Stabilization of Large Membrane-Associated Protein Complexes using Peptide Surfactants

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Statement of Purpose: The stabilization of large protein complexes in a functional manner is of fundamental importance to many technological applications including biosensors and bioactive tissue scaffolds. Of particular interest is the stabilization of large membrane-bound and membrane-associated, protein complexes as they present unique functionalities and could be the basis for novel sensing technologies (i.e. olfactory receptors for bio-artificial 'nose' devices). The control over the backbone structure of peptide detergents (peptergents) offers a powerful tool for the design of protein stabilizing compounds. Recent work has shown that peptergents are able to stabilize complex membrane proteins [1]. However, it is currently unknown if this strategy can be employed for stabilizing membrane associated super-complexes (~ 10 Million Da), composed of several hundred proteins that self-assemble into a highly ordered 3-D structure that is required for function.

For this purpose, a model protein complex, phycobilisomes from cyanobacteria, was selected for stabilization studies. Phycobilisomes are photo-antennae that are associated with the membrane bound reaction center of cyanobacteria. This protein complex presents a unique structure that adsorbs multiple photon energies (450-600 nm), transports this energy to the protein core, where it is fluoresced at a single wavelength (666 nm). It is well known that the energy transport properties of phycobilisomes are severely affected when slightly denatured. Therefore, these unique properties make Phycobilisomes an ideal protein complex to determine the stabilization efficacy of peptergents using simple optical techniques, like fluorescent spectroscopy.

Methods: Native phycobilisomes were isolated from *Porphridium Cruentum*, purified by Martek Biosciences and received in stabilizing buffer (0.75 M PBS, pH ~ 7). Peptergents (ac-A6D and ac-A6K-NH_2) were used as supplied (CPC Scientific) without further purification. Briefly, phycobilisomes (1 ng/mL) were mixed with peptergent-0.75M PBS solutions (0, 1, 2, 4 and 8 critical aggregation concentrations (CAC)). Phycobilisome-peptide solutions were dialyzed (3400 MWCO) against PBS solutions of various molarity (0.6 – 0.01M) for 18 h, in the dark. Room temperature fluorescent spectroscopy of these solutions was conducted using an excitation wavelength of 540 nm and emitted fluorescence (550 – 700 nm) was collected. UV-Vis spectra were obtained to confirm solution concentrations for all acquired fluorescent data.

Results/Discussion: Fig. 1 summarizes one set of acquired fluorescence data for phycobilisomes in various solutions. The stabilized control represents the phycobilisomes in 0.7M PBS buffer, which is well known to yield stabilized phycobilisomes. It is evident that upon changing the buffer to 0.4M PBS, the intensity of the 666 nm fluorescent peak decreases to background. UV-Vis data (not shown) illustrate that the concentration of phycobilisome in the unstable control is only ~ 15% less than that of the stabilized control; thus, the lack of 666 nm signal is not due to the lack of phycobilisome but rather due to protein denaturation. Finally, it is evident that upon addition of both A6D and A6K the phycobilisome maintains its functional structure, yielding 666 nm intensities of 1.3 and 0.7 x10^5 cps. Both A6D and A6K solutions were similar in phycobilisome concentration, both being ~ 15% lower than the concentration of the stabilized control solution. Concentration normalized signal strength for A6D stabilized phycobilisomes is very similar to the stabilized control. However, the presence of a new peak at ~600 nm may indicate a slight perturbation of the phycobilisome structure that is preventing optimal energy transfer to the core of the protein complex.

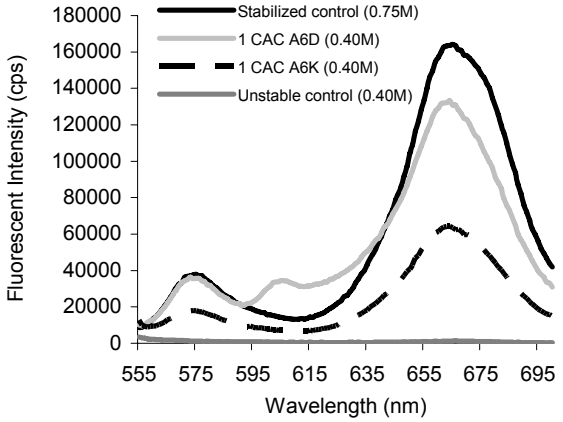

Figure 1. Aquired fluorescent spectra for phycobilisomes in : stabilization buffer (0.75 M PBS,), 1xCAC A6D (0.4 M PBS,), 1xCAC A6K (0.4 M PBS,), unstable control (0.4 M,).

Conclusions: Work is continuing, looking at the mechanisms involved in peptide-surfactant stabilization of large protein complexes. However, the results presented here summarize an elegant method of determining the ability of peptergents to stabilize large protein complexes. These results show that peptergents are powerful molecules that are able to stabilize large, membrane-associated, protein complexes, representing significant progress in the development of these technologies for protein-based biotechnological devices.

References: 1. Kiley, P. *et al.,* PloS Biology. 2005; 3, 1181-1186.