarhus was rented and furnished with laboratory facilities, and in September 1992 Protein Chemistry Laboratory was inaugurated.

From the beginning it was agreed that the work should be basic research and not development of new dairy products. The results should be made available for the Danish dairy industry as well as published in international journals. The work was planned for four years and divided into the following projects: "Structure of the casein micelle", "Proteins of the fat globule membrane", "Whey proteins", and "Proteolytic enzymes in milk". The last of the four projects was not initiated until 1994 as it was a continuation of another project, called the "Plasmin project", which expired in 1993.

When looking at the literature published in the eighties it became clear that the revolution in molecular biology techniques had not been fully implemented within the area of dairy research. The reason for this is most probably due to tradition as the effort has for a long time been focused more on applications than on basic research. Nevertheless, with the high degree of complexity of milk a more thorough knowledge of this physiological fluid would most likely be of benefit not only to the dairy industry but also to the consumer.

From a biological point of view, milk is not only a highly appreciated food ingredient, but also a fundamental substance necessary for the survival of the newborn. The mammals constitute more than 4,000 different species, which by definition produce milk. This group of animals has been able to inhabit most parts of the earth and represent sophisticated living creatures ranging from tiny mice to heavy weight whales. This biological diversity is also reflected in the milk, where the caseins represent one of the fastest diverging protein families.

When working with the molecular biology of milk proteins one will often have to ask questions about the organ which synthesizes the proteins. Increasingly, the mammary gland has been the subject of investigation because of its involvement in so many physiological processes. In the newly established magazine, "Journal of MAMMARY GLAND BIOLOGY and NEOPLASIA", the numerous areas of research linked to the gland have been illustrated by the following figure



PROJECT DESCRIPTIONS

Structure of the casein micelle

Of the four bovine caseins only two contain cysteines, namely α_{s2}-casein and κ-casein, which means that only these two proteins have the possibility of forming disulphide bonds. In the past, most purification methods of the caseins were performed after reduction of the disulphide bonds. Therefore little information has been available regarding the organization of the bridges. Our first work was therefore to isolate the caseins with intact disulphide bonds and characterize their organization. It was shown that the two cysteines in κ-casein were linked in a random fashion so κ-casein formed a multimer of up to 15 chains. Most likely, such a network of κ-casein could be of importance for the stability of the casein micelle.

Similarly, the disulphide bonds in α_{s2}-casein were identified and it was shown that dimers could be formed with both configurations of the sulphide bonds. No disulphide bonds between α_{s2}-casein and κ-casein were identified. Papers 1-5 describe this work.

In the sequence of human κ-casein only one cysteine is present, showing that it could not form multimers through disulphide bonds. Nevertheless, it was found that human κ-casein was present in high molecular complexes. Further investigation identified a new human casein corresponding to α_{s1}-type of other species and this protein chain had three cysteines. Therefore, in the present model of human casein micelles, α_{sl}-casein forms a network to which κ-casein is bound through a single disulphide bond (papers 6-7).

Cross-linking of food proteins is a subject of general interest. One way to form covalent bonds between proteins is by reaction with transglutaminases. This group of enzymes form a bond between the side chain of lysines and the side chain of glutamines. The caseins have for a long time been known as good substrates for transglutaminases. We have therefore begun an investigation of the exact positions in the caseins which can form cross-links (paper 8).

Casein micelles are in general difficult to study because they are large heterogeneous particles of four proteins in a complex with inorganic calcium phosphate. Solid phase NMR spectroscopy is a technique which had not been applied in the studies of the micelle. The advantage of this method is that it can be used with any solid matter which can be filled into the NMR-tube and withstand the centrifugation force generated when the tube is rotated in the magnetic field. As a first step we have obtained ^{31}P spectra of native casein micelles and identified which signals originate from inorganic phosphate and which from the phosphate covalently bound to the protein chains (papers 9-10). Paper number 11 is a summary in Danish of the work performed on the caseins.

