INTRODUCTION
Tropoelastin is known to undergo very little posttranslational modification. Hydroxylation of the proline residues in tropoelastin is reported to occur in some animals to a varying degree by the enzyme prolyl hydroxylase [1]. Whether or not this type of modification has any physiological significance is yet to be known. However, there are reports that cross-linking and the formation of elastin from tropoelastin is reduced by overhydroxylation of the proline residues [2]. The degree of such a modification in human elastin is little investigated. Therefore, in this study, an attempt was made to determine the extent of proline hydroxylation and the locations of potentially hydroxylated proline residues in human skin elastin.

METHODS
Human skin elastin purchased from Elastin Products Company (Owensville, Missouri, USA) was digested with elastase, thermolysin, or pepsin and the resulting peptide mixtures were analyzed using LC-ESI ion-trap and nano-ESI QTOF MS instruments.

The sequences of peptides were determined from the fragment ion spectra of tandem MS experiments following processing of the spectra using Mascot Distiller or MassLynx and searching sequence databases with Mascot search engine and/or de novo sequencing using the software, PEAKS.

For database searching, Swiss-Prot, MSDB, and a home-made database of human tropoelastin developed by considering its splice variants were used.

During searching, hydroxylation of proline was considered.

RESULTS
Over 30 prolines (~ 40% of the prolines in the sequenced regions), where hydroxylation could take place were identified in peptides of human skin elastin produced by enzymatic digestion (Figs. 1 & 2). It was interesting to note that for almost every peptide containing hydroxyproline, there exists a non-hydroxylated counterpart, demonstrating the possibility for partial hydroxylation of tropoelastin.

The hydroxyPro/Pro ratios, as determined from the relative LC-MS chromatogram peak areas of the hydroxylated to non-hydroxylated peptide pairs, differ regionally (Fig. 3). The consistency of these ratios for a specific peptide was confirmed by comparing peptides that existed in the digests of at least two enzymes. The results of three such peptides present in the elastase and thermolysin digests are shown in Fig. 3.

CONCLUSION
Well over 100 peptides were identified by the combination of methods employed. As many as 40% of the prolines in the sequenced segments of this particular elastin sample have a potential for hydroxylation. Due to partial hydroxylation, however, the actual extent of hydroxylation should be clearly less than 40%.

Peptides produced by proteolytic digestion of elastin could be used as biomarkers to quantify elastin. The co-existence of hydroxylated and non-hydroxylated proline containing peptides in elastin digest may be considered in using one form as a natural control of the other for the digestion, LC injection and ionization variability.

References