

Reply to Yamauchi *et al.*: Analyses of lysine aldehyde cross-linking in collagen reveal that the mature cross-link histidinohydroxylysinonorleucine is an artifact

DOI 10.1074/jbc.RL119.010692

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Edited by Gerald W. Hart

Yamauchi *et al.* (1) question the data in our recent paper (2) as support for our conclusions. We disagree and maintain that histidinohydroxylysinonorleucine (HHL)² (3, 4) does not exist as a natural product in collagen.

In Point 1, “these cross-linked peptides” presumably refers to peptides that Mechanic *et al.* (3) thought were linked by HHL based on Edman N-terminal sequencing and amino acid analysis. In our opinion, this did not rule out a mixture of physically associated peptides or a peptide remnant of the C-telopeptide aldol adduct that we conclude creates HHL on acid hydrolysis (see Fig. 7) (2). Quantifying HHL in acid hydrolysates of equal amounts of starting tissue collagen, a proteolytic digest of it, and subsequent fractions is highly instructive. For example, from bovine cornea and dermis, the yields of HHL in a bacterial collagenase digest are 6 and 17% based on the LC/MS assay method used in Fig. 8 (2).³ This is consistent with the low yield of the C-telopeptide aldol adduct (see Figs. 5A and 7) (2) and its HHL artifactual product on LC/MS (see Fig. 8F) (2). Thus, with progressive chromatography under denaturing and acidic conditions, the labile adduct that creates HHL on acid hydrolysis continues to dissociate.

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, histidinohydroxymerodemosine.

³ D. R. Eyre, M. Weis, and J. Rai, unpublished observations.

With regard to Points 2 and 3, we explain above and in Eyre *et al.* (2) why peptides prepared from collagen yield so little HHL. Such concerns do not apply to HHMD because tissue borohydride reduction quantitatively could convert the N-telopeptide dimer pool (see Fig. 5) (2) to HHMD-linked N-telopeptide to $\alpha 1(I)K930$ structures (see Fig. 6) (2). The latter do match exactly the peptide structures shown including the mass of HHMD, as does the peptide in Fig. 7 with its cross-link (Fig. 10) (2).

Finally, the peptides in Fig. 6 are dominant, not minor partial cleavage products, from the N-telopeptide to the helix site under the conditions we use (10% acetonitrile in the digest) to limit the range of products.

References

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