

modifications of BMP-2 do not occur in *E. coli*. These processes are critical for the biological activity of the expressed BMP-2 protein. In recent years, we have successfully developed an *E. coli* expression system to produce rhBMP-2, successfully restoring the biological activity of the BMP-2 protein after expression in *E. coli*. Our studies demonstrate that *E. coli*-expressed rhBMP-2 possesses potent activity inductive of osteoblast differentiation and bone formation *in vitro* and *in vivo*.¹³

Cloning and Expression of rhBMP-2 in *E. coli*

Expression of rhBMP-2

The hBMP-2 cDNA encoding the mature peptide of the BMP-2 protein was amplified from RNA extracted from human osteosarcoma U2-OS cells. DNA sequencing revealed a 321-base pair (bp) DNA fragment encoding C-terminal 107-amino acid of hBMP-2 protein. In the recombinant plasmid, pMX-BMP2, BMP-2 expression was under the direct control of a P_{LPR} thermoinducible promoter (Fig. 1A). RhBMP-2 expression in the DH5 α host cells was induced in incubation at 42°C. SDS-PAGE showed the molecular weight of rhBMP-2 to be about 12 kDa and the expression level of rhBMP-2 more than 15% of the total bacterial proteins after six-hour induction at 42°C.

Purification and renaturation of rhBMP-2

The recombinant human BMP-2 expression in DH5 α cells was in the form of inclusion bodies. The bacteria were harvested by centrifugation at 4°C and lysed by sonication in Tris-buffered saline (TBS). The *E. coli* inclusion bodies containing expression products were collected by precipitation of the bacterial lysates by centrifugation and then washed in 2% Triton X-100 buffer. The proteins in the inclusion bodies were dissolved in 8 M urea buffer and the supernatant then fractionated by DEAE-52 ion-exchange

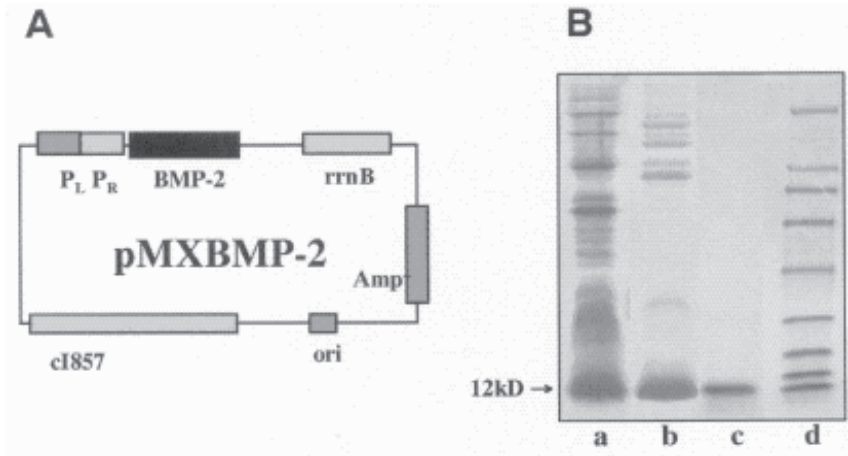


Fig. 1 Expression of rhBMP-2 in *E. coli*. (A) rhBMP-2 expression plasmid. P_LP_R: Thermo-inducible promoter; BMP-2: Coding region of BMP-2 mature peptide; rrnB: Transcription stop signal; and cI857: Coding sequence of aporepressor. (B) SDS-PAGE of rhBMP-2 expressed in *E. coli*. Lane a: Cell lysates from engineered bacteria induced at 42°C; lane b: Inclusion body; and lane c: Purified recombinant protein.

chromatography. Bound proteins were eluted from the column using a 10–1000 mM NaCl gradient in 8 M urea buffer. The BMP-2 fractions were collected and then fractionated on a gel filtration column of Sephacryl S-300. The proteins from whole lysate of bacteria, inclusion bodies and purified materials were electrophoresed on reduced SDS-PAGE gels (Fig. 1B). To restore the activity of the purified BMP-2, several refolding systems have been tested. The purified BMP-2 were collected and dialysed for three days against different refolding buffers at 4°C and then lyophilised. The best dialysis conditions for recovery of BMP-2 activity were against glutathione redox buffer as well as against distilled water at controlled dialysis speed. After these steps, the purity of the rhBMP-2 product was more than 95%, and the overall yield from *E. coli* was about 50–100 mg BMP-2/L *E. coli* culture.