Note

Improved Production of Human Immunoglobulin γ_1 Chain Fc Polypeptides in *Escherichia coli* and Their PropertiesTsukio MASEGI, Satoshi NAKAMURA,*
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Received January 14, 1988

Human immunoglobulin G (IgG) is a major component of the immune system and is important in the biological defence against bacteria and viruses. The Fc region, com-

prising approximately the C-terminal half of the two γ chains, mediates most of the effector functions of the IgG molecule. We have reported the construction of hybrid plasmids which allow the direct expression of human γ_1 chain Fc region genes under the control of the *trp* promoter.¹⁾ Fc polypeptides were generated as monomeric forms in *Escherichia coli* harboring the expression plasmid and their yield was considerably lower. To improve the efficiency of Fc region gene expression, we derived several expression plasmids and examined their expression efficiencies in several *E. coli* hosts. Moreover, the properties of recombinant Fc polypeptides (rFc) were discussed.

pFC203¹⁾ is a plasmid containing the *trp* promoter and the Fc gene. Figure 1 shows the construction of several expression plasmids using the *tac* promoter.²⁾ The Fc gene was inserted just downstream from the *tac* promoter and the SD sequence of pDR540 (Pharmacia) to generate pFC361. A chemically synthesized DNA fragment was used to fuse the tandem stop codons immediately downstream of the Fc gene yielding pFC362. pAA41, a derivative of pYS31N,¹⁾ was used to construct pFC362A

FIG. 1. Construction of Several Expression Plasmids.

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FIG. 2. SDS-PAGE Analysis of rFc from *E. coli* C600r⁻m⁻ (pFC362A).

A SDS-12.5% polyacrylamide gel electrophoresis was done in the absence of 2-mercaptoethanol and stained with Coomassie Brilliant Blue R-250. Lane 1, purified rFc; Lane 2, native Fc polypeptides.

TABLE I. QUANTITATIVE ESTIMATION OF rFc

Plasmid	Promoter	No. of stop codons	<i>trpA</i> terminator	<i>rom</i> gene	Yield (mg/l)
pFC203	<i>trp</i>	1	—	+	0.06
pFC361	<i>tac</i>	1	—	+	0.12
pFC362	<i>tac</i>	2	—	+	0.31
pFC362A	<i>tac</i>	2	+	—	0.92

from pFC362. pFC362A contains the ρ -dependent *trpA* terminator and lacks the *rom* gene which modulates the plasmid replication. The construction of all these plasmids was confirmed by sequencing analysis according to the method of Maxam and Gilbert.³⁾

These derivative plasmids were introduced into *E. coli* C600r⁻m⁻ by the procedure of Norgard *et al.*⁴⁾ *E. coli* C600r⁻m⁻ cells carrying each plasmid were cultured in M9 medium containing 2% casamino acids, 0.2% glucose, and 30 μ g/ml ampicillin. The gene expressions were induced by adding 50 μ g/ml β -indolylacrylic acid [*E. coli* C600r⁻m⁻ (pFC203)] or 50 mM isopropyl- β -D-thiogalactopyranoside (the others). Cell extracts, prepared by sonication and centrifugation, were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE).⁵⁾ Fc polypeptides were detected by Western-blot analysis¹⁾ with rabbit anti human IgG-Fc antibody (Cappel) and measured by densitometric scan analysis of antigenically positive bands compared with the density obtained with varying amounts of native Fc polypeptides. Native Fc polypeptides were obtained by papain hydrolysis⁶⁾ of human IgG (Sigma).

As shown in Table I, replacing the *trp* promoter with the *tac* promoter, addition of tandem stop codons, insertion of *trpA* terminator, and deletion of the *rom* gene were all effective for Fc gene expression. *E. coli* C600r⁻m⁻ (pFC362A) produced 15 times as much Fc polypeptide as *E. coli* C600r⁻m⁻ (pFC203). *E. coli* HB101 (pFC362A) or *E. coli* JM103 (pFC362A) had a lower yield than that of *E. coli* C600r⁻m⁻ (pFC362A). pFC362A was stable in *E. coli*

C600r⁻m⁻ after 7 generations of culture without selective pressure. Miki *et al.*⁷⁾ reported that the copy number of a *rom* gene deleted plasmid was increased in the cells grown at 40°C. In case of *E. coli* C600r⁻m⁻ (pFC362A), more effective expression might be expected in 40°C culture.

rFc were purified from the cell extract of *E. coli* C600r⁻m⁻ (pFC362A) by affinity chromatography using rabbit anti human IgG-Fc antibody and analyzed by SDS-PAGE. As shown in Fig. 2, most of rFc, which were produced as monomeric forms in *E. coli*, were converted to dimeric forms like IgE-Fc polypeptides produced in *E. coli*.⁸⁾ These dimeric rFc moved a little faster than native Fc polypeptides which contained carbohydrate chains. The bands corresponding to rFc were not stained with periodic acid-Schiff reagent⁹⁾ (data not shown). These results suggested rFc were not glycosylated. rFc could also be purified by Protein A Sepharose CL-4B (Pharmacia) column chromatography, indicating that rFc had the same Protein A binding properties as IgG. Further characterization of rFc is in progress.

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