

Analyses of lysine aldehyde cross-linking in collagen reveal that the mature cross-link histidinohydroxylysinonorleucine is an artifact

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Eyre *et al.* (1) recently claimed that the histidine-involved collagen cross-links, histidinohydroxylysinonorleucine (HHL)² (2) and dehydrohistidinohydroxymerodesmosine (HHMD) (3), are both laboratory artifacts. We have several concerns about this study.

1) Failure to identify these cross-linked peptides does not prove their “nonexistence.” The approaches of Eyre *et al.* are very different from ours (4). We prepared the insoluble fraction of bovine dermis, solubilized by repeated denaturation-trypsin treatments, purified the HHL cross-linked peptides by a series of chromatography under dissociative conditions, and further truncated and characterized them. It is highly unlikely that the proposed weakly linked peptides (see Fig. 7) (1) remained together during these processes.

2) Eyre *et al.* provide no direct evidence showing that the proposed peptides generate HHL and HHMD upon acid hydrolysis. Small amounts of HHL and HHMD found in an acid hydrolysate of pooled HPLC fractions (see Fig. 8) (1), a mixture of peptides, do not identify the peptides of their origin.

3) Their proposed peptide/cross-link structures are mostly deduced from the MS analysis. While MS is a powerful analytical tool, the data interpretation becomes challenging when the peptide is complex and large. Indeed, their MS data determine only the partial structure of the proposed peptides (see Figs. 6 and 7) (1) and do not seem to correspond to the theoretical values. In addition, their proposed peptide (see Fig. 6) (1) shows incomplete cleavages by collagenase, like CNBr digestion, implying there can be peptide variants that may have been missed.

Thus, while the proposed ideas are interesting, these data alone are insufficient to support their claims.

References

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The authors declare that they have no conflicts of interest with the contents of this article.

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² The abbreviations used are: HHL, histidinohydroxylysinonorleucine; HHMD, dehydrohistidinohydroxymerodesmosine.