BINDING OF PHTHALOCYANINES TO BETA-LACTOGLOBULIN: FIRST STEP TO INHIBITION OF AMYLOID AGGREGATION

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1. INTRODUCTION

Amyloid aggregation is the process of proteins misfolding, where denatured protein molecules bind together and form rigid insoluble nanosized structures named amyloid fibrils (Fig. 1). This process is mostly known to accompany neurodegenerative deceases (e.g. Alzheimer's or Parkinson's diseases), but takes place also in several other cases, one of them is the food processing. Particularly, beta-lactoglobulin (BLG, Fig. 2) is an important protein of milk whey and, on the other hand, is known to form amyloid aggregates. Since amyloid aggregates of BLG should be avoided in food, the ways of inhibition of such aggregation should be studied.

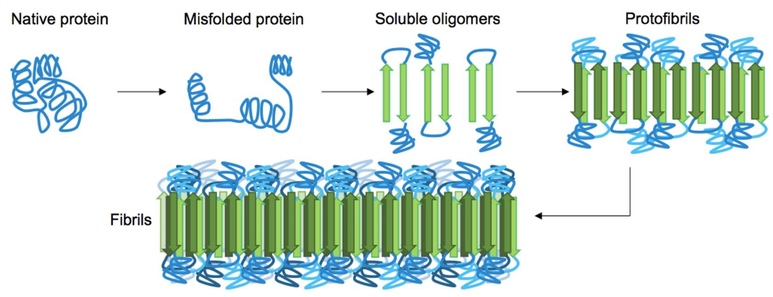

Fig. 1. Formation of amyloid aggregate of the protein

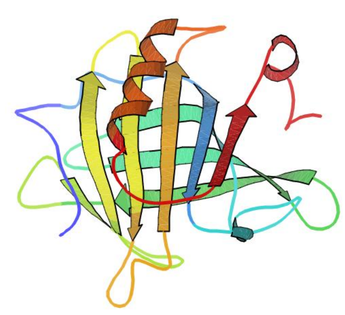

Fig. 2. Beta-lactoglobulin

One of the approaches to inhibit the formation of amyloid aggregates is to form complex between the inhibitor ligand molecule and the native form of the protein (e.g. monomer, dimer or hexamer).

Here we studied binding of BLG in its native (pH 7.9) and denatured (pH 2) form with zirconium phthalocyanines containing out-of-plane ligands bearing negative charge ($PcZrCitr_2$), positive charge ($PcZrLys_2$) and those able to form disulfide bridges ($PcZrS_2$) (Fig. 3).

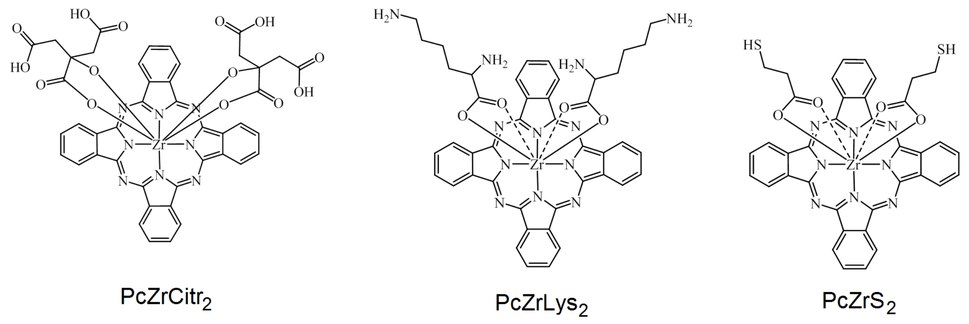

Fig. 3. Structures of the studied phthalocyanines

2. BINDING OF PHTHALOCYANINES TO BLG: EVIDENCE BY ABSORPTION SPECTRA

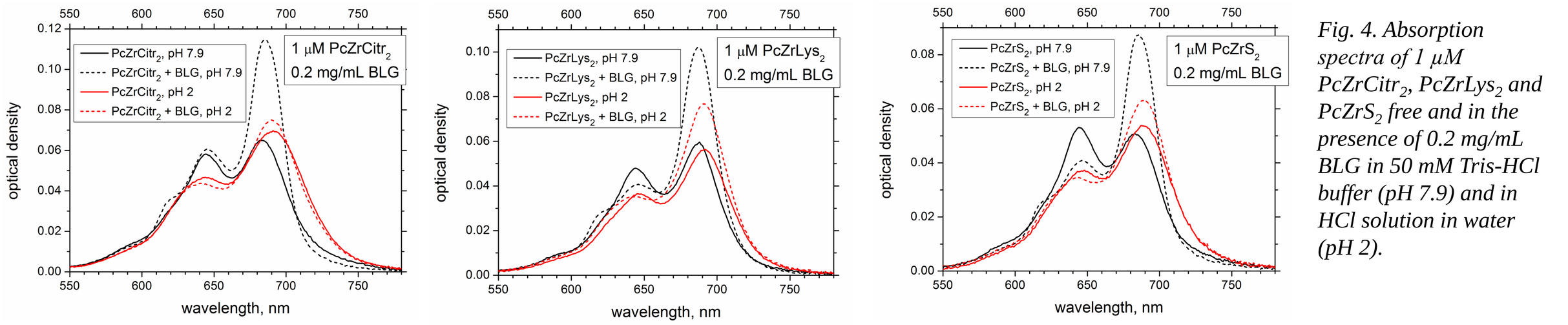

Fig. 4. Absorption spectra of 1 μM $PcZrCitr_2$, $PcZrLys_2$ and $PcZrS_2$ free and in the presence of 0.2 mg/mL BLG in 50 mM Tris-HCl buffer (pH 7.9) and in HCl solution in water (pH 2).