Proteins of the fat globule membrane

As the proteins of the fat globule membrane are relatively less characterized than the other major milk proteins, our strategy has been first to isolate the proteins then to clone the corresponding cDNA before we investigate their functional characteristics. Three of the membrane associated proteins have been treated in this way, namely xanthine oxidase, PAS 6/7. and CD36 (papers 12-15)

Xanthine oxidase is a membrane associated enzyme, which can transfer electrons from an organic substrate to NAD+ or molecular oxygen. The native state prefers NAD+ but by a number of reactions xanthine oxidase can undergo a transformation to prefer molecular oxygen instead. Limited proteolysis of xanthine oxidase is an example of such a reaction. Based on the knowledge of the exact protein sequence we are at present investigating this transformation in molecular detail. The reaction is not only

of academic interest because when oxygen accepts an electron it becomes the highly reactive superoxide radical which can attack a number of molecules. The extent to which xanthine oxidase is involved in the formation of unpleasant flavour in milk products is not clear, but a more precise characterization of xanthine oxidase and the reaction catalyzed by this enzyme will be helpful in elucidating this aspect.

PAS-6/7 is a protein associated with the outer membrane in a rather loose way as it is easily extracted. It is present in two forms as a result of different glycosylation. The function of the protein is not clear but it is present in many tissues other than the mammary gland. At present we are investigating its binding to phospholipids and other proteins.

CD36 is a membrane protein originally identified in blood platelets and later in a number of other tissues. In connection with the fat globule membrane it is often named PAS-4. It binds strongly to the membranes and is not easy to purify. It is heavily modified with carbohydrate and fatty acids. The location and compositions of the carbohydrate groups were determined during the characterization of the proteins.

Vitamin B12 is a valuable nutrient of milk, where it is present in a complex with a protein. We have isolated the protein and characterized it as transcobalamin. Our preparation was well suited for analyzing the binding characteristics of B12 to the protein (papers 16-17). A Danish summary regarding the proteins of the fat globule membrane has been published (paper 18).

VVhey proteins

Whey contains many proteins of which the major ones have been extensively characterized. We have isolated two (new) proteins, PP3 and osteopontin, from the proteose peptone fraction of bovine milk (papers 19-27). Both proteins are phosphorylated and especially osteopontin is rich in phosphate as it contains 27 serine phosphates and 1 threonine phosphate. Osteopontin is a protein of great interest, as it has been linked to a number of physiological reactions especially in connection with calcification. The protein was originally identified in the organic matrix of bones and later in most tissues. It has been difficult to obtain enough of the protein for research purpose, but the discovery of the protein as a component in milk has changed this, and we have sent a number of samples to groups around the world. A Danish summary has been published (paper 28).

When we isolate specific proteins from milk, an unknown peak in a chromatogram occasionally appears. In one event it turned out to be an unknown protein, which we have named EPV20. The protein has been sequenced and a cDNA-clone characterized (paper 29).

Proteolytic enzymes in bovine milk

Our investigation of proteolytic enzymes in milk started before the FØTEK programme was initiated. In the "Plasmin project" we isolated plasminogen and plasmin from milk and characterized plasminogen activators (papers 30-34). The research was continued under the FØTEK-programme (papers 35-36). The major work, namely synthesis of bovine tissue plasminogen activator in a yeast expression system, has not yet been published but a manuscript is in preparation.

Procathepsin D is another proteolytic enzyme in milk, which we have isolated and characterized. It was interesting to discover that the active form cathepsin D is capable of coagulating milk although the concentration should be about ten times higher than the one present in standard milk (papers 37-39).

ORGANIZATION OF THE PROTEIN CHEMISTRY LABORATORY

The laboratory is a part of the Department of Molecular and Structural Biology at the Science Faculty. The research grants dedicated to our work on milk proteins are so-called FIK-funds (research with no commercial activities). The grants are given to the university and administered by the laboratory. The accounts are made by the central administration of the university. The salary of employees is university standard.

As part of the university we have been teaching in the master of science programme for students of biology and molecular biology. This also includes students in their last year (masters thesis) and ph.d. students. Titles of their theses are listed separately.

COMMUNICATION OF RESULTS TO THE DAIRY INDUSTRY

As most of our grants are obtained for projects performed in collaboration with the Danish Dairy Board, emphasis has been put on the communication of the results to the dairy industry. This is done by holding a meeting twice a year, where the last results are presented for a committee appointed by the Danish Dairy Board. Later, when the results are ready for publication, the manuscripts are presented to the chairman of the committee. Furthermore, our results have been presented at a number of meetings arranged either by the Danish Dairy Board or by LMC (Centre for Advanced Food Studies) at The Royal Veterinary and Agricultural University, Copenhagen.

