Binding of the kringle-2 domain of human plasminogen to streptococcal PAM-type M-protein causes dissociation of PAM dimers

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Abstract

M-protein (PAM) largely contributes to the pathogenesis of Pattern D Group A Streptococcus pyogenes (GAS). However, the mechanism of complex formation is unknown. In a system consisting of a Class II PAM from Pattern D GAS isolate NS88.2 (PAMNS88.2), with one K2hPg binding a-repeat in its A-domain, we employed biophysical techniques to analyze the mechanism of the K2hPg/PAMNS88.2 interaction. We show that apo-PAMNS88.2 is a coiled-coil homodimer (M.Wt. ˜80 kDa) at 4°C - 25°C, and is monomeric (M.Wt. ˜40 kDa) at 37°C, demonstrating a temperature-dependent dissociation of PAMNS88.2 over a narrow temperature range. PAMNS88.2 displayed a single tight binding site for K2hPg at 4°C, which progressively increased at 25°C through 37°C. We isolated the K2hPg/PAMNS88.2 complexes at 4°C, 25°C, and 37°C and found molecular weights of ˜50 kDa at each temperature, corresponding to a 1:1 (m:m) K2hPg/PAMNS88.2 monomer complex. hPg activation experiments by streptokinase demonstrated that the hPg/PAMNS88.2 monomer complexes are fully functional. The data show that PAM dimers dissociate into functional monomers at physiological temperatures or when presented with the active hPg module (K2hPg) showing that PAM is a functional monomer at 37°C.

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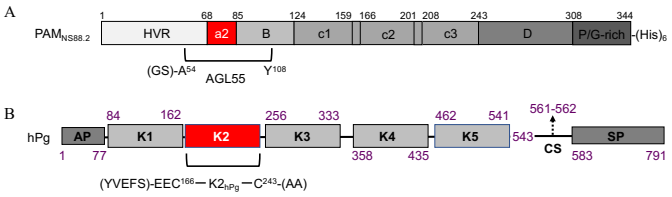

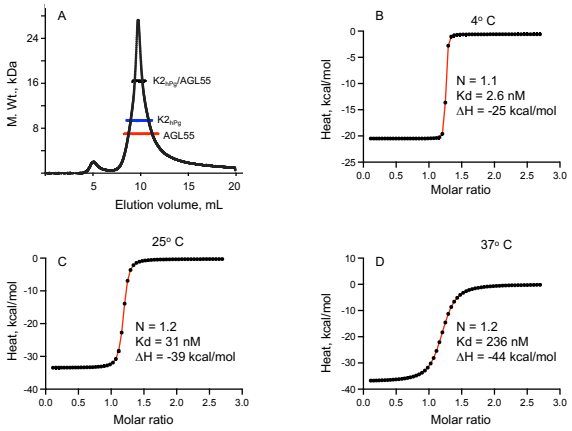

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