Different α-casein association states and their interaction with lipid vesicles to study antibacterial activity

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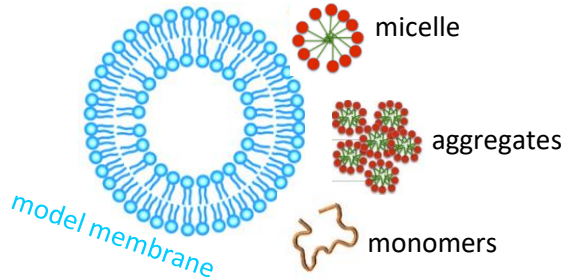
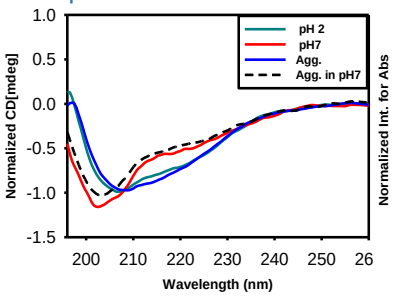
Abstract

The interactions between caseins and lipid membranes are fundamental for the physiological function of these proteins. Moreover, the understanding of the underlying molecular mechanisms is of great interest for the development of new casein derived antimicrobial peptides. Indeed, it was already shown that peptides derived from caseins possess antibacterial activity but their mechanisms of action is still debated. Here, we present an experimental study on the interaction between model lipid membranes and α-casein by means of spectroscopy and fluorescence microscopy techniques. α-casein is an unfolded protein that due to its amphiphilic nature is known to self-assembly into non-stable micellar structures whose presence, diameter and compactness depend on environmental conditions. Presented experiments are aimed at assessing the effects of this protein in different states (monomeric, micellar and aggregated) on the membranes highlighting the role of micelles.The association state of α-casein at different pH and temperatures was analysed by fluorescence spectroscopy, circular dichroism and dynamic light scattering. Then, α-casein in different states was added to giant lipid vesicles and fluorescence microscopy and spectroscopy techniques were used to map and quantify induced modifications on the membrane. Our results indicate that, depending on the specific properties of the added protein state, different membrane structure and morphology changes occur. Interestingly, the most effective species in altering membranes is constituted by highly hydrophobic oligomers originating from larger aggregates disassembly.

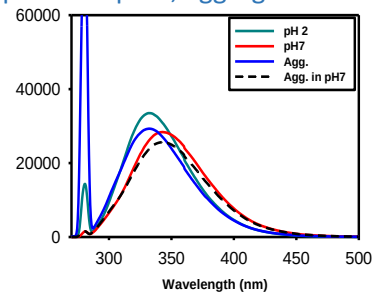


Characterization of protein association states

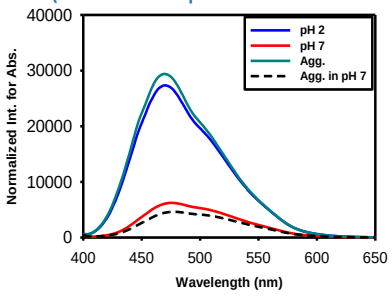
Samples: α-casein in solution at pH 2 and pH 7, aggregated α-casein (obtained upon incubation at 70°C for 24 hours) and aggregates dissolved in solution at pH 7



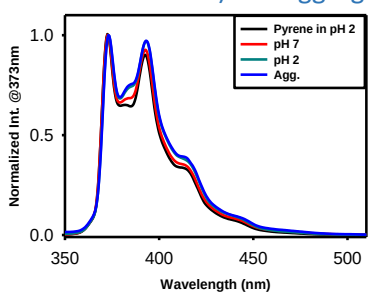
Far-UV CD spectra: secondary structure



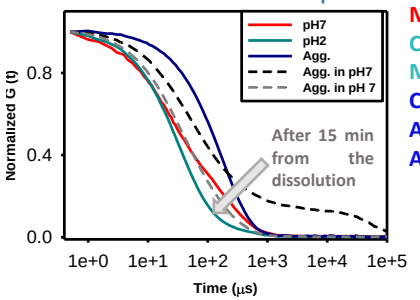
Intrinsic fluorescence emission spectra (λex = 280 nm): tertiary structure



