

Biochemical Characterisation of *E. coli*-Expressed rhBMP-2

HPLC elution profile

To determine the physicochemical properties of the rhBMP-2 expressed in *E. coli* and to compare them with those of rhBMP-2 expressed in mammalian cells, we examined the HPLC elution profiles of rhBMP-2 expressed in *E. coli* and CHO cells. The purified rhBMP-2 from both sources was dissolved in 0.1% trifluoroacetic acid (TFA), loaded onto a C₄ reversed-phase HPLC column and washed with 0.1% TFA. Bound BMP-2 was eluted from the column with a 17.5–45.5% acetonitrile gradient in 0.1% TFA and monitored by UV at 214 nm. Both recombinant proteins eluted at 31.5% acetonitrile with the same retention time. The purity of the two recombinant proteins was similar. The CHO cell-expressed rhBMP-2 was obtained from Dr. Anthony Celeste at the Genetics Institute, MA.

Western blot analysis

In order to determine whether the rhBMP-2 expressed in *E. coli* is recognisable by an anti-human BMP-2 antibody (R&D Systems, Inc., Minneapolis, MN), we performed Western blot analysis. The rhBMP-2 expressed in *E. coli* was detected by goat anti-human BMP-2 polyclonal antibody. The molecular weight of rhBMP-2 on Western blot was about 12 kDa. However, when the same antibody was used to detect CHO cell-expressed rhBMP-2, an 18 kDa band was detected. The sequence of the BMP-2 cDNA clone indicated the presence of a potential asparagine-linked glycosylation site in the mature region of the BMP-2 protein. This very likely explains the difference in molecular weight between rhBMP-2 expressed in CHO cells and that expressed in *E. coli*. Using purified *E. coli*-expressed rhBMP-2, we have also developed an anti-BMP-2 monoclonal antibody, which has been used to detect BMP-2 in ELISA. The detection limit for this antibody was as low as 20 pg.

Mass spectrometric analysis

To determine the precise molecular weight of the rhBMP-2 expressed in *E. coli*, we performed matrix-assisted laser desorption/ionisation (MALDI) time-of-flight mass spectrometry (TOF/MS) analysis. The molecular weight of rhBMP-2 shown in TOF/MS was 11 989 Daltons. The calculated molecular weight based on rhBMP-2 amino acid composition is 11 977 Daltons. The molecular weight of rhBMP-2 determined by mass spectrometry is within a range of $\pm 0.2\%$ error for mass spectrometry analysis. This assay provides further evidence that the rhBMP-2 expressed in *E. coli* has the appropriate amino acid sequence and molecular weight.

N-terminal sequence

We further determined the amino acid sequence of the rhBMP-2 expressed in *E. coli*. The N-terminal sequences determined using the purified BMP-2 protein is identical to that of the rhBMP-2 expressed in mammalian cells.

Functional Analysis of *E. coli*-Expressed rhBMP-2

Effects of rhBMP-2 on osteoblast proliferation and differentiation *in vitro*

To examine the effects of rhBMP-2 on osteoblast differentiation *in vitro*, we used a clonal osteoblast precursor cell line, 2T3, as model system. 2T3 cells were derived from the calvaria of a transgenic mouse expressing large T-antigen driven by the BMP-2 promoter. These cells are primarily specified to differentiate into mature osteoblasts³ and their phenotypic properties have been extensively characterised. 2T3 cells express BMP-2, -4 and -6, and types IA, IB and type II BMP receptors constitutively.² They respond to