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Assembly of functional antibodies from immunoglobulin heavy and light chains synthesised in *E. coli*

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ABSTRACT

Genes for a murine μ heavy chain and a λ light chain immunoglobulin have been inserted into bacterial expression plasmids containing the Escherichia coli trp promoter and ribosome binding site. Induction of transcription from the trp promoter results in accumulation of both light and heavy chain polypeptides in appropriate host strains. Both proteins were found as insoluble products. Following extraction and purification of the immunoglobulin containing fractions, antigen binding activity was recovered. The activity demonstrates essentially the same properties as the antibody from the hybridoma from which the genes were cloned.

INTRODUCTION

Immunoglobulin genes and their products represent one of the most extensively studied families of eukaryotic macromolecules. Immunoglobulin polypeptides are secreted proteins and are synthesised with an amino-terminal signal peptide which is cleaved to yield the mature protein. The expression of immunoglobulin genes in E. coli forms the initial stage in the production of antibodies produced via recombinant DNA techniques. Such antibodies would have many uses. For example, detailed studies on antigen-antibody interactions following alterations of the antigen combining site by site directed mutagenesis could be carried out, or the Fc regions of the molecules could be altered for specific uses such as binding to matrices for immunopurification. Thus, it is surprising that with the many studies on expression of eukaryotic genes in E. coli (1), little has been done on immunoglobulin genes. So far immunoglobulin genes have been expressed in modified forms at low levels in E. coli, usually as incomplete amino-terminal fusion proteins (2,3,4). In one case, a trpE-IgE fusion has been expressed at 10% total E. coli protein (5).

Here we describe the bacterial expression of a murine μ heavy chain and a murine λ light chain immunoglobulin cDNA. The Ig μ and λ genes used in these studies are from cDNA clones isolated from the hybridomas B1-8 and S43

(6), respectively. These hybridomas were raised to the hapten 4-hydroxy-3-nitrophenyl acetyl (NP) and produce IgM antibodies which are termed heteroclitic, that is binding a related hapten e.g. 4-hydroxy-5-iodo-3 nitrophenyl acetyl (NIP) more strongly than NP (7). The μ gene was cloned from the hybridoma line B1-8 (8) and the λ gene was cloned from the related hybridoma S43 (9). However, the sequence of the λ from S43 varied by only two amino acids from B1-8 λ sequence, assuming that B1-8 λ has germ-line sequence (9). Both changes were conservative and outside of the complementarity determining regions. So in effect, the antibody from B1-8 can be used to represent the parental monoclonal antibody.

The two polypeptides were synthesised in E. coli as native proteins lacking eukaryotic signal sequences and presumably possessing amino-terminal methionine residues. High levels of expression were achieved using the E. coli K12 strain E103S or E. coli B but only low levels of expression occurred in HB101 (10). Following solubilisation of μ and λ polypeptides expressed in the same cell or different cells, the protein products were purified and antigen binding activity recovered. This activity demonstrates essentially the same properties as those found for an NP binding IgM hybridoma antibody.

MATERIALS AND METHODS

Chemicals and Cloning Procedures

Materials were purchased as follows: restriction enzymes (Bethesda Research Laboratories and New England Biolabs), T4 DNA polymerase (P-L Biochemicals), DNase I (Sigma), radioisotopes (Amersham), rabbit anti-mouse IgM (Bionetics), rabbit anti-IgM (Tago), rabbit anti-λ (Miles), MOPC 104E an IgM ($\mu\lambda_1$) myeloma protein (Bionetics), calf intestinal alkaline phosphatase and S1 nuclease (Boehringer Mannheim). Unless otherwise stated cloning procedures were as described (10).

Oligodeoxyribonucleotides were synthesised by the phosphotriester procedures (11) and were designed to have the sequences; 5'-GATCAATGCAGGCTGTTGTG-3' (R45), and 5'-ATTCCTGAGTCACAACAGCC-3' (R44).