ANS fluorescence spectra (λex = 280 nm): exposure of hydrophobic regions



Fluorescence spectra of pyrene (λ_{ex}=330nm): micelles formation



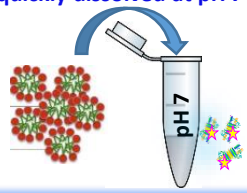
Dynamic light Scattering: sample characteristic size

Casein pH 7 : unfolded protein, mostly in MONOMERIC form

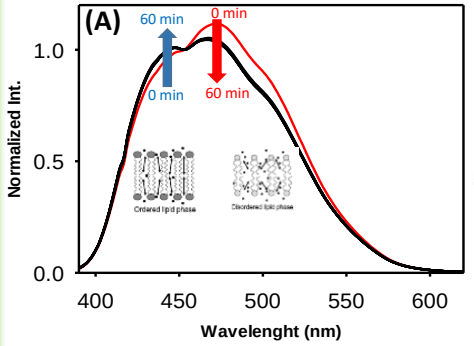
Casein pH 2 : intermolecular β- structures, MICELLEs

Casein in pH 2 incubated at 70°C for 24h: MICELLEs AGGREGATES , hydrophobic surface.

Aggregates quickly dissolved at pH 7

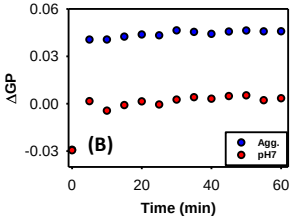


Laurdan Generalized Polarization (Cuvette)



$$GP = \frac{I_{440} - I_{490}}{I_{440} + I_{490}}$$

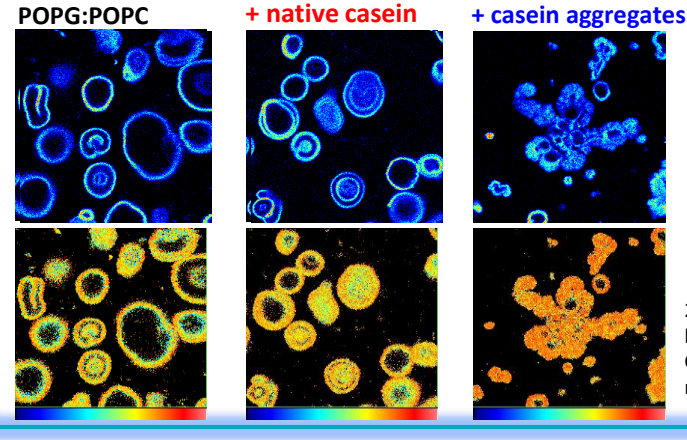
GP= -1 liquid disordered phase
GP= +1 liquid ordered phase



- **FAST CHANGE (WITHIN 5 MIN)**
- **GP GROWTH**
- **STIFFENING AND DEHYDRATATION OF VESICLES**
- **AGGREGATED FORM HAS LARGER EFFECT**

(A) Laurdan fluorescence spectra (λ_{ex}=380nm) before and after the addition of 0.9mg/ml of casein aggregates pH2. (B) GP variation of Laurdan emission after addition of protein samples as a function of the time.

Laurdan Generalized Polarization Microscopy



THE PRODUCTS OF THE DISAGGREGATION OF CASEIN AGGREGATES INDUCE :

- **CHANGES IN LIPOSOMES MORPHOLOGY**
- **VESICLES COLLAPSE**
- **VESICLES BUDDING**

256x256 pixel intensity (top line) and GP-maps (lower line) before and after addition of native and casein aggregates. Color bar indicates the value of GP in each pixel. GP values range from -1(blue) to +1 (red)