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The fluorescent monomeric protein Kusabira Orange. Pressure effect on its structure and stability

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ABSTRACT

The structure and stability of the fluorescent protein monomeric Kusabira Orange (mKO), a GFP-like protein, was studied under different pressure levels and in different chemical environments. At different pH values (between pH 7.4 and pH 4.0) and under a pressure up to 600 MPa (at 25 °C), mKO did not show significant fluorescence spectral changes, indicating a structural stability of the protein. In more extreme chemical conditions (at pH 4.0 in the presence of 0.8 M guanidine hydrochloride), a marked reduction of mKO fluorescence intensity emission was observed at pressures above 300 MPa. This fluorescence emission quenching may be due to the loss of the intermolecular bonds and, consequently, to the de-structuration of the mKO chromophore structure. Since the electrostatic and hydrophobic interactions as well as the salt bridges present in proteins are usually perturbed under high pressure, the reduction of mKO fluorescence intensity emission is associated to the perturbation of the protein salt bridges network.

1. Introduction

Many cnidarians utilize fluorescent proteins as energy-transfer acceptors in bioluminescence. These highly fluorescent proteins are unique due to the chemical nature of their chromophore, which is composed of modified amino acid residues within the polypeptide. For example, by changing the amino acid residue 66 and/or the amino acid residues close to the chromophore, the color and fluorescence intensity of GFP (Green Fluorescent Protein) can be modified [1–3].

Many different biological and ecological functions have been proposed for bioluminescence [4,5], but the most parsimonious explanation is that bioluminescence is used as an anti-predator defensive stratagem. Sudden flashes in dark surroundings have been shown to startle, deter, and stun the prey. Bioluminescence induced by multifarious stimuli has long been observed and remains under investigation because of its great complexity. Many cnidarians emit light when they are mechanically disturbed. Proteins involved in the light emission process should resist to external perturbations, because the three-dimensional structure of protein molecules can exert a strong influence over the protein active site leading to inactivation of the light emission process [6].

The mKO is the monomeric version of Kusabira Orange, a GFP-like protein, isolated from the stony coral *Fungia concinna* and emitting bright orange fluorescence. The mKO structure is a typical beta barrel architecture with a fully mature chromophore containing a 3-thiazoline ring as a third ring that accounts for the fluorescence properties of the protein [7]. Fig. 1a shows a schematic representation of the secondary structure topology, consisting of 15 secondary structure elements, mainly beta strands. Fig. 1b shows the 3D structure in two orientations, with secondary structures elements and the chromophore in evidence. The structural stability of mKO appears enhanced, in comparison to beta barrels structures of other proteins analyzed as counterparts, by the presence of a higher number of salt bridges and H-bonds. Table 1 shows the number of H-bonds and salt bridges observed into the structure of mKO and of bovine and porcine Odorant Binding Protein (bOBP and pOBP, respectively). In fact, OBPs belong to the lipocaline family. This class of proteins displays a beta barrel tertiary structure organization, 70–80% of the total number of amino acid residues [8], even if OBPs do not share a significant primary structure similarity. From a general point of view, this observation suggests that mKO may be more stable than bOBP and pOBP despite that these proteins are very stable to the denaturing action of GdnHCl [9] and reveal a particularly high thermostability having a denaturation temperature of 90 °C [10]. A more detailed analysis of the position of the salt bridges may offer additional information. The interactions that constitute the network of salt