THE FØTEK 2 PROGRAMME

The continuation of the FØTEK programme in FØTEK 2 made it possible to apply for further research funds for our work on milk proteins. Again in collaboration with the Danish Dairy Board. three projects have been granted for the period July 1996 until June 1999. The three projects are:

a) Whey proteins and casein micelles,
b) Proteins of the fat globule membrane, and
c) Proteolytic enzymes and bioactive peptides in milk.

From The Danish Research Councils (FELFO) we have obtained support for the project

Molecular biology of milk proteins.

which has allowed us to purchase advanced equipment for protein purification and DNA sequencing. In autumn 1996 a group of four people was appointed by The Danish Research Councils to follow our work. Two from this group are from the industry and the other two from the universities. We have not yet had the opportunity to discuss the project, but it will probably be arranged in spring 1997. In conclusion, with the FØTEK 2 projects we have obtained support for continuation of the basic research of milk proteins, which was initiated in 1992.

LIST OF EMPLOYEES Date of employment

Head of laborarory

Torben Ellebæk Petersen, Assoc.Prof.	Science Faculty

Academic staff

Mads Bak, MSc	15.09.96
Lars Erik Berglund, PhD	01.08.92
Serguei Nikolaevich Fedossov, PhD	01.09.93
Christian Würtz Heegaard, PhD	01.02.96
Jane Hvarregaard, MSc	01.10.95-31.12.95
Laust Bruun Johnsen, MSc	01.03.95
Lotte Bach Larsen, PhD	01.01.94-31.12.95
Lone Kjær Rasmussen, PhD	01.07.92
Jan Trige Rasmussen, PhD	01.01.93
Peter Ravn, engineer	01.11.92-31.01.95
Charlotte Brandt Sørensen. MSc	01.01.96-30.06.96
Esben Skipper Sørensen, PhD	01.03.93
Jens Kaalby Thomsen. MSc	01.01.95-31.08.96

Technicians

Marian Dyrberg Andersen	01.10.92
Anni Boisen	01.01.94
Hanne Aude Due	01.09.02-30.06.96
Lise Møller	01.08.92
Margit Skriver Rasmussen	Science Faculty
Mitra Shamsali, apprentice	01.08.96
Dorte Simonsen	16.01.95-30.06.95
Maria Vinther	01.09.95

Administrative staff

Gert Johansen Almind, secretary	01.10.92
Roy Erwin Guldberg, engineer	01.04.94
Gorm Nørgaard Pedersen, engineer	01.01.94-28.02.94

Current MSc students

Mikkel Holmen Andersen
Maiken Borup-Mikkelsen
Ida Margaret Bonnevie Lister (ERASMUS-student). Imperial College, London

Current PhD students

Connie Benfeldt. MD Foods Research and Development Centre, Brabrand
Laust Bruun Johnsen

MSc AND PhD THESES

MSc theses

Christensen, B.M. (1995). Localization of transglutaminase-reactive glutamine residues in bovine caseins. Cand.scient.-thesis, University of Aarhus, November (pp. 1-71).

Hvarregaard, J. (1995). Isolation and characterization of PAS-6 and PAS-7 major glycoproteins of bovine milk fat globule membrane. Cand.scient.-thesis, University of Aarhus, August (pp. 1-87).

Johnsen. LB. (1995). Genomic and cDNA cloning of two milk protein genes: Bovine PP3 and human asr-casein. Cand.scient.-thesis, University of Aarhus, March (pp. 1-79).

PhD theses

Larsen, L.B. (1995). Zymogens for proteolytic enzymes in milk: procathepsin D and plasminogen. Ph.D.-thesis, University of Aarhus, February (pp. 1-207).

Sørensen, E. S. (1995). Osteopontin and component PP3: characterization of two multiphosphorylated proteins from bovine milk. Ph.D.-thesis, University of Aarhus, January (pp. 2-111).

