

IgA plantibody against carbohydrate binding subunits of Shiga toxin

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Shiga toxins (Stx) are virulence factors of enterohemorrhagic *Escherichia coli* strains serotype O157:H7. Stx-specific secretory immunoglobulin A (IgA) is expected to prevent diseases by inhibiting toxin entry from the mucosal surface such as intestine. We have established hybridomas producing IgA and IgG monoclonal antibodies (mAb) specific for the carbohydrate binding subunit of Stx1 (Stx1B). After isolating cDNAs encoding these monoclonal antibodies, we have also made hybrid immunoglobulin genes with the variable region of IgG mAb and the heavy chain constant region of IgA mAb (termed hybrid-IgA). Upon expression in Chinese hamster ovary cells, we revealed toxin neutralization activity of the hybrid-IgA. If these mAbs can be expressed in plant, therapeutic or preventive effects would be expected by eating these plants containing “plantibody.” We attempted to make transgenic plants that express hybrid-IgA specific for Stx1B. DNA fragments corresponding to the heavy and light chains were subcloned into different binary vectors, and then introduced into *Arabidopsis thaliana* by floral dip method. After appropriate selection, several transgenic plants were obtained. When *IgA* genes in the transgenic plants were amplified by PCR, an alpha chain-specific as well as a kappa chain-specific DNA fragment was detected. Transcription of heavy and light chain genes were verified by RT-PCR method. As for protein synthesis, the hybrid-IgA was detected in the extracts of the transgenic plants by means of a sandwich ELISA. Furthermore, the plantibody was shown to bind to Stx1B. Finally, protein assembly of the hybrid-IgA was revealed by an immunoblot analysis. These results indicated that a monomeric functional hybrid-IgA was produced in an *Arabidopsis* system.