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Research paper

Ribosome display of antibodies: expression, specificity and recovery in a eukaryotic system

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Abstract

In ribosome display, proteins are linked to their encoding genetic material as protein-ribosome-mRNA complexes. The technology has been applied to the isolation of antibodies and other proteins from large PCR-derived libraries. Here we demonstrate the specificity of eukaryotic ribosome complexes and investigate recovery and display procedures using a single chain version of the anti-progesterone monoclonal antibody DB3. Complexes are formed by deletion of the 3′ stop codon in a coupled rabbit reticulocyte system. Using inhibition with different steroid probes, we show that the fine specificity of the combining site expressed as a nascent protein is closely similar to the native monoclonal, indicating correct folding and function while bound to the ribosome. We have demonstrated that the 3′ end of the mRNA is blocked by the stalled ribosome and unavailable to primers. Moreover, we show that an in situ RT-PCR recovery procedure, carried out on intact complexes, is more efficient than ribosome disruption and isolation of mRNA followed by RT-PCR. We also explore the Mg^{2+} and DTT concentrations and time required for efficient production of complexes. Our findings confirm the effectiveness of the eukaryotic ribosome display system and define conditions for efficient selection of single chain antibodies.

Keywords: Ribosome display; Single-chain antibody; Cell-free expression

1. Introduction

Library display technologies select individual proteins from large populations in vitro through coupling of genotype and phenotype (Smith, 1985; Winter et al., 1994). Typically, foreign proteins are expressed clonally on the surface of organisms (phage, bacteria, yeast, etc.); selection of a protein isolates the individual organisms carrying it and subsequently the specific encoding genes can be

Abbreviations: ARM, antibody-ribosome-mRNA complex; PBS, phosphate buffered saline; Prog-11α-HMS, progesterone-11α-hemisuccinate; Prog-11α-HMS-BSA, progesterone-11α-hemisuccinyl-BSA; Prog-3-CMO, progesterone-3-carboxymethyloxime; Prog-6β-HMS, progesterone-6β-hemisuccinate; Prog-21-HMS, progesterone-21-hemisuccinate; E3G-OVA, estrone-3-glucuronide-ovalbumin.

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recovered, manipulated and expressed. Such methods can also be used to improve protein function through repeated cycles of mutation and selection and, by integration with screening technologies such as protein microarrays, enable high throughput identification of protein–ligand interactions (Hayhurst and Georgiou, 2001).

As well as cell based methods, various means have been found of associating proteins directly, covalently or noncovalently, with their individual encoding mRNA or DNA using cell free transcription and translation systems (Jackson et al., 2004).

Ribosome display is one such method, in which stalling of ribosomes on mRNA molecules at the end of translation produces stable linkages of nascent proteins and their encoding mRNA molecules as ternary protein-ribosome-mRNA complexes (Mattheakis et al., 1994; Hanes and Plückthun, 1997; He and Taussig, 1997, 2002; Schaffitzel et al., 1999). Stalling may be achieved by deletion of the 3′ stop codon from the mRNA (Hanes and Plückthun, 1997; He and Taussig, 1997) or through use of antibiotics (Mattheakis et al., 1994). From a library of complexes, selection of an individual protein through ligand binding simultaneously selects the encoding mRNA, which can then be recovered as cDNA by RT-PCR. Cycles of selection and recovery can be reiterated to enrich initially rare ligand-binding molecules. Since the libraries used in cell free systems are often created by PCR, without requiring cell transformation, they have the benefits of very large size and avoidance of cellular toxicity or insolubility, hence improving the chances of protein discovery.

Ribosome display has been exemplified in both prokaryotic and eukaryotic systems and successfully used for selection and evolution of single-chain antibodies (Hanes and Plückthun, 1997; He and Taussig, 1997; Schaffitzel et al., 1999; He et al., 1999, 2004) ligand-binding proteins (Lamla and Erdmann, 2001; Irving et al., 2001; Binz et al., 2004), peptides (Mattheakis et al., 1994, Lamla and Erdmann, 2003) and enzymes (Amstutz et al., 2002). Our eukaryotic method uses rabbit reticulocyte lysate for coupled transcription and translation and deletion of the stop codon to create complexes (He and Taussig, 1997; He et al., 2004). When applied to antibody selection, this technology has been termed ARM (antibody-ribosome-mRNA) display. A distinctive feature is the use of an in situ RT-PCR procedure for DNA recovery, carried out on intact ribosome complexes and avoiding the need to isolate mRNA separately, a specific element of other ribosome display methods. Using as a model the single-chain, three domain V_H/K form of the DB3 anti-progesterone monoclonal antibody (Arevalo et al., 1993a; He et al., 1995), we report here further studies on the specificity and optimisation of the ribosome display process.

2. Materials and methods

2.1. Primers

T7Ab:5′GCAGC*TAATACGACTCACTATAG*GAAGAACAGACCACC**ATG**(C/G)AGGT(G/C)CA(G/C)CTCGAG(C/G)AGTCTGG-3′. The italicised sequence encodes the T7 promoter for in vitro gene expression, ATG (bold) encodes the initiation methionine and the underlined sequence is an *Xho*I site for cloning.

D1: 5′-GCTCTAGAACACTCTCCCCTGTTGAAGCT-3′, where the underlined sequence is an *Xba*I site.

D2: 5′-CGTGAGGGTGCTGCTCAT-3′

D3: 5′-GGGGTAGAAGTTGTTCAAGAAG-3′

2.2. Reagents

TNT T7 Quick kit (rabbit reticulocyte cell free extract) was from Promega; one-tube single step RT-PCR kit (Titan™) and Taq polymerase (Expanded™ high fidelity PCR system) were from Boehringer Mannheim; dNTP stock solutions (10 mM or 2.5 mM of each dNTP) were from Sigma; In vitro mRNA generation kit was from Stratagene; RNase-free DNase I was from Roche (UK); progesterone-11α-hemisuccinate (Prog-11α-HMS) and progesterone-11α-hemisuccinyl-BSA (Prog-11α-HMS-BSA) were from Sigma; progesterone-3-carboxymethyloxime (Prog-3-CMO), progesterone-6β-hemisuccinate (Prog-6β-HMS), progesterone-21-hemisuccinate (Prog-21-HMS), and estrone-3-glucuronide-ovalbumin (E3G-OVA) were from Steraloids, Wilton, NH.