NON-CLASSICAL EXPORT OF SIGNAL PEPTIDE-LESS PROTEINS STUDIED VIA VIBRATIONAL SPECTROSCOPY AND LIPOSOME DESTABILIZATION TECHNIQUES

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A growing number of secreted proteins have been demonstrated to be devoid of classical signal sequences in their primary structures and thus are unable to utilize the endoplasmic reticulum (ER)-Golgi pathway for their release. The signal peptide-less (SPL) proteins however play a significant role in the regulation of a variety of critical cellular activities such as growth, differentiation, and migration. Elucidation of the transport mechanism may potentially result in the design of therapeutics targeted at regulating transport and hence the management of clinical disorders induced by SPL polypeptide transport. For example, the fibroblast growth factor (FGF) gene family includes the prototype members FGF-1 and FGF-2 that lack signal peptides, are major regulators of embryogenesis, and play critical roles in angiogenesis, certain types of cancer, and restenosis. Structural deformations of lipid hybrid bilayer membranes induced by signal peptideless (SPL) proteins have been studied using the inherently surface specific nonlinear optical technique of sum frequency generation vibrational spectroscopy (SFS). Specifically, deformations of 1,2 distearoylphosphatidylglycerol (DSPG) membranes induced by interaction with FGF-1 have been investigated. FGF-1 was found to induce lipid alkyl chain deformations in previously highly ordered DSPG membranes at the extremely low concentration of 1 nM at $60\ ^{\circ}$C. The deformation process was shown to exhibit a degree of reversibility upon removal of the protein by rinsing with buffer solution. This work has been complemented with biochemical studies utilizing mutant versions of SPL proteins; specifically FGF-1 and the SPL proteins that mediate its release. Liposome destabilization techniques along with protein localization and secretion studies have shown that the phospholipid binding region of each SPL polypeptide is critical for protein localization to the cellular periphery prior to transport. Point mutations in the phospholipid binding pocket drastically attenuated the proteins secretion in live cells as well as the ability of the protein to destabilize liposomes containing fluorescent molecules.