Interactions between Orange Carotenoid Protein and mesoporous silica. From fundamental research to the development of photoactivable nanodevices for imaging and artificial photosynthesis

Project to be developed under the supervision of Dr Alberto Mezzetti (Sorbonne University, Paris, France) in collaboration with Dr Diana Kirilovsky (Paris-Saclay University, Gif-sur-Yvette, France).

The study of the interactions between proteins and inorganic mesoporous surfaces, notably silica, is a relevant research field for several disciplines, ranging from chromatography to biocatalysis, biosensors and drug delivery [1, 2]. Nevertheless, several aspects of this kind of interactions are still not well understood, in particular how the matrix can influence the dynamics or the working mechanism of the matrix.
In this project, we propose to study the photoactivable Orange Carotenoid Protein (OCP) in silica mesoporous matrices. On the one hand, this subject has a fundamental research objective, as OCP represents a model system for its simplicity, its versatility and because it is a photo-activated protein (with a light-induced modification of its physico-chemical properties and of its shape), an aspect that simplifies its investigation. On the other hand, the peculiar optical and antioxidant properties make the OCP@SiO2 system very promising for future applications, in particular in the development of nano-optical systems for imaging, in artificial photosynthesis and in system for targeted protein delivery.

Detailed projet

OCP is a water soluble protein playing a key photoprotective role in cyanobacteria. OCP can interact with the photosynthetic membrane to dissipate the light energy in excess, which could entail a photo-induced oxidative stress. OCP is made up of a polypeptidic chain of ~300 amino acids (AAs) and of a carotenoid, 3'-hydroxyechinenone. Under continuous light, OCP can undergo, with a low quantum yield, a photoconversion from the stable orange form OCP^O to a photoactivated red form OCP^R. When light exposure ends, OCP^R can slowly relax to the OCP^O form. For its relevance in cyanobacterial photoprotection, but also for its simplicity, OCP has been the object of increasing interest not only in photosynthesis, but also in physical chemistry. Recently, its excellent antioxidant properties have also been demonstrated [4] and a protocol for OCP production in *E. coli* has been developed, which makes possible its large-scale production [5].
OCP photoconversion mechanism has been studied by different techniques [6-10]. Notably, it has been observed that OCP^R has a much more elongated form compared to OCP^O. This is a consequence of the separation of the two domains of OCP, the N-terminal domain (NTD) and the C-terminal domain (CTD), as well as of the movement of the carotenoid that in the OCP^O form connects the NTD and the CTD, whereas in OCP^R is completely buried inside the NTD [6, 7]. In addition, OCP^R surface is likely to be much more hydrophilic than the one of OCP^O [7].

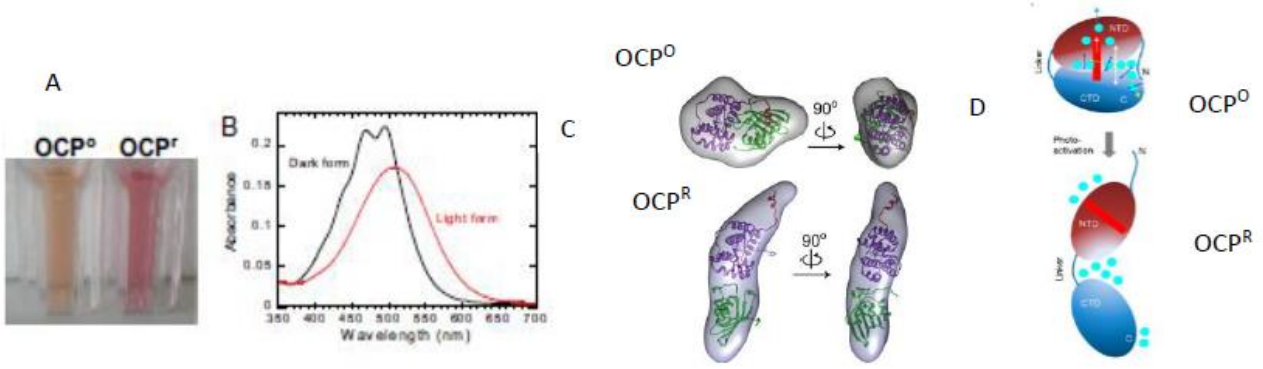

Figure 1. A: OCP color in its two forms. B: UV-Vis spectrum of OCP^O (black trace) and OCP^R (red trace). C: Shape of OCP^O and OCP^R in solution, according to [7]. D: proposed mechanism for the $OCP^O \rightarrow OCP^R$ transition. 3'-hydroxychinenone is show as a red stick.