FUNDING x 1,000

FØTEK 1 July 1992 - June 1996 *(Actual costs)*

50% funded by the Danish Dairy Board, and 50% funded by the Ministry of Agriculture, the Ministry of Industry, and The Danish Research Councils.

The Structure of the Casein Micelle	
Salaries	3,212
Equipment	485
Running costs	2,527
Proteins of the Milk Fat Globule Membrane	
Salaries	5,426
Equipment	1,333
Running costs	3,228
Whey Proteins	
Salaries	2,252
Equipment	450
Running costs	1,617
Proteolytic Enzymes in Milk (January 1994 - June 1996)	
Salaries	2,641
Equipment	56
Running costs	1,611

FØTEK 2 July 1996 - June 1999 *(Budget figures)*

50% funded by the Danish Dairy Board, and 50% funded by the Ministry of Agriculture and The Danish Research Councils.

Structure of the Casein Micelle and Whey Proteins	
Salaries	2,801
Equipment	177
Running costs	1,327
Proteins of the Milk Fat Globule Membrane	
Salaries	2,801
Equipment	177
Running costs	1,147
Proteolytic Enzymes and Bioactiye Peptides in Milk	
Salaries	3,126
Equipment	177
Running costs	1,572

FELFO July 1996 - June 1998 *(Budget figures)* x 1.000

100 % funded by The Danish Research Councils

Milk Proteins, core unit	
Salaries	1,375
Equipment	1,165
Running costs	1,660

(All figures in Danish currency)

EQUIPMENT AND OTHER FACILITIES

The laboratory has at the moment a total of 562.2 m^2 available at the Science Park Aarhus. Laboratories and offices are 288.6 m^2 and 155.4 m^2, respectively, and available store rooms in the basement are a total of 118.2 m^2.

The following equipment purchased during the FØTEK 1 research programme:

Camspec M350 Double Beam Spectrophotometer with extras

New Brunswick Innova 4000 Incubator Shaker

New Brunswick Innova 4300 Incubator Shaker

Bruker Biflex Time-of-Flight Mass Spectrometer

Struers EL 311S Mikroplate AutoReader

Dionex PED-2 Pulsed Electrochemical Detector with extras

Spectra-Physics Integrator SP4400 with extras

Spectra-Physics P200/AS300 Amino Acid Analyzer

Pharmacia/LKB HPLC equipment

The following equipment purchased on basis of other funding:

Applied Biosystems 477 A Protein Sequencer with 120A Analyzer

Hewlett-Packard AminoQuant/Fluorescence Detector

The following equipment purchased recently on basis of the FELFO core unit (FØTEK 2 programme) :

Pharmacia-Biotech SMART Micro HPLC System

Perkin Elmer ABI Prism 310 Genetic Analyzer with extras

Server and updated software

Fermentation system *(to be delivered)*

In addition the laboratory has various high-speed and preparative centrifuges plus standard protein and DNA equipment.

CONGRESS **PARTICIPATION**

Cologne Spring Meeting, Protein-DNA Recognition and Gene Control, Cologne, Germany, February 24-26, 1993.

Gordon Research Conference on Mammary Gland Biology, New London, New Hampshire, USA, June 20-25, 1993.

7th Protein Society Symposium, San Diego, Califomien, USA, July 23-28, 1993.

Cold Spring Harbor Meeting, Cancer Cells: Regulation of Eukaryotic mRNA Transcription, Cold Spring Harbor, New York, USA, September 1-5, 1993.

5th International Conference on Aspartic Proteinases, Nagoya, Japan, September 19-24, 1993.

2nd IUBMB Conference, Biochemistry of Cell Membranes, Bari, Italy, September 29 - October 3, 1993.

Fall Meeting of the Mass Spectrometry Club of the Swedish Chemical Society, Stockholm, Sweden, November 22-23, 1993.

FEBS '94 Congress, Helsinki, Finland, June 26-30, 1994.

10th International Conference on Methods in Protein Structure Analysis, Snowbird, Utah, USA, September 8-13, 1994.

Osteopontin: Role in Cell Signalling and Adhesion. The New York Academy of Sciences, New Brunswick, New Jersey, USA, October 21-23, 1994.

