ANALYTICAL CHARACTERIZATION AND FORMULATION ASSESSMENT OF MODEL SECRETORY-IMMUNOGLOBULIN-A (sIgAs) FOR THEIR POTENTIAL USE AS LOW COST, ORALLY DELIVERED sIgAs

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Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of bacterial diarrheal disease in developing countries, especially among children and infants. ETEC is estimated to cause 280-400 million diarrheal episodes per year in children <5 years of age, resulting in 300,000 to 500,000 deaths.[1] Despite the need for a vaccine, there are currently no licensed vaccines against ETEC. Alternatively, passive immunization by oral delivery of pathogen-specific immunoglobulins is another promising approach to provide "instant" protection against ETEC. The potential advantages of oral delivery are reduced cost, simplicity of administration and localized treatment within the GI tract. Secretory IgA (sIgA) is of particular interest because it is naturally found in the mucosal surfaces within the GI tract, relatively more resistant to proteolysis by digestive enzymes (vs. IgGs), and can protect against enteric bacteria by directly neutralizing virulence factors.[2] One major challenge of this approach is the instability of protein molecules during oral delivery (in the digestive tract) as well as during long-term storage (in various formulations). In this study, two proteins, sIgA1 and sIgA2 against heat labile toxin (LT, one of the major virulence factors of ETEC), were used as model sIgA molecules for developing analytical techniques and assessing stability (physicochemical as well as *in vitro* binding) under various conditions. A combination of biochemical and biophysical methods were employed to comprehensively characterize the sIgA1 and sIgA2 model proteins including primary structure, post translational modifications (i.e., N-linked glycans), size, apparent solubility, higher order structure and conformational stability as well as *in vitro* antigen binding. Using these characterization and stability indicating methods, we are monitoring the stability of these two model sIgAs both in an *in vitro* digestion model (to mimic *in vivo* degradation conditions), and during accelerated stability studies (to assess storage stability). Our goal is to use the information gained by these aforementioned methods and stability studies to design stable, low-cost liquid formulations for oral delivery of sIgAs in the developing world.

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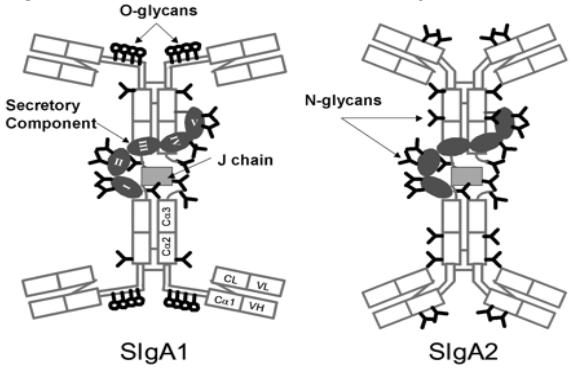

Figure 1: Structure of sIgA [3]