

Casein		
Component	% Total	%Casein
Casein	83	
α -s Casein	45	55
β -casein	21	25
κ -casein	12	15
γ -casein	4	5

Casein

Those proteins that are precipitated from raw skim milk by acidification to pH 4.6 at 20° C

Mellander showed by free boundary electrophoresis that casein could be divided into three fractions:

α β γ

Waugh

Separated casein into two fractions

One fraction was sensitive to the presence of calcium ions and called α_s casein

One fraction insensitive to calcium called κ - casein

α_s casein

The fraction of whole casein precipitating at 0.4 M CaCl_2 and at 4° C

Stabilized against precipitation by 0.02 M CaCl_2 by κ -casein

Precipitated from 6.6 M urea by 12% TCA or pH 1.3 to 1.5

Properties

Properties of α_s casein	
% P	1.01
% N	15.66
Isoionic Point	5.05
Monomer Charge	25± 2

Charges of α_s casein

At pH 6.6, binds 8 Ca^{++}

Net charge was -24

16 + Charges bound

Charge at pH 6.6 in presence of calcium is -8

Molecule precipitates

α_s casein

Contains 199 amino acids
Molecular weight 23,644
H ϕ ave 1170
In the presence of calcium undergoes a rapid, non temperature dependent aggregation - dissociates at pH = 12
Contains 17 prolines - randomly distributed

α_s casein

Contains three large hydrophobic regions 1-44, 90-113, 132-199
Contains two highly charged regions 45-89 and 114-131
Segment 45-89 contains all 8 phosphate groups + 12 COO⁻
Has a net charge of -22.7

α_s casein

Random distribution of proline suggests little if any secondary structure
Three hydrophobic areas are buried in a common compact area
Residues 45-89 are very hydrated and occupy a large hydrodynamic volume

β -Casein

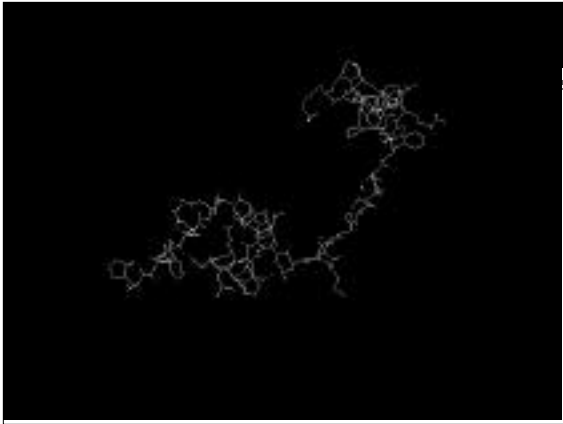
% P	0.65
% N	15.72
Isoionic Point	5.3
Net Charge at pH 6.6	-13
Molecular Weight	23,980

β -Casein Properties

Contains 209 amino acids
4 PO₄ groups very close
Residues 13-21 in β are identical to residues 62-70 in α except Ala/Val and Ile/Leu

β -Casein Properties

Temperature dependent association
Temp dependence of Ca⁺⁺ sensitivity
Precipitates at pH 5.4 - net charge = -6
Contains 5 PO₄ groups



β -Casein Properties

Molecule is very asymmetric

Intrinsic viscosity doesn't change in urea

May contain some poly proline or small amounts of α -helix

Contains 17% proline

β -Casein Properties

The N-terminal residues, 1-21 have a net negative charge of -12 at pH 6.6

Rest of molecule has no net charge at same pH

Contains a highly hydrated portion and a very hydrophobic region

Protein has been described as "soap-like"

β -Casein Association

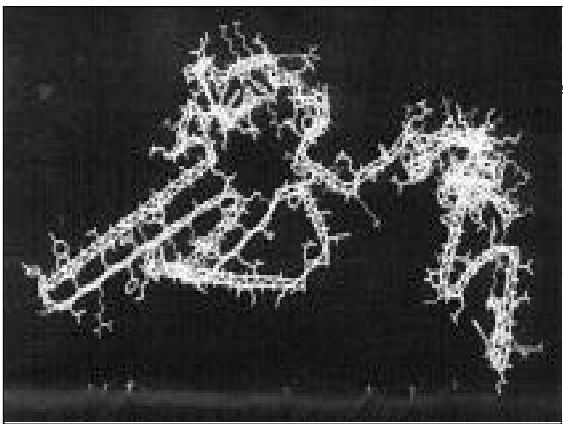
In presence of Ca^{++} $n\text{b} \leftrightarrow \text{b}_n$ $n = 23$

