PROTEOLYSIS OF NATIVE BONE COLLAGEN BY CATHEPSIN K: QUANTITATIVE RELEASE OF THE CROSS-LINKED N-TELOPEPTIDE NEOEPITOPE (NTX).

Introduction
The immunoassay of cross-linked N-telopeptides of type I collagen (NTx) s in urine provides a sensitive index of osteoclast-mediated bone resorption (1). Our studies have focused on identifying the osteoclast-specific protease(s) that cleave bone collagen to generate immunoreactive NTx (Figure 1). Of particular interest has been the role of the cysteine proteinase, cathepsin K, since there is growing evidence that cathepsin K is specific to osteoclasts and is essential for mineralized collagen degradation during bone resorption (2-4).

Since there is growing evidence that cathepsin K is specific to osteoclasts and essential for mineralized collagen degradation during bone resorption (2-4), we have also shown that cathepsin K rapidly and quantitatively releases immunoreactive NTx from denatured extracts of human bone collagen in vitro (5). The aim of this study was to determine if recombinant cathepsin K, without the cooperation of other proteases, was able to degrade demineralized bone matrix and release the NTx epitope.

Methods
The cDNA for human procathepsin K was expressed in the methylotropic yeast Pichia pastoris as an alpha-factor fusion construct. On methanol induction, the proenzyme was secreted into the culture medium. Autoprocessing of the proenzyme was initiated by reducing the pH of the medium to 5.0 by dialysis. The mature active enzyme was purified by anion exchange chromatography and its homogeneity demonstrated by SDS/polyacrylamide gel electrophoresis (PAGE). Preliminary sequence data suggest that the protease involved in osteoclast-mediated bone resorption.

Results and Discussion
The core peptide recognized by monoclonal antibody 1H11 is the α2(I) N-telopeptide sequence to release latent NTx at 37°C, they will be denatured. The finding that cathepsin K alone can degrade cross-linked fibrillar bone collagen to low molecular weight peptides was confirmed by SDS/PAGE and reverse-phase-HPLC analysis of the digestion products. The findings support the concept that cathepsin K is the critical protease involved in osteoclast-mediated bone resorption.

References

Figure 1: Molecular origin of the NTx analyte in type I collagen fibrils. Urinary NTx consists of telopeptide fragments still attached to a pyridinoline cross-linking residue.

Figure 2: Timecourse for the release of immunoreactive NTx from bone collagen by cathepsin K or bacterial collagenase.

We have shown that cathepsin K, without the activity of other proteases, can solubilize human bone matrix and quantitatively release the NTx epitope. Given the specificity of cathepsin K to osteoclasts (4), this observation provides a molecular explanation for the responsiveness and specificity of NTx as a clinical marker of bone resorption, and for the observed ability of osteoclasts to generate NTx quantitatively when resorbing bone in vitro (6). Further work is underway to purify immunoreactive NTx from cathepsin K digests of bone collagen and to identify the key cleavage sites in the telopeptide and helical domains. Preliminary sequence data suggest that the main 1H11-immunoreactive peptides comprise the α2(I) chain octapeptide linked to a short segment of the α1(I) N-telopeptide and a fragment of the α1(I) or α2(I) helix. The finding that cathepsin K alone can degrade cross-linked fibrillar bone collagen to low molecular weight peptides was confirmed by SDS/PAGE and reverse-phase-HPLC analysis of the digestion products. The findings support the concept that cathepsin K is the critical protease involved in osteoclast-mediated bone resorption.