

47 kDa Protein of *Orientia tsutsugamushi* Do a Critical Role in Invasion to Eucaryotic Cells by Binding to Cell Surface Heparan Sulfate

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Heparan sulfate proteoglycan can act as a receptor for many microorganisms including bacteria, virus, and protozoan. In the previous study we investigated the role of heparan sulfate in *Orientia tsutsugamushi* infection, and we showed that *O. tsutsugamushi* bound to heparin and cell surface heparan sulfate was critical for *O. tsutsugamushi* infection. To analyze the invasion mechanism of *O. tsutsugamushi*, identification of heparin-binding protein of *O. tsutsugamushi* was performed.

To determine the heparin-binding protein of *O. tsutsugamushi*, the outer membrane fraction of *O. tsutsugamushi* was reacted with heparin-Sepharose, and eluted with column buffer containing various concentrations of NaCl. 47 kDa protein, one of major outer membrane proteins of *O. tsutsugamushi* was revealed to bind heparin by this heparin affinity-chromatography. Thus the function of 47 kDa protein as a cytoadhesin was directly investigated by using recombinant 47 kDa protein

expressed in *E. coli*. 47 kDa encoding gene was cloned into pTYB12 vector and overexpressed in *E. coli* ER2566, and confirmed by SDS-PAGE and western blot. Binding of this recombinant protein to heparin was confirmed by dot blot with radiolabeled heparin. Recombinant 47 kDa protein were bound to heparin and eluted by 1 M NaCl in a similar manner with intact 47 kDa protein in heparin affinity-chromatography. In addition to binding to heparin, recombinant 47 kDa protein inhibited *O. tsutsugamushi* infection into L929 fibroblast cells in a dose-dependent manner, however recombinant 56 kDa protein, one of the major outer membrane proteins of *O. tsutsugamushi*, did not inhibit.

These results suggest that 47 kDa protein of *O. tsutsugamushi* do a critical role in *O. tsutsugamushi* infection as cytoadhesin by binding with cell surface heparan sulfate.