Nevertheless, several steps of the photocycle are still unknown, notably the link between the initial carotenoid photophysics and the large-scale conformational change described above. For the point of view of molecular biophysics, OCP is particularly interesting as it is a photoactivable protein and its photoconversion entails a huge change in the shape of the protein, associated also to a change of physicochemical properties such as hydrophilicity. In addition, OCP is a simple system, which can be easily characterized by biophysical techniques such as FTIR difference spectroscopy (FTIR-DS) [11]. This technique make it possible to study at an atomic level conformational changes of proteins, and it can be used in a time-resolved way and it can be applied to proteins in different conditions (hydration state, temperature, interaction with a surface etc) [12]. Resonance Raman spectroscopy [9] is also very useful as it allows to study selectively the structure and the conformation of the carotenoid.
Encapsulation in the SiO_2 matrix will make it possible to better understand the influence of the surrounding environment on OCP photocycle. As far as we know, this approach has never been applied to OCP. Different matrices with pores of different size will be used, and the influence of the water content inside the pores will be investigated, following previous approaches used on photosynthetic films [13].
We will investigate the molecular details of the photocycle for OCP@SiO_2 by time-resolved FTIR-DS, using site-specific mutants and isotopic exchange.

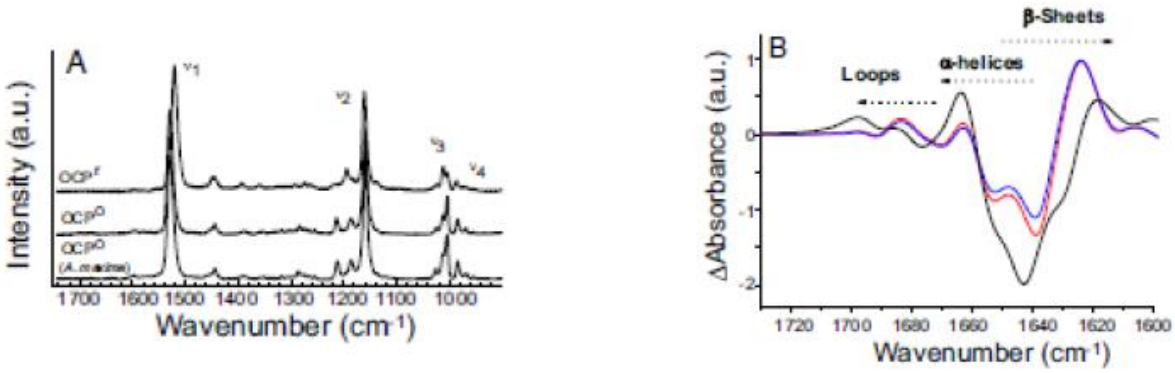

Figure 2. A Resonance Raman spectra of 3'-hydroxyechinenone in OCP^O and OCP^R. B FTIR difference spectra of OCP after exposure to light, in H_2O (black trace) and D_2O (blue trace, after 5 seconds of exposure, red after 50 seconds of exposure).

Concerning more long-term goals, photochromic and photo-dissipative properties of OCP have been recently exploited in an artificial system [14]. OCP has been used as an on/off switch capable of modulating the energy transfer between two organic dyes. Under high irradiance, OCP^O becomes OCP^R which can dissipate the excess of energy. We will investigate if OCP@SiO_2 can also act in the same way. This could be the proof of concept for the development of nano-optical devices based on OCP, acting simultaneously as a switch and as a photoprotection element under strong illumination. On the other hand, relying of OCP antioxidant properties, OCP@SiO_2 could possibly be used for OCP targeted delivery in a biological system. In principle, some matrices could even allow only the delivery in just one of the two forms, the photoactivated OCP^R or the dark-adapted OCP^O.
Dr Mezzetti and Dr Kirilovsky collaboration on the investigation of OCP mechanism by vibrational spectroscopy is well-established (see [12] ; a manuscript will be submitted in the coming weeks). Dr Mezzetti has a long-standing experience in Raman and time-resolved FTIR applied to photo-activable proteins and carotenoids [12, 13, 15 and refs. therein], and has already gained experience in characterization of biomolecules at interfaces [17, 18]. Dr Kirilovsky's team is probably the most active on OCP research

worldwide (see for instance [3-6 ; 11, 16]). It should be mentioned that Dr Kirilovsky discovered in 2008 the photo-activation of OCP and its relevance for cyanobacterial photoprotection [16].

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