A Constrained Peptide that Targets the TLR4/MD2 Interaction

&

Investigating the Mechanism of Ultra-stability in Bacterial Chemosensory Arrays

by

Peter F. Slivka

B.S., Moravian College, 2007

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Doctor of Philosophy

Department of Chemistry and Biochemistry

2012

This thesis entitled:
A Constrained Peptide that Targets the TLR4/MD2 Interaction
Investigating the Mechanism of Ultra-stability in Bacterial Chemosensory Arrays
written by Peter F. Slivka
has been approved for the Department of Chemistry and Biochemistry

_____
Joseph J. Falke

_____
Hang (Hubert) Yin

Date_____

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

Slivka, Peter F. (Ph.D., Chemistry)

A Constrained Peptide that Targets the TLR4/MD2 Interaction

Investigating the Mechanism of Ultra-stability in Bacterial Chemosensory Arrays

Thesis directed by Professor Joseph J. Falke

Pathological pain is a serious health problem that is initiated and perpetuated by Toll-like Receptors (TLRs) on glial cells. Among the TLRs, Toll-like Receptor 4 (TLR4) is one of most studied and most significant members of the TLR family that organizes an innate immune response by recognizing exogenous and endogenous danger signals. Specifically, TLR4 recognizes lipopolysaccharide (LPS) from the cell wall of Gram-negative bacteria, as well as endogenous signals such as HSP70, HSP90, and HMGB-1. Agonism of the receptor is dependent upon the accessory protein MD2 which is responsible for binding LPS and mediating the interaction between TLR4 receptors in an active signaling unit. The recent crystal structures of the TLR4/MD2 complex demonstrate that all of the critical residues for the MD2 interaction with TLR4 are localized to one stretch (C95-E111) of MD2. Moreover, this stretch of amino acids is constrained by a crucial disulfide bond (C95-C105). The proximity of these critical features suggests that an MD2 based synthetic peptide incorporating these critical elements could compete with full-length MD2 for the TLR4 binding interface and subsequently prevent signaling. This study investigates the feasibility of such an approach and demonstrates that a 17 residue peptide based on the TLR4 binding region of MD2 can prevent full-length MD2 from associating with TLR4 and subsequently prevent TLR4 signaling.

Bacteria utilize large multi-protein chemosensory arrays to sense attractants and repellants in their environment. The essential components of these arrays are hexagonally

arranged core units consisting of receptor trimer-of-dimers, CheA histidine kinase, and CheW coupling protein. Incorporation of these units into arrays has several advantages including strong cooperativity and high sensitivity in ligand sensing, a large dynamic range and rapid signal transduction through the signalling circuit.

Another unique advantage of the array architecture is a striking ultra-stability *in vitro*: arrays retain kinase activity, attractant sensitivity and bound components for weeks. This work examines this remarkable ultra-stability and its origin in more detail. The results of this study demonstrate that arrays are not homogenous, but rather exhibit two major populations. One population is quasi-stable with a lifetime of 1-2 days, and loss of this population is highly correlated with proteolytic degradation of CheA kinase. The second population is truly ultra-stable with a lifetime of 20 days or more, and is less accessible to proteolysis. Following degradation of the less stable population, the cooperativity of the array increases, arguing that the less stable regions of array are not as well ordered and cooperative as the ultra-stable regions. To test the hypothesis that a well-ordered array is required for ultra-stability, we have introduced a small density of defects into the complex through chemical modification. Notably, even a very low degree of packing defects can abolish array ultra-stability, supporting the hypothesis. These findings are consistent with a model in which cooperativity and ultra-stability arise from extensive interconnectivity between multiple components within a well-ordered array.