Enzymatic Reversibility of Nonenzymatic Glucosylation?

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Dear Sir,

The chemistry of the glycosylation of proteins, the reaction between proteins and carbohydrate, is shown below,

H H

I I

H-C=O H-C=N-Pr H-C-N-Pr

I I I

(H-C-OH)₄+H₂N-Pr → (H-C-OH)₄ - - * C=O

! ← I ← I

CH₂OH CH₂OH (H-C-OH)₃

CH₂OH I II III

(Pr= Protein)

The article 'Enzymatic Reversibility of Nonenzymatic Glycosylation of the Glomerular Basement Membrane' [1] contains some errors in basic chemistry. Lubec et al. [1, p. 26] write: 'The basic reaction involves the formation of an aldimine, the formation of a ketoamine undergoing the Amadori rearrangement ...'. It is the aldimine (II) which undergoes the Amadori rearrangement producing the ketoamine (III).

They then go on and write: 'This N-glycosidic linkage ...' (p. 27). In the ketoamine (III) the carbohydrate portion is linked via a C³-group to the nitrogen atom of the protein. Therefore the product of the Amadori rearrangement, the ketoamine (III), is an N-alkylated compound and not a glycoside and there is no glycosidic linkage.

The removal of the carbohydrate moiety attached to the nitrogen atom, which their extract does, might be due to an oxidative reaction finally producing an aldehyde. The reaction is essentially an oxidative dealkylation of nitrogen and has its analogues in N-demethylases.

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It is also possible that the enzyme complex in the extract is reversing the Amadori reaction producing an aldimine (II) which is hydrolyzed to the starting materials glucose (I) and protein. When the fate of the nitrogen-containing groups on the protein to which the carbohydrate had been bound are known then, and only then, can the enzyme removing the carbohydrate be named. It is premature to call the enzyme a hydrolase.

Lubec et al. [1] also report that no fructose, only glucose (1) was found on examination of the hydrolysates. It is possible that the reversibility of ketoamine formation [2] goes through an intermediate in which the asymmetry of C-2 is destroyed. No fructose would be found but

mannose would be present as was shown by acid hydrolysis [3]. No mention was made by Lubec et al. [1] of a search for mannose.

References

- Lubec, G.; Vycudilik, W.; Coradello, H.; Pollak, A.: Enzymatic reversibility of nonenzymatic glycosylation of the glomerular basement membrane. *Nephron* 33:26 (1983).
- Mortensen, H.B.; Christophersen, C: Glycosylation of human hemoglobin A. Kinetics and mechanisms studied by isoelectric focusing. *Biochim. biophys. Acta* 707:154 (1982).
- Bunn, H.F.; Haney, D.N.; Gabbey, K.H.; Gallop, P.M.: Further identification of the nature and linkage of the carbohydrate in hemoglobin Alc. *Biochem. biophys. Res. Commun.* 67: 103 (1975).

Note added in proof

Monnier [1] has published a short communication in which he indicates the analytical errors made by Lubec et al. [2] in a paper using analytical techniques similar to those used in the *Nephron* paper [3] which I have here criticized.

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Mester et al. [4] have discussed the reversibility in vitro of 3Maillard-type substances in sheep microsomal preparations. They indicate the possible involvement of cytochrome P450 and activation by NADPH.

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Lubec, G.; Vycudilik, W.; Coradello, H.; Pollak, A.: Enzymatic reversibility of nonenzymatic glycosylation of the glomerular basement membrane. *Nephron* 33:26 (1983).

Mester, L.; Szabados, L.; Mester, M.: Enzymic cleavage of in vitro formed Maillard-type compounds involved in hemostasis (Abstract). *Thromb. Haemostasis* 46:393 (1981).

References

Monnier, V.M.: Is there an enzymatic reversibility of nonenzymatic glucosylation of hemoglobin? *Clin.Chem.* 29:1997(1983).