TECHNIQUES AND APPLICATIONS

S-layers put to therapeutic use?

Microbeads coated with a common component of bacterial cell envelopes could be at least 20 times more effective at removing auto-antibodies from the sera of autoimmune patients than are commercially available immunoadsorbents, according to results presented in a recent issue of *Applied and Environmental Microbiology*.

In many Bacteria and Archaea, the outermost component of the cell envelope comprises a two-dimensional crystalline array known as an S-layer. S-layers are composed of identical protein or glycoprotein subunits, which are linked non-covalently to each other and to the underlying cell envelope. The regular, high-density arrangement of functional groups, combined with the ability to self-assemble in suspension and recrystallize on a solid support, makes S-layers prime candidates for biotechnological manipulation.

In this work, Völlenkle *et al.* created a fusion protein construct that contained the coding sequence for two copies of a synthetic domain based on immunoglobulin G (IgG)-binding domain B from *Staphylococcus aureus* protein A, a short linker region and the coding region from the 3′ end of the gene encoding a truncated form of an S-layer protein from *Bacillus sphaericus*. The S-layer protein sequence encompassed a binding region for a cell wall component known as a secondary cell wall polymer (SCWP) and a self-assembly domain.

The fusion protein was overexpressed in *Escherichia coli* and purified. After checking that it could crystallize on a solid surface and retain its IgG-binding capacity, Völlenkle *et al.* went on to recrystallize the fusion protein on the surface of cellulose-based SCWP-coated microbeads, which can be used in a plasma apheresis system. Surface plasmon resonance analysis revealed that the IgG-binding capacity of microbeads coated with a fusion protein monolayer was 78% of the theoretical saturation capacity, and 65% of the theoretical saturation capacity if the fusion protein monolayer was crosslinked. Batch adsorption experiments demonstrated that the IgG-binding capacity of microbeads that were coated with the fusion protein and crosslinked was more than 20 times greater than that of commercially available immunoadsorbent particles. Additionally, tests proved that both the cytotoxicity and the levels of endotoxin in the microbead preparations were low.

In this proof-of-concept study, Völlenkle *et al.* have exploited the ability of S-layer proteins to recrystallize into closed monolayers on a solid surface to create a novel immunoadsorbent capable of binding IgG. The increased binding capacity and biocompatibility of the coated microbeads, together with the fact that IgG can be eluted from the beads without decreasing their binding capacity, makes this technology an attractive prospect for making the transition from the laboratory to the clinic.

Sheilagh Clarkson

References and links

ORIGINAL RESEARCH PAPER Völlenkle, C. *et al.* Construction of a functional S-layer protein comprising an immunoglobulin G-binding domain for development of specific adsorbents for extracorporeal blood purification. *Appl. Environ. Microbiol.* **70**, 1514–1521 (2004)