

Functional Interaction Analysis of GM1-Related Carbohydrates and *Vibrio cholerae* TOxins using Carbohydrates Microarray

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The development of analytical tools is important for understanding the infection mechanism of pathogenic bacteria or viruесе. In the present work, a functional carbohydrate microarray combined with a fluorescence immunoassay was developed to analyze the interactions of *Vibrio cholerae* toxin (ctx) proteins and GM1-related carbohydrates. Ctx proteins were loaded onto the surface-immobilized GM1 pentasaccharide and six related carbohydrates, and their binding affinities were detected immunologically. The analysis of the ctx-carbohydrate interactions revealed that the intrinsic selectivity of ctx was GM1 pentasaccharide >> GM2 tetrasaccharide > asialo GM1 tetrasaccharide > GM3 trisaccharide, indicating that a two-finger grip formation and the terminal monosaccharides play important roles in the ctx-GM1 interaction. In addition, whole cholera toxin (ctxAB5) had a stricter substrate specificity and a stronger binding affinity than only the cholera toxin B subunit (ctxB). Based on the quantitative analysis, the carbohydrate microarray showed the sensitivity of detection of the ctxAB5-GM1 interaction with a limit-of-detection (LOD) of 2 ngml⁻¹ (23pM).