Bacterial strains and Plasmids

Plasmids were transformed into E. coli strain HB101, DH1, E. coli B (10) and E. coli K12 strain E103S (Dr. Lee Simon, Waksman Institute of Microbiology, Piscataway, New Jersey 08854-0759, personal communication), and grown in L-broth containing 0.1g carbenicillin per litre. Plasmids pABμ-11 (8) and pABλ1-15 (9) were a gift from Drs. A. Bothwell and D.

Baltimore. B1-8 proteins were gifts from Drs. M. Neuberger and T.Imanishi-Kari.

Pulse Chase Analysis

For pulse chase analysis inductions were set up as described above, except that the medium used consisted of: proline (0.3g/L), leucine (0.1g/L), Difco methionine assay medium (5g/L), glucose (60mg/L), thiamine (10mg/L), $CaCl_2$ (22mg/L), $MgSO_4$ (0.25g/L)m and carbenicillin (0.1g/L). During exponential growth cells were pulse labelled with 30 µCi/ml L-[^{35}S] methionine for 2 minutes, after which unlabelled methione (100 µg/ml) was added and the incubation continued for the times indicated.

Other Methods

Procedures used for bacterial lysis and fractionation were as described (12), as were procedures for inductions and protein assays (13).

Protein Purification

For further purification of the λ light chain, the cell debris were dissolved in 10mM Tris-HCl pH8.0, 25% formamide, 7M urea, 1mM EDTA and 2mM dithiothreitol. This material was loaded onto a DEAE Sephacel column (Pharmacia) (1 x 25cm at a flow rate of 5ml/hr) which had been equilibrated in 9M urea, 10mM Tris-HCl pH8.0, 1mM EDTA and 2mM DTT. The DEAE Sephacel column was developed using a 0-150mM NaCl gradient in loading buffer. The eluted peak of λ light chain immunoreactivity, corresponding to the major peak of protein was diluted to a final concentration of 2.25M urea, 10mM Tris-HCl pH8.0, 1mM EDTA, 2mM DTT and loaded onto an octyl-Sepharose column (Pharmacia) (2.5 x 10cm). Material was eluted by use of a urea gradient of 2.25-9M urea. The peak material was pooled, dialysed into ammonium bicarbonate and lyophilised.

The µ heavy chain was purified from 9M urea solubilised pellets by anion exchange chromatography and chromatofocussing (Pharmacia).

Reconstitution of Activity

Production of functional antibodies from E. coli expressing both heavy and light chains was achieved by lysing the cells and clarifying the supernatant by centrifugation. The insoluble material was washed, followed by sonication (3 times for 3 minutes), and finally dissolved in 9M urea, 50mM glycine-NaOH pH10.8, 1mM EDTA, and 20mM 2-mercaptoethanol. This extract was dialysed for 40 hours against 3 changes of 20 vols. of 100mM KC1, 50mM glycine-NaOH pH10.8, 5% glycerol, 0.05mM EDTA, 0.5mM reduced glutathione and 0.1mM oxidised glutathione. The dialysate was cleared by centrifugation at 30,000g for 15 minutes and loaded directly onto DEAE Sephacel, followed by

development with a 0-0.5M KC1 linear gradient in 10mM Tris-HC1, 0.5mM EDTA, pH8.0.

The purified Ig μ and λ were treated as above, except that no anion exchange chromatography was carried out. The preparation was finally dialysed into phosphate buffered saline, 5% glycerol, 0.01% sodium azide and 0.5mM EDTA pH7.4.

RESULTS

Construction of Plasmids for Expression of λ Light Chain

We chose to express the λ gene in E. coli by direct expression of the gene lacking the eukaryotic signal peptide but containing a methionine initiator residue at the amino-terminus (met lambda). The approach used for bacterial synthesis of met-λ was to reconstruct the gene in vitro from restriction fragments of a cDNA clone and to utilise synthetic DNA fragments for insertion into the bacterial plasmid pCT54 (12) (figure 1). As a source of light chain we used the plasmid pABλ1-15 which contains a full-length λ_1 light chain cDNA cloned into the PstI site of pBR322 (9). We have previously outlined the construction of plasmids for the expression of λ light chain (14).

