INTERACTION OF BETA-CASEIN WITH KAPPA-CASEIN FIBRILS

I. Portnaya, S. Avni , E. Kesselman, R. Khalfin, U. Cogan and D. Danino

Department of Biotechnology and Food Engineering, Technion,
Haifa 32000, Israel, portnaya@tx.technion.ac.il

ABSTRACT

Amyloid protein fibrils are associated with numerous degenerative diseases. Kappa-casein (κCN) at physiological conditions is known to form amyloid-like fibrils. Although these are not considered disease-related, understanding fibrillization and inhibition of this process may assist studying fibril formation phenomena in these diseases. Therefore, the associative behaviour of κCN, especially fibrillization, and ways to suppress it are currently of great interest. Recently, the possibility of inhibition of κCN fibrillization by another milk protein, beta-casein (βCN), was suggested [1-2]. The mechanism of this impact is not yet clear.

Keywords: Kappa-casein, fibrils, beta-casein, interactions.

1 MATIRIALS AND METHODS

1.1 Characterization of the main objects

βCN is characterized by a highly polar, negatively charged N-terminal domain and a highly nonpolar C-terminal domain, and displays a pronounced self-association behavior. Micellization of βCN is a reversible process depending on temperature and pH [3-5].

κCN is also amphiphilic protein, possessing a predominantly hydrophilic C-terminal block and a hydrophobic N-terminal end. However, κCN self-association has been found to be a more complex process. This protein contains two cysteine residues (Cys11 and Cys88).

In the native form of κCN (N-κCN), intra- and inter-molecular disulfide bonds lead to the formation of multimeric species ranging from monomers to decamers, followed by further association of the subunits into micelles.

In contrast, κCN, in which the disulfides were reduced (R-κCN), exhibits a monomer-micelle equilibrium.

Both native κCN and R-κCN are known to also form not reversible fibrils. Their tendency to fibrillization increases with increasing of temperature and time of incubation, wherein R-κCN forms fibrils much more readily than N-κCN. The Figure 1 clearly demonstrates foregoing tendencies.

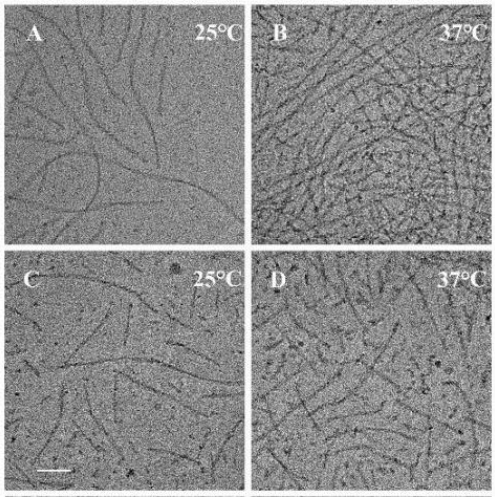


Figure 1: Cryo-TEM images of R-κCN (A, B) and N-κCN (C, D) after a week of incubation at different temperatures. Scale bar is 100 nm.

1.1 Methods

Isothermal Titration Calorimetry (ITC). The interactions between the two casein proteins were characterized using a VP-ITC calorimeter (MicroCal). κ βCN (40 mg/ml) was loaded to an injector–stirrer syringe (289 mL), then injected into the reaction cell with R-κCN and N-κCN solutions of different incubation times (κCN fibrils suspension) in 28 steps of 10 mL each, and the heat flow was measured. Conditions during the titration were: stirring speed - 310 rpm, injection duration - 20 s, equilibration time between consecutive injections - 3 min.

Cryogenic-Transmission Electron Microscopy (cryo-TEM). Solutions of N-κCN, R-κCN, and their mixtures with βCN were examined. All samples were studied under low-dose conditions in an FEI T12 G2 TEM, operated at 120 kV. Images were recorded on a Gatan US1000 2k x 2k high-resolution cooled CCD camera[47-48] using Digital Micrograph.

Small-Angle X-Ray Scattering (SAXS). SAXS data were obtained using a slit collimated Kratky camera with a one-dimensional sensitive detector (Ni-filtered, Cu Kα radiation, operating at 40 kV and 25 mA). Radius distribution parameters were obtained by the fitting of the experimental scattering by Gaussian distribution (in short radius interval) of very long cylinders

Fluorescent microscopy. Samples of κCN , βCN and their mixtures were visualized with fluorescent microscopy (Zeiss Cell observer with an ORCA camera of Hamamatsu) with filter band of EFGP (ex 470/20 and Em 525/50) for Fluorescein with the exposure time for all samples. Representative images were obtained for the entire sample width.

2 RESULTS AND DISCUSSION

To elucidate the mechanism of inhibiting fibrils formation we studied the interactions between κCN (in its native and reduced forms) with βCN micellar solutions at different temperatures and incubation periods.

Two modes of operation that lead to inhibition of κCN fibrillization were found. The first mode is caused by mixed micellization. It is more effective at low temperatures and short incubation periods, notably in presence of sufficient κCN monomers.