The XXIVth European Symposium on Calcified Tissue, Aarhus, May 27-30, 1995.

Symposium on Ripening and Quality of Cheeses, Besan<;on, France, February 26-28, 1996.

Current Topics in Gene Expression Systems, 1996 Pichia pastoris, San Diego, Californien, USA, March 3-6, 1996.

XIIIlth International Congress on Fibrinolysis and Thrombolysis, Barcelona, Spain, June 24-28, 1996.

12th International Symposium of Flavins and Flavoproteins, Calgary, Canada, June 30 - July 6, 1996.

Tenth Annual Symposium of the Protein Society, San Jose, Californien, USA, August 3-7, 1996.

Third joint EAAP/ASAS Workshop on the Biology of Lactation in Farm Animals, Lillehammer. Norway, August 24-25, 1996.

4th European Symposium on Vitamin B12 and B12-Proteins, Innsbruck, Austria, September 2-6, 1996.

PUBLICATIONS

1. Rasmussen. LK., and Petersen, T.E. (1991). Purification of disulphide-linked α_{s2}- and κ-casein from bovine milk. J. Dairy Res. 58, 187-193.

2. Rasmussen, LK., Højrup, P., and Petersen, T.E. (1992). Localization of two interchain disulfide bridges in dimers of bovine α_{s2}-casein. Parallel and antiparallel alignments of the polypeptide chains. Eur. J. Biochem. 203, 381-386.

3. Rasmussen, LK., Højrup, P., and Petersen, T .E. (1992). The multimeric structure and disulfide-bonding pattern of bovine κ-casein. Eur. J. Biochem. 207, 215-222.

4. Rasmussen, LK., Højrup, P., and Petersen, T.E. (1994). Disulphide arrangement in bovine caseins: localization of intrachain disulphide bridges in monomers of κ- and α_{s2}-casein from bovine milk. J. Dairy Res. 61, 485-493.

5. Rasmussen, LK., and Petersen, T.E. (1993). Characterization of disulfide-linked caseins from bovine milk. In: Proceedings of IDF Seminar, Munich, August 25-28, 1992, Protein and fat globule modifications by heat treatment, homogenization and other technological means for high quality dairy products, IDF Special Issue 9303, pp. 88-95, International Dairy Federation. 41 Square Vergote, B-I040 Brussels, Belgium.

6. Rasmussen, LK., Due, H.A., and Petersen, T.E. (1995). Human α_{s1}-casein: purification and characterization. Comp. Biochem. Physiol 111B, 75-81.

7. Johnsen, LB.. Rasmussen, LK., Petersen, T.E., and Berglund, L (1995). Characterization of three types of human α_{sl}-casein mRNA transcripts. Biochem J. 309, 237-242.

8. Christensen, B.M., Sørensen, E.S., Højrup , P., Petersen, T.E., and Rasmussen, LK. (1996). Localization of potential transglutaminase cross-linking sites in bovine caseins. J. Agric. Food Chem. 44, 1943-1947.

9. Thomsen, J.K., Jakobsen, H.J., Nielsen, N.C., Petersen, T.E., and Rasmussen. LK. (1995). Solid-state magicangle spinning ^{31}P-NMR studies of native casein micelles. Eur. J. Biochem. 230,454-459.

10. Rasmussen, LK., Sørensen, E.S., Petersen, T.E., Nielsen, N.C. and Thomsen, J.K. (1997). Characterization of phosphate sites in native ovine, caprine, and bovine casein micelles and their caseinomacropeptides: a solidstate phosphorus-3l nuclear magnetic resonance and sequence and mass spectrometric study. J. Dairy Res., *in press.*

11. Rasmussen, LK., and Petersen, T.E. (1996). Kaseinmicellen - et unikt arrangement af proteiner og kalciumfosfat. Mælkeritidende 19, 426-427.

12. Berglund, L, Rasmussen, J.T., Andersen, M.D., Rasmussen, MS, and Petersen, T.E. (1996). Purification of the bovine xanthine oxidoreductase from milk fat globule membranes and cloning of complementary deoxyribonucleic acid. J. Dairy Sci. 79, 198-204.