A plasmid was isolated (designated pCT54 19-1) and shown to have the anticipated sequence except that there was an alteration at the fifth codon from GTG to ATG, changing the amino acid at this point from valine to methionine (figure 1). Valine is an invariant residue at this position in mouse λ chains. Methionine, however, is the residue most frequently found in mouse κ chains at this position (15).

As most E. coli mRNAs have 6-11 nucleotides between the Shine-Dalgarno (SD) sequence and the AUG (16) the distance in pCT54 19-1 was reduced by modification at the Cla1 site. Altering the distance between the SD sequence and the ATG has been demonstrated to alter the expression of a number of genes (13,14,17-20) presumably by placing the SD and ATG sequences in the optimal configuration for formation of the initiation complex. pCT54 19-1 was cut with Cla1 and incubated with S1 nuclease. The amount of S1 nuclease was adjusted so that some DNA molecules would lose 1-2 extra base pairs as a result of 'nibbling' by the enzyme. This DNA on religation with T4 DNA ligase and transformation into E. coli strain HB101 gave rise to a number of plasmids which had lost the Cla1 site. The nucleotide sequence across the modified region of two of these plasmids was determined (figure 1). pNP4 and pNP3 were shorter than pCT54 19-1 by 5 and 4 nucleotides

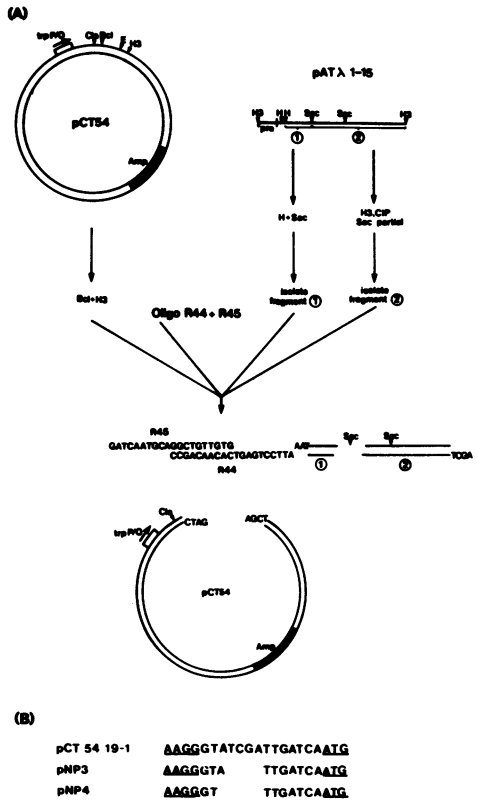

Figure 1. Construction of plasmids for the direct synthesis of λ light chain in E. coli.

Plasmid pATλ1-15 contains the λ gene inserted into the HindIII site of pAT153. (A) 5' HinfI - SacI fragment 1 was isolated by polyacrylamide gel electrophoresis. The 3' fragment 2 of the gene was isolated as a SacI - HindIII fragment. pCT54 was cut with BclI + HindIII and the λ gene fragments together with oligodeoxyribonucleotides R45 and R44 ligated to yield plasmid pCT54 19-1. (B) Digestion of pCT54 19-1 with ClaI and S1 nuclease produced plasmids pNP3 and pNP4, with reduced SD-ATG distances, E, EcoRI; H, HinfI; H3, HindIII.

respectively, giving SD-ATG distances of 9 and 10 nucleotides. Secondary structure analysis, as described (13), revealed no hairpin loop sequestering the SD or initiation codon into double-stranded regions of the mRNA of either pCT54 19-1 or the S1 derivatives. Such base pairing interactions have been shown drastically to reduce translational efficiency of a number of genes, notably those for phageλ cro (17), fibroblast and leukocyte

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