The present study is primarily focused on the second mode, which is more pronounced in presence of a considerable number of fibrils.

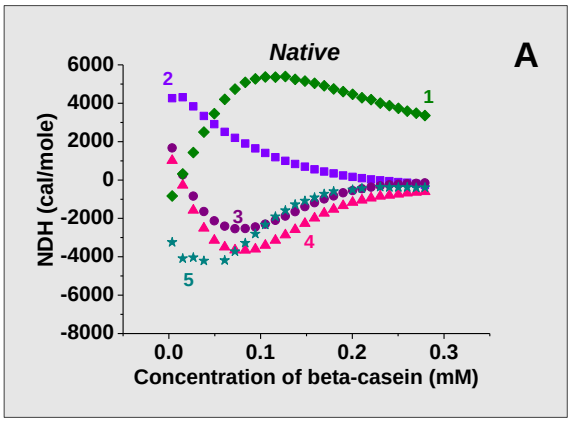
2.1 ITC study

For revealing of βCN ability to influence on κCN fibrils which already exist, we carried out the ITC study of βCN (40mg/ml) titration into R-κCN and N-κCN solutions of different incubation times. As possible to see in the Figures 2 A and 2 B almost all the shapes of the enthalpy thermograms are rather like. An exception is the thermogram related to βCN titration into N-κCN without incubation (Figure 2 A2). The shape of this thermogram describes completely endothermic process typical for micellization of N-κCN and its mixtures with βCN. Therefore, in this case as well as in the mentioned ones just κCN determines the mode of mixed micellization due to abundance of no fibril species.

The rest of curves are characterized by two parts: at the beginning they demonstrate the enthalpy changes describing dilution, initial stage of demicellization of βCN and the mixed micellization. Then, the curves change to distinct S-shape exothermic thermograms typical for binding processes.

We propose that at this stage of titration, along with forming of βCN micelles, an interaction between all βCN species (monomers and micelles) and the fibrils is realized. The differences between the thermograms of titration of βCN into κCN solutions of the same concentration but with different incubation times, are explained by increasing of number of κCN fibrils (Figure 1).

ITC results, obtained for titrations of βCN into incubated native and reduced κCN solutions (Figure 2A and 2B), indicate strong interactions between the fibrils and βCN species (monomers and micelles) in both stuied cases.



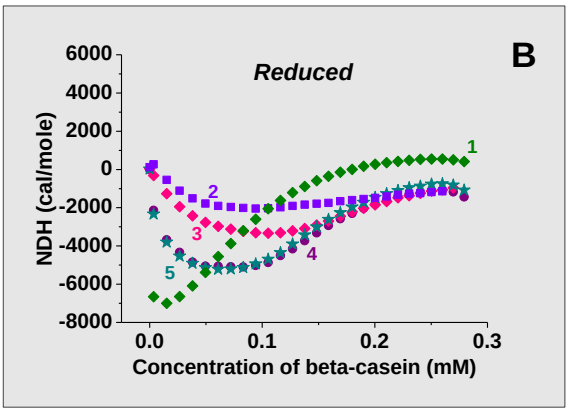


Figure 2: ITC titrations of 40 mg/ml βCN into native (A) and reduced (B) 10 mg/ml κCN, preliminary incubated at 37°C during different time periods , at neutral pH & 25°C: A : 1 – into the buffer (blank); 2 – without incubation; 3 – one day; 4 – one week; 5 – two weeks B: 1 into - the buffer with reducing agent (blank); 2 - without incubation; 3 – four hours; 4 - one day; 5 – five days.

Table 1 clearly demonstrates an increasing of enthalpy of κCN/βCN interaction with increasing of time of incubation, i.e with extension of the fibrillization.

Time of Incub.	N-κCN ΔH, cal/mole	Time of Incub.	R-κCN ΔH, cal/mole
One day	2343	Four hours	905
One week	3025	One day	2378
Two weeks	3896	Five days	3997

Table 1: Enthalpy of interaction between κCN fibrils and beta-casein species at 37°C

2.2 Cryo-TEM, SAXS and Fluorescent Microscopy results.

Cryo-TEM (Figure 3) , SAXS (Figure 4) and Fluorescence Microscopy (Figure 5) support this.

