Acceleration of glucose-mediated crosslinking of collagen by free lysine

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Summary. Crosslinking occurred in collagen when it was incubated with glucose. Free lysine accelerated the crosslinking markedly.
Key words. Collagen; crosslink; Maillard reaction.

It is known that reducing sugars such as glucose react nonenzymatically with amino groups in proteins and the condensation products then undergo multiple chemical reactions to form various products, including brown pigments and protein crosslinks[1]. The reactions have received attention from food chemists, since stored and heat-treated foods undergo these reactions and their bioavailability is thus decreased. More recently, it has been shown that the glucose-mediated crosslinking also occurs in vivo. The crosslinking occurs appreciably in the long-lived proteins such as collagen and lens crystallins during aging[2,3]. The reaction appears to be accelerated in diabetes[4]. However, the mechanism of the glucose-mediated crosslinking of proteins has not been elucidated well. This paper reports the acceleration of the glucose-mediated crosslinking of collagen in vitro by free lysine.

Materials and methods. Tibias and femurs were obtained from 6-week-old chicks. The diaphysis was cleaned of marrow, cut into small pieces, washed with a large volume of 0.5 M NaCl at 4 °C, and subjected to demineralization. The demineralized bone was homogenized by a Physcotron homogenizer.

Bone collagen (100 mg wet wt.) was suspended in 0.1 M sodium phosphate buffer, pH 7.2 (1.5 ml) in the presence or absence of D-glucose and L-amino acids at 37 °C for 4 weeks. One drop of toluene was added to inhibit bacterial growth. After incubation bone collagen was collected, washed with water and then digested with cyanogen bromide in 70% formic acid at 20 °C for 3 h[2]. After digestion, each sample was diluted 10-fold in water and centrifuged at 10,000 × g for 20 min. The supernatant was evaporated under reduced pressure and the dried sample was hydrolyzed with 6 M HCl at 110 °C for 24 h. The precipitate was also hydrolyzed in the same manner. Hydroxyproline in the hydrolysate was determined[5] and the amount of collagen was estimated.

Results and discussion. The level of crosslinking in the collagen was assessed by measuring the amount of solubilized peptides after cyanogen bromide digestion[2]. The results are summarized in the table. The chick bone collagen incubated without glucose was solubilized completely by cyanogen bromide treatment. The collagen incubated with glucose alone was also solubilized completely, although SDS-polyacrylamide gel electrophoresis of the solubilized peptides indicated that crosslinking occurred to some extent (data not shown). The collagen incubated with glucose in the presence of free lysine was markedly resistant to solubilization by cyanogen bromide, indicating that the collagen was highly crosslinked and remained insoluble even after cyanogen bromide had cleaved several bonds. The degree of the insolubility depended on the concentration of lysine. Other amino acids (alanine and arginine) had a similar, but lesser effect. The result indicates that free amino acids, particularly lysine, accelerate the glucose-mediated crosslinking in collagen.

The mechanism of the glucose-mediated crosslinking in proteins has not been elucidated well. Pongor et al. proposed an imidazole compound incorporating two glucose molecules and two lysine residues as a possible crosslinking group[6]. Oritani et al. suggested that the crosslinking may be caused by dicarbonyl compounds formed from glucose by the reaction with amino groups[7]. The present finding may favor the latter mechanism. The reaction between glucose and free amino acids also produces dicarbonyl compounds, resulting in the acceleration of crosslinking in the protein.

The glucose-mediated crosslinking may be involved in the aging process of human body[2,3] and in diabetes[4]. It has been thought so far that only the concentration of glucose in the body fluids affects the rate of the crosslinking. The present study suggests that the concentration of free amino acids, particularly that of lysine, may affect the rate of the crosslinking, too.

Cyanogen bromide digestibility of collagen after in vitro incubation

Incubation conditions			Amount solubilized by cyanogen bromide digestion (%)
Glucose (mM)	Amino acid	(mM)	
0	–	0	100
200	–	0	100
200	Lysine	20	79
200	Lysine	60	26
200	Lysine	200	16
20	Lysine	200	100
200	Alanine	200	71
200	Arginine	200	49

Results are the mean of duplicates.

1 Mauron, J., Prog. Fd Nutr. Sci. *5* (1981) 5.
2 Fujimoto, D., Biomed. Res. *5* (1984) 279.
3 Monnier, V.M., and Cerami, A., Science *211* (1981) 491.
4 Monnier, V.M., Kohn, R.R., and Cerami, A., Proc. natn. Acad. Sci. *81* (1984) 583.
5 Woessner, J.F., Archs Biochem. Biophys. *93* (1961) 440.
6 Pongor, S., Ulrich, P.C., Bencsath, F.A., and Cerami, A., Proc. natn. Acad. Sci. *81* (1984) 2684.
7 Okitani, A., Cho, R.K., and Kato, H., Agric. biol. Chem. *48* (1984) 1801.

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Phospholipid composition of cardiac ($Na^+ + K^+$)-ATPases from various species

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Summary. There is a difference in phospholipid composition of cardiac ($Na^+ + K^+$)-ATPase preparations between species which are sensitive to ouabain and those which are not. Sphingomyelin is higher and phosphatidylcholine is lower in the enzymes from sensitive species than in those from insensitive ones. Lysophosphatidylcholine is detectable only in the latter preparations.
Key words. ($Na^+ + K^+$)-ATPase; phospholipid; ouabain.