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LETTER TO THE EDITOR

Efficient endotoxin removal from T7 phage preparations by a mild detergent treatment followed by ultrafiltration

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Phage display technology has been playing a crucial role in disease diagnostics, vaccine development, tumor imaging and targeted gene and drug delivery. However, phage preparations contain large amounts of bacterial LPS that are released upon cell lysis. Even minute amounts of LPS from protein preparations (1 ng/ml) can induce pyrogenic reactions and septic shock in humans and experimental animals. LPS monomers with an estimated molecular weight of 10–20 kDa, form highly stable micelles and vesicles (>1000 kDa). Lipid A, the toxic moiety of the LPS molecule, is extremely stable compared to proteins, resisting extreme pH and temperatures. Furthermore, LPS shows a remarkable capability to interact with proteins mainly through electrostatic interactions (*1*). Therefore, efficient removal of LPS is an ongoing challenge in biological research and pharmaceutical industry and a lot of research is being focused on this issue. So far, various techniques including phase separation (*2*), anion exchange and affinity chromatography (*3–6*) have been investigated to remove LPS from recombinant protein solutions with varying degrees of success.

In this study, a combination of DOC treatment and UF was used to remove bacterial LPS from T7-S-tag phage preparations. The oligonucleotide sequence encoding S-tag peptide (KETAAAKFERQHMDS) plus a glycine-glycine-glycine-serine (GGGS) spacer was cloned into *Eco*RI/*Hind*III digested and dephosphorylated T7Select415-1b vector (Novagen, USA). Then, ligated genome was *in vitro* packaged using T7 phage packaging extract (Novagen, USA) and titer was determined by plaque assay according to manufacturer's instructions. Plaques derived from *in vitro* packaging reaction were screened for S-tag peptide display by a standard plaque lift assay (*7*). Correct insertion of S-tag sequence into T7 phage genome was further confirmed by PCR and sequencing using Taq DNA polymerase (Fermentas) and the primers provided by the manufacturer. Structural proteins of T7-S-tag phages were characterized by SDS-PAGE and western blot. T7-S-tag phages were amplified in *Escherichia coli* BL21 culture and precipitated by PEG/NaCl as described (*7*). Considering unique resistance of T7 phage against detergents (*8*), samples of T7-S-tag phage preparation were treated with 1% DOC detergent and incubated with shaking at 37°C for 60 min to dissociate tightly bound LPS molecules and disrupt the vesicles and micelles to monomers and small aggregates removable by UF. An important advantage of de-

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Abbreviations: DOC = deoxycholate; LPS = lipopolysaccharid; MWCO = molecular weight cut-off; MMW = micellar molecular weight; UF = ultrafiltration; VLP = virus-like particle

Table 1. Efficiency of DOC treatment and ultrafiltration in removal of endotoxin from T7 phage preparations and preservation of their titer

Cycles of DOC treatment and UF			Non-treated	Characteristic assayed
3rd	2nd	1st		
0.83	537	1×10^4	1.7×10^6	LPS (EU/ml)
5×10^{11}	6.5×10^{11}	8×10^{11}	9×10^{11}	phage titer (PFU/ml)

oxycholate is its small MMW (4.2 kDa) which makes it easy to remove by UF. The DOC-treated T7-S-tag phage sample was filtered through a Triton X-100-passivated polyethersulfone membrane with 100 kDa MWCO (Sartorius, Germany) using centrifugation at 5000 x g. LPS concentration was measured in the retenate using a sensitive colorimetric Limulus Amebocyte Lysate QCL-1000® kit (Lonza, USA) and phage recovery was analyzed by standard plaque assay. A previously described phase separation method using Triton X-114 detergent was used to compare the efficiency of our LPS removal strategy (*2*).

The S-tag peptide was successfully displayed as a fusion to 10B capsomers of T7 phage as shown by plaque lift assay, SDS-PAGE and western blot analysis (data not shown). A single round of DOC treatment and UF was able to reduce LP concentration by 42% without any significant loss of the phage recovery (Table 1). After three cycles of DOC treatment and UF, LPS was reduced to 0.83 EU/ml which is in acceptable range for *in vivo* applications. However, UF alone reduced LPS concentration by 9% (data not shown). This indicates that a major fraction of LPS molecules in the T7-S-tag phage preparation existed as large micelles and vesicles which could be disrupted by DOC treatment. A two-phase separation method using Triton X-114 was able to remove LPS from T7-S-tag preparations efficiently. However, four sequential cycles of phase separation were required to reduce endotoxin level to 0.8 EU/ml at the expense of 10% reduction in the recovered volume and 2-fold reduction in the titer of T7-S-tag phage (data not shown).

In conclusion, our data show that treatment of T7 phage preparations with DOC detergent followed by UF removes LPS efficiently without significant loss of phages. Furthermore, DOC was easily removed from the T7 phage preparation by UF. Taken together, our data suggest that this procedure would be worth to be tested for removing LPS from other types of phage particles such as Lambda and T4 or various VLPs produced in *E. coli* with the aim of cell culture and *in vivo* applications.

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