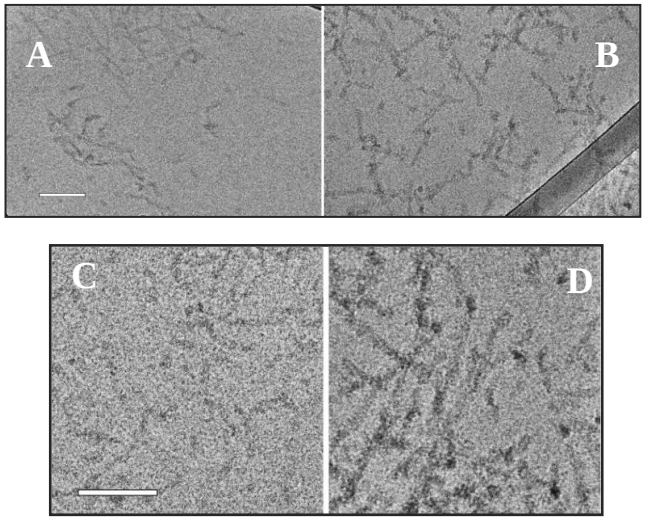


Figure 3: Cryo-TEM images of individual 10 mg/ml N-κCN gel after 12 days of incubation and subsequent centrifugation (A&C), and its mixture gel with βCN, obtained as result of 7 days preliminary κCN incubation , adding of βCN (molar ratio 1:1), 5-days mixture incubation and subsequent centrifugation (B&D). Scale bar is 100 nm.

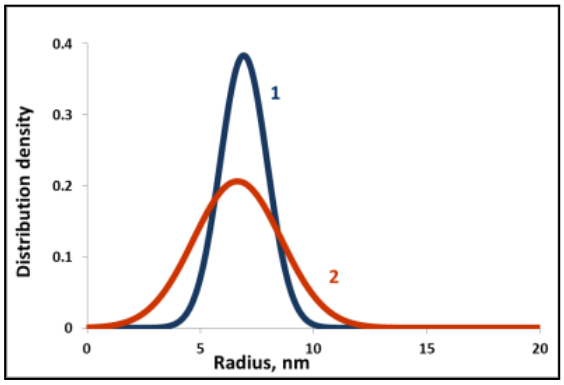


Figure 4: Fibrils radius distribution in the 10 mg/ml N–κCN solution, incubated during 12 days (1), and the system, obtained as result of 7 day's preliminary κCN incubation , adding of βCN (molar ratio 1:1) and following additional 5-day's incubation .

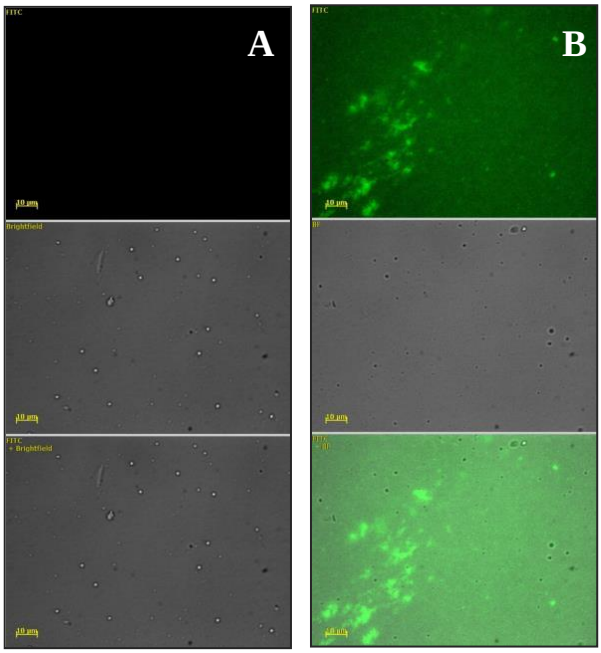


Figure 5: FITC and bright field imaging of the mixture gel: A– galleries of κCN with no labeled βCN; B - galleries of κCN with βCN, labeled by Fluorescein.

TechConnect Briefs 2016, TechConnect.org, ISBN 978-0-9975-1170-3

3 CONCLUSIONS

By combining time-resolution studies using ITC and cryo-TEM with SAXS and Fluorescence Microscopy, we revealed the ability of βCN species (monomers and micelles) to be adsorbed onto already existing fibrils.

Adsorption of βCN on the κCN fibrils inhibits their subsequent growth and, thereby, inhibits extension of fibrillization in the native and reduced κCN/βCN mixtures.

REFERENCES:

[1] Ecroyd, H.; Thorn, D. C.; Liu, Y.; Carver, J. A. *The Biochemical journal* **2010,** *429* (2), 251-60.

[2] Thorn, D. C.; Meehan, S.; Sunde, M.; Rekas, A.; Gras, S. L.; MacPhee, C. E.; Dobson, C. M.; Wilson, M. R.; Carver, J. A. *Biochemistry* **2005,** *44* (51), 17027-36.

[3] Portnaya, I.; Cogan, U.; Livney, Y. D.; Ramon, O.; Shimoni, K.; Rosenberg, M.; Danino, D. **2006,** *54* (15), 5555-61.

[4] Portnaya, I.; Ben-Shoshan, E.; Cogan, U.; Khalfin R.; Fass, D.; Ramon, O.; Danino, D. **2008,** *56* (6), 2192-8.

[5] Moitzi, C.; Portnaya, I.; Glatter, O.; Ramon, O.; Danino, D. *Langmuir : the ACS journal of surfaces and colloids* **2008,** *24* (7), 3020-9.