Absorption spectra (Fig. 4) show than the presence of BLG results in the change in the absorption spectrum of phthalocyanine, that is manifested mostly as the increase in the contribution of the short-wavelength Q-band (near 645 nm) into the spectrum with corresponding decrease of that of long-wavelength one (near 690 nm). **This could point to the destruction of the phthalocyanine aggregates formed in the solution that accompanies the binding of its monomers with both native and denatured protein.**

3. BINDING OF PHTHALOCYANINES TO BLG: EVIDENCE BY FLUORESCENCE SPECTRA

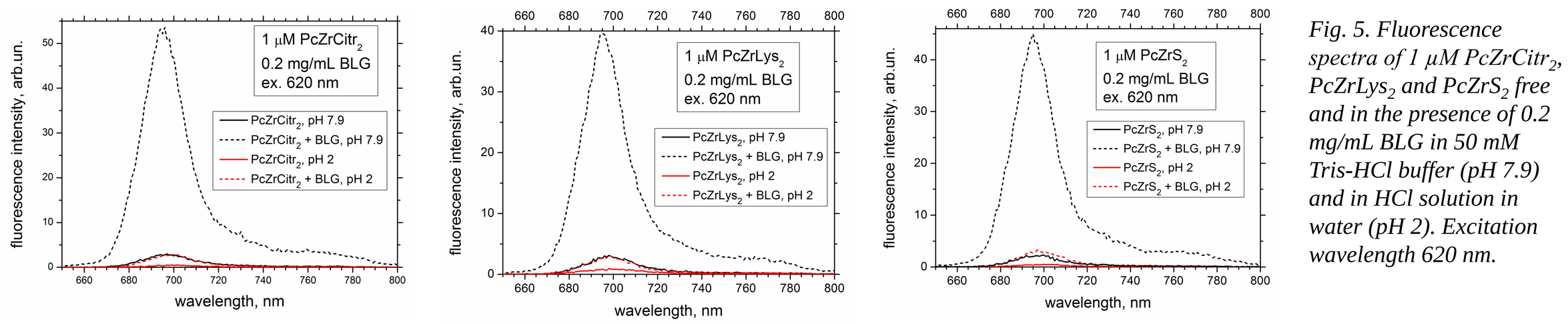

Fig. 5. Fluorescence spectra of 1 μM $PcZrCitr_2$, $PcZrLys_2$ and $PcZrS_2$ free and in the presence of 0.2 mg/mL BLG in 50 mM Tris-HCl buffer (pH 7.9) and in HCl solution in water (pH 2). Excitation wavelength 620 nm.

Fluorescence spectra (Fig. 5) demonstrated that $PcZrCitr_2$, $PcZrLys_2$ and $PcZrS_2$ when free reveal weak fluorescence emission in both neutral and acidic media; fluorescence intensity of phthalocyanines at pH 7.9 exceeds corresponding value at pH 2 in 3.5-6 times. At the same time, addition of BLG leads to the increase of fluorescence intensity of all studied phthalocyanines in 13-21 times in neutral medium and in 3.0-6.3 times in neutral one. **This means that phthalocyanines bind to BLG in its both native (at pH 7.9) and denatured (at pH 2) conformations.**

4. MORE RESULTS OF FLUORESCENCE STUDIES

- Fluorescence excitation spectra showed the **absence of excitation energy transfer from tryptophan residues of BLG to phthalocyanines**; thus they bind to BLG at either big distance or perpendicular orientation of transition dipole moments with respect to those of its tryptophan residues (or both these factors).
- Equilibrium constant of phthalocyanine-BLG binding at pH 7.9 was estimated; its value was close for all studied compounds (near 3×10^5 M^{-1}). Thus we could suppose that the **phthalocyanine binding to native BLG at pH 7.9 is mainly due to tetraindole macrocycle of phthalocyanine** with only little effect of out-of-plane ligands.

CONCLUSIONS:

• Phthalocyanines containing out-of-plane ligands bearing negative charge, positive charge and those able to form disulfide bridges bind to BLG in its both native (at pH 7.9) and denatured (at pH 2) conformations.
• Phthalocyanines bind to BLG at either big distance or perpendicular orientation of transition dipole moments with respect to those of its tryptophan residues (or both these factors).
• Phthalocyanines bind to native BLG at pH 7.9 mainly due to tetraindole macrocycle with only little effect of out-of-plane ligands.

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