13. Kristensen, D., Nylander. T., Rasmussen, J.T., Paulsson, M., and Carisson, A. (1996). Bovine milk sphingomyelin at the air/water interface and its interaction with xanthine oxidase. Langmuir, *in press*.

14. Hvarregaard, J., Andersen, M.H., Berglund, L, Rasmussen, LT., and Petersen, T.E. (1996). Characterization of glycoprotein PAS-6/7 from membranes of bovine milk fat globules. Eur. J. Biochem. 240, 628-636.

15. Berglund, L, Petersen, T.E., and Rasmussen, J.T. (1996). Structural characterization of bovine CD36 from the milk fat globule membrane. Biochim. Biophys. Acta 1309, 63-68.

16. Fedosov, S.N., Petersen, T.E., and New, E. (1996). Transcobalamin from cow milk: isolation and physicochemical properties. Biochim. Biophys. Acta 1292, 113-119.

17. Fedosov. S.N., Petersen, T.E., and Nexø, E. (1995). Binding of cobalamin and cobinamide to transcobalamin from bovine milk. Biochemistry 49, 16082-16087.

18. Rasmussen, J. T., Berglund, L, and Petersen, T.E. (1996). En kompleks membran omgiver mælkens fedtkugler. Mælkeritidende 19,437-439.

19. Sørensen, E.S., and Petersen, T.E. (1993). Purification and characterization of three proteins isolated from the proteose peptone fraction of bovine milk. J. Dairy Res. 60, 189-197.

20. Sørensen, E.S.. and Petersen. T.E. (1993). Phosphorylation, glycosylation and amino acid sequence of component PP3 from the proteose peptone fraction of bovine milk. J. Dairy Res. 60, 535-542.

21. Johnsen. LB., Sørensen. E.S., Petersen, T.E., and Berglund, L (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.

22. Johnsen, LB., Petersen, T .E., and Berglund, L (1996). The bovine PP3 gene is homologous to the murine GlyCAM-1 gene. Gene 169,297-198.

23. Sørensen, E.S., Rasmussen, LK., Møller, L, Jensen, P.H., Højrup, P., and Petersen, T.E. (1994). Localization of transglutaminase-reactive glutamine residues in bovine osteopontin. Biochem. J. 304, 13-16.

24. Sørensen, E.S., and Petersen, T.E. (1994). Identification of two phosphorylation motifs in bovine osteopontin. Biochem. Biophys. Res. Commun. 198, 200-205.

25. Sørensen, E.S., Højrup, P., and Petersen, T.E. (1995) Posttranslational modifications of bovine osteopontin: identification of twenty-eight phosphorylation- and three *O*-glycosylation sites. Protein Sci. 4, 2040-2049.

26. Sørensen, E. S., and Petersen. T.E. (1995). Phosphorylation-, glycosylation- and transglutaminase sites in bovine osteopontin. Ann. N.Y. Acad. Sci. 760, 363-366.

27. Sørensen, E.S., Rasmussen, LK., Højrup, P., and Petersen, T.E. (1995). Localization of *in vivo* phosphorylation sites in multiphosphorylated proteins: application of S-ethylcysteine derivatization and mass spectrometric analysis. In: Methods in Protein Structure Analysis (eds. Atassi, M.Z., and Appella, E.), pp. 217-225, Plenum Press, New York.

28. Sørensen. E.S., and Petersen, T.E. (1996). Proteose-pepton fraktionen fra mælk indeholder hidtil ubeskrevne proteiner. Mælkeritidende 21, 531-533.

29. Larsen, LB., Ravn, P., Boisen, A., Berglund, L, and Petersen, T.E. (1996). Primary structure of EPV20, a secretory glycoprotein containing a previously uncharacterized type of domain. Eur. J. Biochem., *in press.*

30. Benfeldt, C., Larsen, LB., Rasmussen, J.T., Andreasen, P.A., and Petersen, T.E. (1995). Isolation and characterization of plasminogen and plasmin from bovine milk. Int. Dairy J. 5, 577-592.

31. Berglund, L., Andersen, M.D., and Petersen, T.E. (1995). Cloning and characterization of the bovine plasminogen cDNA. Int. Dairy J. 5, 593-603.

