APPLICATION NOTE

AN1614 Multi-protein complexes identified by SEC-MALS

Bradford Powell, Ph.D., Jeffrey Enama, Ph.D. and Jeffrey Adamovicz, Ph.D., USAMRIID

Introduction

The Yersinia pestis injectosome is a multi-protein apparatus that transfers bacterial virulence factors into targeted host phagocytes. This is an essential part of plague pathogenesis and contributes to the evasion of mammalian immune defenses. The Y. pestis injectosome consists of a basal structure, the type III secretion system (T3SS) spanning both membranes of the bacterial envelope, upon which assembles a hollow needle-like structure. On specific adhesion and docking with a phagocyte, the injectosome transfers a set of secreted anti-host effector proteins called Yersinia outer proteins (Yops) directly into the host cell, some of which prevent bacterial engulfment and block the normal production of proinflammatory cytokines. Genetic regulation of these virulence factors is known to include heat-inducible and calcium-repressible pathways, so that their production increases at 37°C and by dilution of solution calcium to less than millimolar content—the low calcium response (lcr). The gene products LcrG and LcrV comprise an important part of a proposed molecular gate which controls the efflux of Yops and allows their release only after successful docking with the host cell. The biochemical mechanism of gating the transfer of Yops is not yet understood, but is also suggested to involve calcium.

Analytical size exclusion chromatography coupled to absolute molar mass measurement by multi-angle light scattering (SEC-MALS) with a DAWN® multi-angle light scattering (MALS) detector demonstrates that each of these component proteins exhibits its predicted molar mass at 37°C, including purified monomeric and dimeric forms of LcrV (Fig. 1).

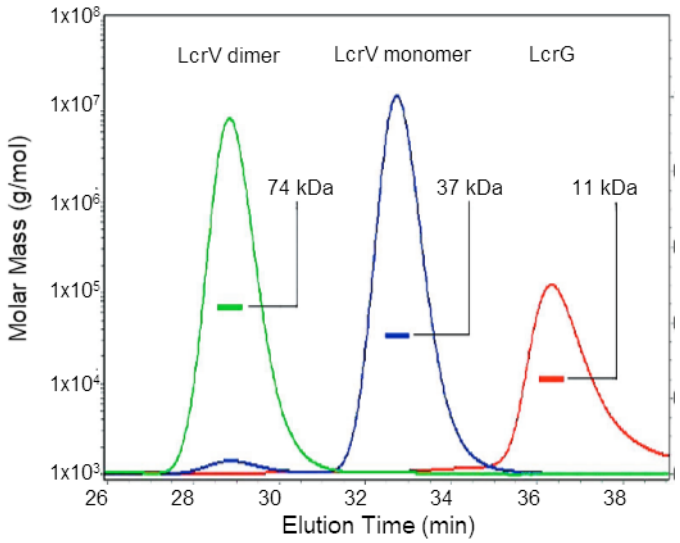

Figure 1. Overlay of SEC-MALS analyses of individual purified virulence proteins. Red curve, pure LcrG; blue curve pure LcrV monomer; green curve, pure LcrV dimer. Left y-axis, molar mass; Right y-axis, incremental refractive index and relative concentration of chromatographic peaks; x-axis, elution time; thick lines, measured mass (italics) for each peak.

Results

After mixing equal weight amounts of LcrG and dimeric LcrV, SEC-MALS shows an abundance of the 48 kDa heterodimeric LcrV-LcrG form (Fig. 2, blue curve, peak 3) compared to the individual components (peaks 2 and 4).

Although LcrG clearly breaks the covalent LcrV dimer (peak 2), no free monomeric LcrV is observed and excess LcrV appears to associate into a trimeric complex (peak 1). Interestingly, the presence of 5 mM $CaCl_2$ inhibits formation of the LcrV-LcrG heterodimer (red vs. blue curves, peaks 2 & 3). Even though LcrG is in molar excess (peak 4), a notable portion of LcrV remains dimeric (peak 2) and apparently does not react under either of these conditions. This suggests regulation via threshold change

in component equilibrium, or the participation of factors or conditions not present in these experiments.

This study was facilitated by the capability of the SEC-MALS system to provide direct and absolute mass measurements while allowing adjustments to be made in experimental temperature, solvent chemical composition, and protein concentration.

These results are helping to deconstruct the mechanism of gating by the Y. pestis injectosome, and similar SEC-MALS applications can provide invaluable insight for understanding other soluble protein-protein associations.

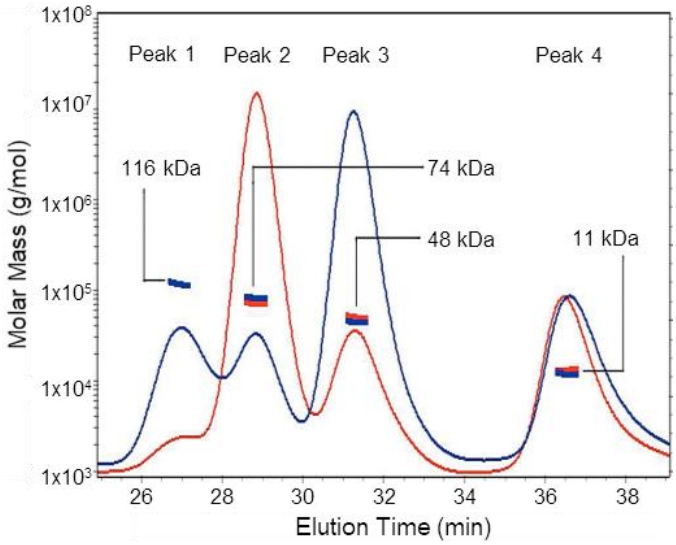

Figure 2. Comparison of SEC-MALS of multi-protein complex formation as a function of solution content. Blue curve, buffer contains no $CaCl_2$; red curve, buffer contains 5 mM $CaCl_2$. Chromatographic peaks are numbered sequentially and measured molar masses labeled (italics).