PROTOCOL

Expression and Purification Strategies for the Production of Single-Chain Antibody and T-Cell Receptor Fragments in E. coli

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Abstract
This work describes protocols for the production of single-chain antibody and T-cell receptor fragments in E. coli. A choice of methods is given for the purification of the recombinant fragments that rely on the use of either immunoaffinity or metal chelate affinity chromatography. The TCR fragments may have to be denatured and refolded before the fragments attain their proper conformation.

Index Entries: scAb; antibody fragments; immunoglobulins; scTCR; T-cell receptors; metal chelate affinity chromatography; bacterial expression systems.

1. Introduction
There has recently been an upsurge of interest in producing immunoglobulins as recombinant fragments expressed in Escherichia coli. These fragments are intended for a variety of uses which take advantage of their relatively low molecular weight. Medical uses range from tissue imaging to the specific delivery of therapeutic drugs to tumors. They have also been used to study antibody–antigen interactions at the molecular level and have been suggested as possible environmental clean-up agents (1). Perhaps their widest use, however, will be for the display of immunoglobulin repertoires on the surface of filamentous phage libraries as a more rapid alternative to standard hybridoma technology (2).

Large numbers of antibody fragments have been expressed in the form of single-chain antibodies (scFv), disulfide-linked Fvs, Fab, and disulfide linked Fab fragments (3). As a result, the conditions for the efficient production of soluble, functional antibody fragments in E. coli are well established (4). Advances in the production of antibody fragments in E. coli have led to the application of this technology to the production of recombinant T-cell receptor (TCR) fragments, as it is thought that both molecules are likely to fold in a similar manner. The generation of a complete panel of anti-TCR antibodies would be extremely useful to facilitate investigation of the human TCR repertoire. Soluble TCR molecules may be exploited in the production of these antibodies. Soluble TCRs could also provide an important contribution to the advancement of disease immunotherapy. For example, Acha-Orbea and colleagues were able to reverse and prevent experimental allergic encephalopathy in a murine model using an anti-TCR monoclonal antibody (5). As TCRs are naturally membrane bound, soluble recombinant TCRs will also facilitate the study of the molecular interactions between the TCR, major histocompatibility complex molecules, and peptide.

The conditions for the efficient production of single-chain TCRs have, however, not proved as easy to establish. Two cases of the production of soluble single-chain TCR in the periplasm have been reported (6, 7), but in most cases single-chain
Fig. 1. A schematic diagram of vectors pPM1-His and pD3-TCR that we use to express scAb and scTCR, respectively. The construction of pPM1-His is described in ref. (8). pD3-TCR contains a (Gly4 Ser)3 linker and was derived from Pet 22 (Novagen, Madison, WI), which is under the transcriptional and translational control of the T7 RNA polymerase promoter. This vector was constructed in a similar manner to pPM1-His. Both single chain molecules are expressed with a Pel B (Pb) leader sequence. Useful restriction sites and endogenous immunoglobulin and TCR variable domain genes are also shown on both vectors.

TCRs have been found to form inclusion bodies that must be resolubilized and the protein refolded.

In this work we describe the vectors and protocols we use routinely to produce recombinant single-chain antibody fragments and TCRs. As our single-chain antibody expression vector (pPM1-His, Fig. 1) contains a human \( \kappa \) constant region, we refer to the product of this vector as a scAb (Fig. 2) to underline its structural difference from single-chain Fv molecules that contain no constant domains. The \( \kappa \) constant region is present to facilitate immunological detection and purification of the scAb fragment.

2. Equipment and Materials

Specific reagents and equipment are available from a variety of suppliers. Here we have listed those used by our laboratory. All \( \text{H}_2\text{O} \) has been treated in a Milli-Q water purification system (Millipore Ltd., London, UK) and all solutions with the exception of the antibiotics and solutions used in gel electrophoresis and Western blotting, are routinely sterilized in an autoclave (model EV150 Prior Clave Ltd., London, UK) at 120°C and 2 bars for 15 min.

2.1. Bacterial Strains and Plasmids

1. \( E. \text{coli} \) XL1blue (supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lacF' [proAB + lacIq lacZ M15 Tn10 (ter)] ) was used as host for expression of scAb. \( E. \text{coli} \) BL21 (DE3) (F-, ompT, \( rB^- \), \( mB^- \)) was used as host for the expression of TCR.
2. Plasmid pPM1-His (8) was used as the expression vectors for the production of single-chain antibody fragments (Fig. 1).
3. Plasmid pET-22 (NBL Gene Sciences Ltd., Cramlington, UK) was used to construct the TCR expression vector pD3-TCR (Fig. 1).

2.2. Bacterial Growth and Expression

1. Controlled environmental orbital shaker (New Brunswick Scientific Ltd., Hatfield, UK).
2. LKB Biochrom ultraspectrophotometer 4050 (Pharmacia-Biotech Ltd., St. Albans, UK).
3. Sorvall RC-5B refrigerated superspeed centrifuge with GS3 and SS34 rotors (DuPont Ltd., Stevenage, UK).
4. 2X YT broth: 16 g bactotryptone, 10 g bactoyeast extract, 5 g NaCl made up to 1 L with distilled water. 1.5% (w/v) agar was added to the broth prior to autoclaving when solid media were required.
5. 5X M9 salts: 33.9 g Na2HP O, 15 g KH2PO4, 2.5 g NaCl, 5 g NH4Cl made up to 1 L with distilled water.
6. 1M MgSO4.
7. 1M CaCl2.
8. 1% (w/v) thiamine in distilled water: Sterile filter the solution by passing it through a 0.2-\( \mu \)m disposable filter (Sartorius Ltd., Belmont, UK).
9. 20% (w/v) glucose.
10. 50 mg/mL ampicillin in distilled water: Sterile filter the solution by passing it through a 0.2-\( \mu \)m disposable filter.
11. 12.5 mg/mL tetracycline in ethanol: No sterilization required.
12. 0.84M isopropylthio-\( \beta \)-D-thiogalactopyranoside (IPTG) sterile filtered through a 0.2-\( \mu \)m disposable filter.