32. Heegaard, C.W., Rasmussen, LK., and Andreasen, P.A. (1994). The plasminogen activation system in bovine milk: Differential localization of tissue-type plasminogen activator and urokinase in milk fractions is caused by binding to casein and urokinase receptor. Biochim. Biophys. Acta. 1222, 45-55.

33. Heegaard, C.W., Christensen, T., Rasmussen, M.D., Benfeldt, C., Jensen, N.E., Sejrsen, K., Petersen, T.E., and Andreasen, P.A. (1994). Plasminogen activators in bovine milk during mastitis, an inflammatory disease. Fibrinolysis 8, 22-30.

34. Ravn. P., Berglund, L. and Petersen, T.E. (1995). Cloning and charcacterization of the bovine plasminogen activators uPA and tPA. Int. Dairy J. 5, 605-617.

35. Heegaard, C.W., Andreasen, P.A., Petersen, T.E., and Rasmussen, LK. (1997). Binding of plasminogen and tissue-type plasminogen activator to dimeric α_{s2}-casein accelerates plasmin generation. Fibrinolysis, *in press.*

36. Heegaard. C.W., Larsen, LB., Rasmussen, LK., Højberg, K.-E., Petersen, T.E., and Andreasen, P.A. (1997). The plasminogen activation system in human milk. J. Pediatr. Gastroenterol. Nutr., *in press.*

37. Larsen, LB., Boisen, A., and Petersen, T.E. (1993). Procathepsin D cannot autoactivate to cathepsin D at acid pH. FEBS Lett. 319, 54-58.

38. Larsen, LB., and Petersen, T. E. (1995). Identification of five molecular forms of cathepsin D in bovine milk. In: Aspartic proteinases: Structure, function, biology and biomedical implications (ed. Takahashi, K.). AEMB Vol. 362, pp. 279-283, Plenum Press, New York.

39. Larsen, LB., Benfeldt, C., Rasmussen, LK., and Petersen, T.E. (1996). Bovine milk procathepsin D and cathepsin D: coagulation and milk protein degradation. 1. Dairy Res. 63, 119-130.

OTHER PUBLICATIONS

Heegaard. C.W., White, J.H., Zavizion, B., Turner, J.D., and Politis, 1. (1994). Production of various forms of plasminogen activator and plasminogen activator inhibitor by cultured mammary epithelial cells. J. Dairy Sci. 77, 2949-2958.

Rasmussen, J.T., Berglund, L, and Petersen, T.E. (1997). Structural characterization of bovine xanthine oxidoreductase from the milk fat globule membrane. In: Flavins and Flavoproteins 1996 (eds. Stevenson, K.J., Massey, V., and Williams Jr., C.H.), pp. xx-xx, University of Calgary Press, Calgary, Canada, *in press.*

Rasmussen, LK., Larsen, LB., Johnsen, LB., Andreasen, P.A., Petersen, T.E., and Heegaard, C.W. (1996). Casein, a novel matrix for tPA-catalyzed plasminogen activation. Fibrinolysis 10, Suppl. 3, 31 *(abstract).*

Sørensen, E.S., Rasmussen, LK., Jensen, P.H., Højrup, J., and Petersen, T.E. (1994). Identification of posttranslational modifications in bovine osteopontin: localization of 28 phosphorylation sites, 3-*O*-g1ycosylation sites and 2 transglutaminase reactive glutamines. J. Protein Chem. 13,539 *(abstract).*

Sørensen, E.S., and Petersen, T.E. (1995). Localization of posttranslational modifications in bovine osteopontin. Calc. Tissue Int. 56, 465 *(abstract).*

Sørensen, E.S., and Petersen, T .E. (1993). Phosphoproteins. Protein Sci. 2, Suppl. 1, 111 *(abstract).*

Zavizion. B.. Heegaard. C.W.. White, J.. Cheli, F., and Politis, L (1996). Synthesis of plasminogen activator inhibitor I by bovine mammary epithelial and myoepithelial cell lines. J. Dairy Res. 63, 451-458.

Mejeriforeningen • Danish Dairy Board • Frederiks Allé 22 • DK-8000 Århus C
Telefon +45 8731 2000 • Fax +45 8731 2001 • ddb@mejeri.dk • www.mejeri.dk