Short Communication

Expression of Synthetic Human Lysozyme Gene in *Escherichia coli*

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Human lysozyme is a hydrolytic enzyme acting on β-(1→4) glycosidic bonds of the polysaccharide of bacterial cell walls or chitin and its physico-chemical and enzymatical properties have been investigated by many researchers.1) From a medical point of view human lysozyme has a potential as a non-allergic anti-inflammation drug and so on.2) At present it is mainly obtained from human milk or placenta on a small scale, so it is industrially valuable to produce it in large quantities in microorganisms using recombinant DNA techniques. In this communication, we describe the summarized results of the chemical synthesis of the human lysozyme gene and its expression in *E. coli.*

Figure 1 shows the amino acids and nucleotide sequence of the synthetic gene for human lysozyme used in this work. We intend to express the synthesized gene in bacteria and yeast. Because the bias of codon usage in *E. coli* is not so strict as that in *S. cerevisiae,*20 the coding region was designed using the most frequently used codons in the genes of abundant proteins in *S. cerevisiae.* But when the choice disrupted the strategic location of restriction cleavage sites, other codons were used. For example, the codon GGC was used instead of GGT as a codon for Gly-72 to construct the restriction cleavage site, Bbel.

The oligomers were chemically synthesized by the phosphotriester method on a polymer support2) and ligated to give the total gene enzymatically, then cloned into the BamHI site of pBR322. The resulting plasmid was named pHLY 1 (Fig. 2). The sequence of the cloned gene was confirmed to be the same as the designed sequence by the Maxam–Gilbert method.5) The synthetic gene fragment digested from pHLY 1 was inserted into the BamHI site of pMY12-6 Amp-1, an expression vector plasmid in *E. coli* containing the tandem promoter structure of lambda pR and lambda pL, and the cl857 gene.6) The cl857 gene produces a temperature-sensitive repressor against lambda pL promoter. The resulting recombinant plasmid, which has the synthetic gene fragment in the ordered direction to the lambda pL promoter, was named pPL HLY-1 (Fig. 2).

*E. coli* SK1077 (lac mal rha xyl leu thr thy rna-19 pnp-7 str8) was transformed with pPL HLY-1. When the bacteria harboring pPL HLY-1 grown at 28°C in the early-log phase (OD660 =0.3) were transferred to grow at 42°C for 2 hr, a 14.7 K dalton protein, corresponding to human lysozyme, accumulated and the amount reached several percent of the total cellular proteins (Fig. 3). During this heat induction, the growth rate of bacteria harboring pPL HLY-1 was somewhat reduced, but neither lysis nor the death of cells was observed. The fact that the synthesized human lysozyme was found mainly in the cell debris fraction, but scarcely in the supernatant fraction of the sonicated *E. coli* cells suggests the presence of the protein in an insoluble and inactive form in the cell (Fig. 3).

The insoluble product was solubilized by 10% SDS at room temperature and the solubilized product was immunologically active to anti-human lysozyme rabbit antisera (Green Cross Co., Japan) by the conventional immunoprecipitation method (data not shown). The solubilized product was purified by gel filtration and reverse phase HPLC. The N-terminal 13 amino acid sequence was found to
be Met-Lys-Val-Phe-Glu-Arg-Cys-Glu-Leu-Ala-Arg-Thr-Leu- using an automatic gas phase protein sequencer. This sequence is the same as that of mature human lysozyme except that there is an additional methionine residue at the N-terminal of the protein due to the translational initiation codon.

As reported, eukaryotic proteins expressed at high levels in E. coli generally exist in a biologically inactive form because of the formation of inclusion bodies with incorrectly formed intra and intermolecular disulfide bonds. Therefore it is important to establish a method to solubilize the inclusion body and regenerate the biological activity of the protein synthesized in bacteria. However, since the active form of human lysozyme is potentially lethal to E. coli, the formation of an inactive protein in the cell may be advantageous to the industrial purpose, if the enzymatic activity is successfully regenerated by chemical treatments. Wetlaufer et al. reported the successful recovery of the biological activity of reduced human lysozyme using a rapid non-enzymatic regeneration system, and its application to our human lysozyme synthesized in E. coli is now under investigation.

REFERENCES
1) R. E. Canfield, S. Kammerman, J. H. Sobel and F. J.
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Fig. 2. Construction of Recombinant Plasmids, pHLY1 and pPL HLY-1.

Fig. 3. Expression of the Synthetic Human Lysozyme Gene in E. coli.