$\Delta H = 30$ Kcal/mole

$\Delta S = 100$ eu

At 4°C , $\Delta G = + 3$ Kcal/mole

At 37°C , $\Delta G = -1$ Kcal/mole



γ -Casein Formation

Bovine milk contains traces of the serum protease, plasminogen

Can cleave β -casein near specific lysine residues

Results in the formation of γ -caseins

Range in size from 22,000 to 12,000

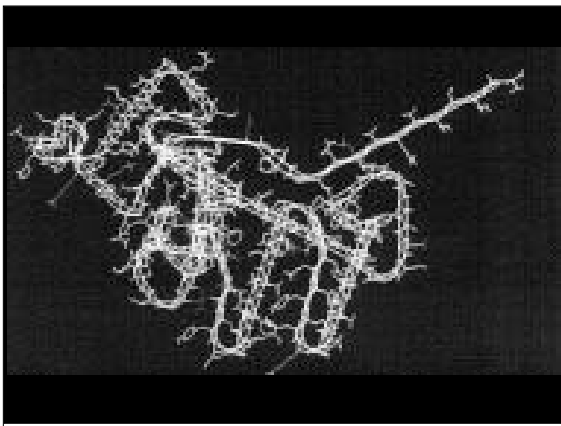
Very hydrophobic

κ -Casein Properties

Soluble in the presence of Ca^{++}
Stabilizes other caseins
Rennin hydrolyzes one very reactive bond at position 105 (phe/met)
Contains variable amounts of carbohydrate
Contains 1 PO_4

κ -Casein Properties

% P	0.16
% N	15.5
Isoionic Point	5.37
Charge at pH 6.6	- 4.3
Molecular Weight	17,800
Normal state is trimer	
Contains 1 -SH group	



Casein Phosphorylation

Phosphorous added in Golgi
Post-translational change
Protein kinase adds phosphates
Recognizes sequences:
Ser or Thr/ X / Glu or Sep-P

Micelle Observations

At 37° C and low Ca^{++} - both free α_s - Ca^{++} and α_s - κ exist
Stable micelles form only at Ca^{++} levels higher than required to precipitate α_s
Large micelles have less κ than small micelles
Complete stabilization at α_s - κ ratios up to 10 - some stability at higher ratios

Micelle Observations

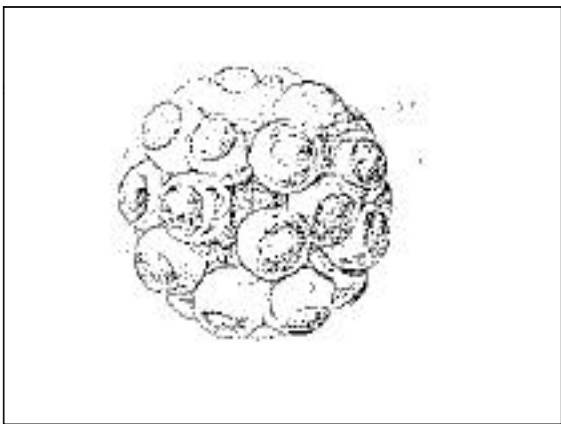
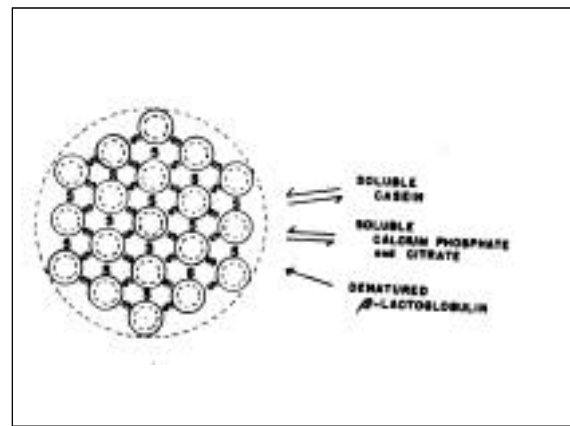
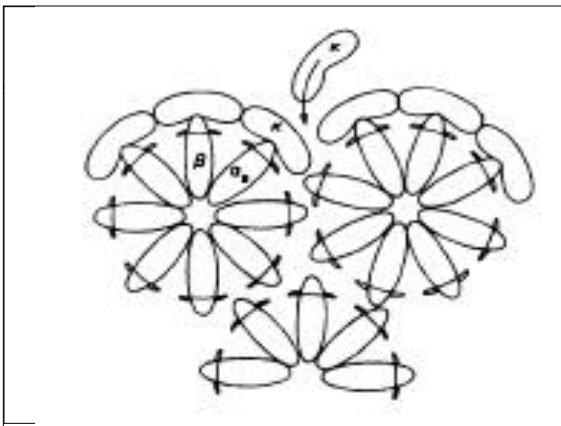
If κ is added to micelles, the average micelle size decreases
Micelles are highly solvated as α_s - κ ration increases, solvation decreases
Casein Micelles - Properties
Precipitated by rennin
Memory

Micelle Observations

High concentration of Ca and PO_4
Increase of additional κ -casein results in a decrease in average micelle
Highly hydrated - over 3g $\text{H}_2\text{O}/\text{g}$
Calcium sensitivity
Effects of citrate and phosphate on stability
Porous to large molecules

Sub Micelles

Composed of from 20 to 25 monomers
10 - 20 nm in diameter
Used in micelle models
Models proposed by: Waugh, Morr, Slattery



Functional Properties

High heat stability
Viscous in absence of calcium
Turbid in presence of calcium
Easily unfolds at interfaces
Forms Ca^{++} and acid gels
"Melts"