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**Downstream Purification and Analysis  
of the Recombinant Human Myelin Basic Protein  
Produced in the Milk of Transgenic Cows**

**A thesis presented in partial fulfillment of the requirements for the degree of**

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## Abstract

Downstream purification and analysis of a model biopharmaceutical protein (recombinant human myelin basic protein) is described. The recombinant protein was expressed in the milk of transgenic cows and was found exclusively associated with the casein micellar phase. Binding of milk calcium to the active sites of a cation exchanger resin was used beneficially in this study in order to gently disrupt the casein micelles and liberate the recombinant protein. This approach was found superior to the conventional micelle disruption procedures with respect to product recovery, resin fouling due to milk components and column hydrodynamic properties. Further purification was carried out using  $\text{Ni}^{2+}$  affinity chromatography and resulted in purity more than 90% and a total recovery of 78%. A capillary electrophoresis total protein assay employing large volume sample stacking and a microsphere-based, sandwich-type immunoassay were developed and validated. Both methods were successfully integrated with the downstream purification protocol in order to evaluate various quality attributes of the recombinant protein. A one-step capillary isoelectric focusing protocol was developed in order to monitor the recombinant protein in milk samples. The results showed extra protein bands in the transgenic milk that had isoelectric points significantly lower than the theoretically calculated one which indicated that the protein had been modified during expression. The association between the recombinant protein and bovine milk caseins was explored at the molecular level using the surface plasmon resonance technique. Results showed a calcium-mediated interaction between the recombinant protein and the phosphorylated caseins. This selective interaction was not noted between the human myelin basic protein and milk caseins which indicated mammary gland-related posttranslational modifications, most likely phosphorylation. The co-expression of the recombinant protein and caseins in the mammary gland, along with the ability of the recombinant protein to form calcium bridges with caseins explained its association with the casein micellar phase in the transgenic milk. Despite this and owing to the low expression levels of the recombinant protein in milk, light scattering investigations using diffusing wave spectroscopy showed no significant differences between the transgenic and the non-transgenic milk samples with respect to the average micelle size and the micelle surface charges.

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## List of Abbreviations

<b>Abbreviation</b>	<b>Definition</b>
$\Delta t_m$	Difference in migration time
A	Integrated peak area
ANOVA	Analysis of variance
BA	Benzyl alcohol
BGE	Background electrolyte
BME	$\beta$ -Mercaptoethanol
BSA	Bovine serum albumin
CA	Carrier ampholytes
CAP	Calcium phosphate nanoparticles
CB	Comassie Blue protein stain
cDNA	Complementary deoxyribonucleic acid
CE	Capillary electrophoresis
CIEF	Capillary isoelectric focusing
CIP	Clean-in-place
CK	Cholecystokinin flanking peptide
CM	Carboxymethyl
CMC	Critical micelle concentration
CN	Casein
CNF	Casein fraction
CNM	Casein micelles
CS	Carbonic anhydrase II
cv	Column volume
CZE	Capillary zone electrophoresis
DAD	Diode array detector
DP	Deep Purple protein stain
DWS	Diffusing wave spectroscopy
EDC	1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride

EDTA	Ethylenediaminetetraacetic acid disodium salt dihydrate
ELISA	Enzyme linked immunosorbent assay
EOF	Electroosmotic flow
EtA	Ethanolamine
Fc	Flow cell
FS	Fused silica
GDL	Glucono- $\delta$ -lactone
GST	Glutathione-S-transferase
h	Peak height
HEPES	4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid
His tag	An amino acid motif in proteins which consists of polyhistidine residues, often at the C- or N-terminus.
hMBP	Human myelin basic protein
HRP	Horse radish peroxidase
HSDC	High sensitivity detection cell
I.D.	Internal diameter
ICH	International conference on harmonization of technical requirements for registration of pharmaceuticals for human use
IEF	Isoelectric focusing
IMAC	Immobilized metal affinity chromatography
LA	Lactalbumin
LF	Lactoferrin
LG	$\beta$ -Lactoglobulin
LLOQ	Lower limit of quantitation
LOD	Limit of detection
LP	Lactoperoxidase
LVSS	Large volume sample stacking
mAb	Monoclonal antibody
MBP	Myelin basic protein
MFGM	Milk fat globule membrane
MFI	Median fluorescence intensity

mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
MSD	Mean square displacement
MW	Molecular weight
MWCO	Molecular weight cut off
NC	Nitrocellulose
NHS	N-Hydroxysuccinimide
O.D.	Outer diameter
OG	Orange G
PA	Migration time-corrected integrated peak area
PBS	Phosphate buffered saline
PEO	Polyethylene oxide
PQ	ProQ Diamond protein stain
PTM	Posttranslational modifications
QC	Quality control
R <sup>2</sup>	Correlation coefficient
rhMBP	Recombinant human myelin basic protein
RN	Ribonuclease A
RT	Room temperature
RU	Resonance unit
SA-PE	Streptavidin-coupled <i>R</i> -phycoerythrin
SDS	Sodium dodecylsulfate
SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SF	Serum fraction
S-NHS	N-Hydroxysulfosuccinimide
SPBB	Sulfopropyl Sepharose big beads
SPR	Surface plasmon resonance
Tg	Transgenic
TGmilk	Milk obtained from transgenic cows by natural lactation
TGmilk <sub>h</sub>	Milk obtained from transgenic heifers by hormonal induction
tm	Migration time