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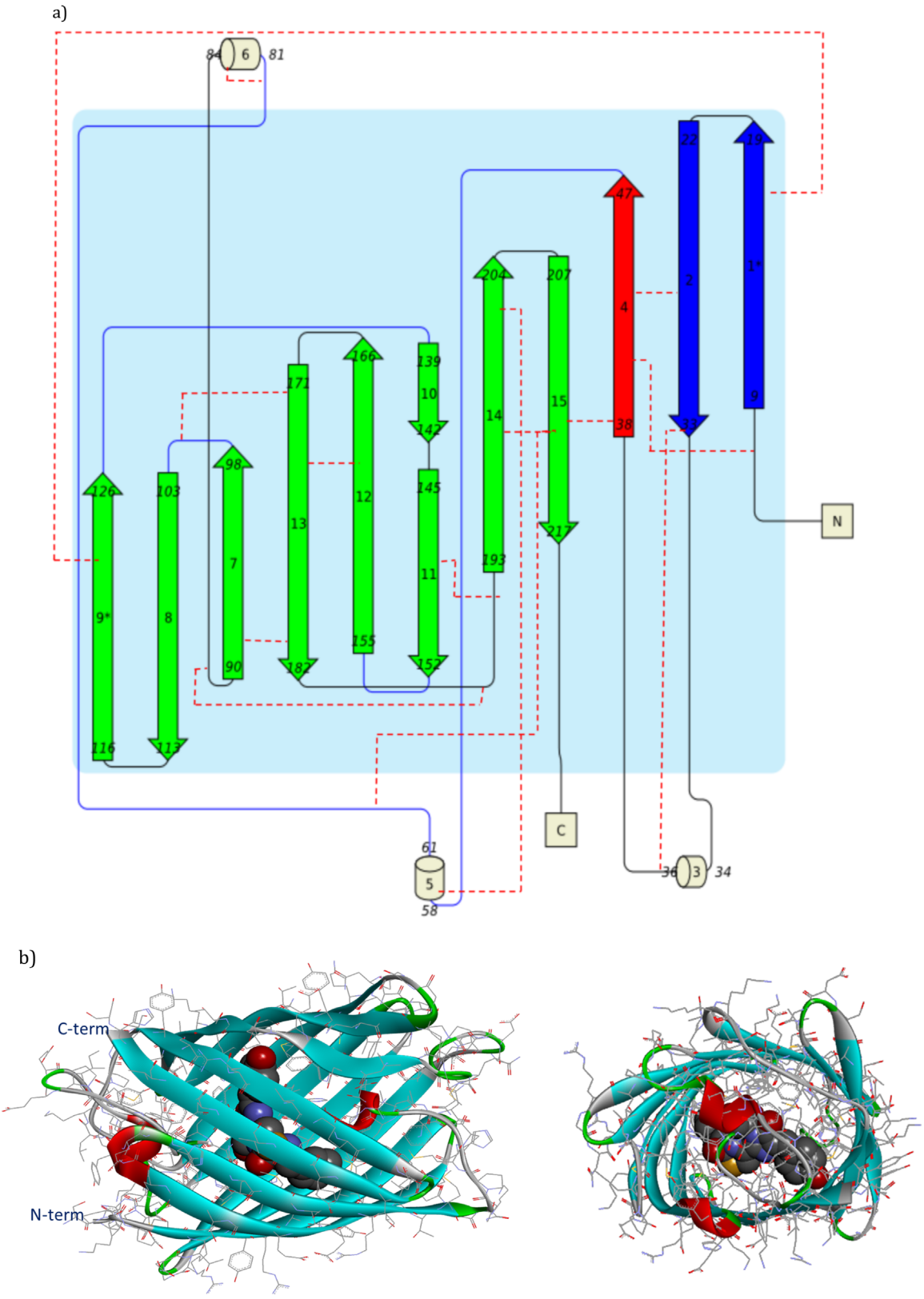

Fig. 1. (a) Secondary structure schematization of mKO. Each secondary structure element has a progressive number in the middle, and the first and last residue numbers at extremities. Arrows indicate beta strands, cylinders indicate helices. Dashed lines indicated the network of predicted salt bridges. (b) 3D structure of mKO, with two different orientations. Secondary structure is represented with beta strands (arrows colored in cyan) and helices (cylinders colored in red), while the chromophore in the middle of the structure is represented as spacefill atoms with standard colors (C=gray, N=blue, O=red, S=yellow). 3D structure of mKO protein from *VerrilloFungia concinna* extracted from the Protein Data Bank archive (PDB code: 3MGF). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)