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Glycation[1] of H1-Histone

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H1-histone (H1) purified from calf thymus was subjected to nonenzymatic glycation *in vitro*. A lysine residue in a restricted environment, with an apparent pK_a of 6.9, was glycated, notwithstanding the high lysine content of H1. The extent of glycation per lysine residue was 2.4 times larger than that of human serum albumin. Fluorescence at Em. 420 nm (Ex. 340 nm) was closely correlated to the degree of glycation.

Keywords——glycation; H1-histone; nonenzymatic glycosylation; fluorescence

Glucose and other reducing sugars react nonenzymatically with proteins to form stable covalent adducts. Nonenzymatic glycation has been studied mainly in relation to diabetic complications. Insulin-independent tissues such as erythrocytes,[2] lens crystalline,[3] nerves[4] and connective tissue collagens[5] undergo accelerated glycation of their proteins in diabetes accompanied with hyperglycemia. Physicochemical and functional changes observed in these proteins are similar to those that occur with aging.[6] One cause of the functional decrement characteristic of cellular senescence is considered to be progressive accumulation of genetic injury. Nonenzymatic glycation is a first-order process with respect to sugar concentration,[7] and glucose bound to the ε-amino group of a lysine residue or the α-amino group of the N-terminus produces 5-hydroxymethylfurfural(5-HMF) following weak acid hydrolysis. Chang and Noble[8] reported that renal glomerular proteins in the diabetic rat, in which the nuclear fraction contains 40% of the total 5-HMF, show higher 5-HMF values than the control. Histone is a major component of chromatin and is considered to regulate the beginning of transcription of deoxyribonucleic acid (DNA).[9] As Hl-histone(Hl) is a lysine-rich protein and is located outside the nucleosome, we supposed that Hl is likely to be more glycated than other histones.

Materials and Methods

Hl was extracted from calf thymus according to the method of Johns[10] with slight modifications. Briefly, a suspension of calf thymus homogenate in 5% perchloric acid was fractionated by the addition of trichloroacetic acid. The precipitate was washed with acetone–HCl and then acetone. The purification of Hl was confirmed by 15% polyacrylamide gel electrophoresis[11] and by the absence of the fluorescence of tryptophan (Hl contains no tryptophan residue). Solutions of Hl in 1/15 M phosphate buffer (pH 5—9) containing up to 150 mM glucose were sterilized by filtration through a 0.45 μm Millipore filter and subsequent incubation for up to 10 d at 37 °C. The extent of glycation was estimated colorimetrically by the use of the thiobarbituric acid reaction[12] as 5-HMF/Hl (mol/mol). The molecular weight of Hl was taken as 21500.[13] Fluorescence and absorption spectra were obtained with a Hitachi MPF-4 spectrofluorometer and a Hitachi 557 spectrophotometer.

Results and Discussion

Figure 1 shows the *in vitro* glycation of Hl under various conditions of incubation period(a), glucose concentration(b), and pH(c). The extent of glycation was proportional to

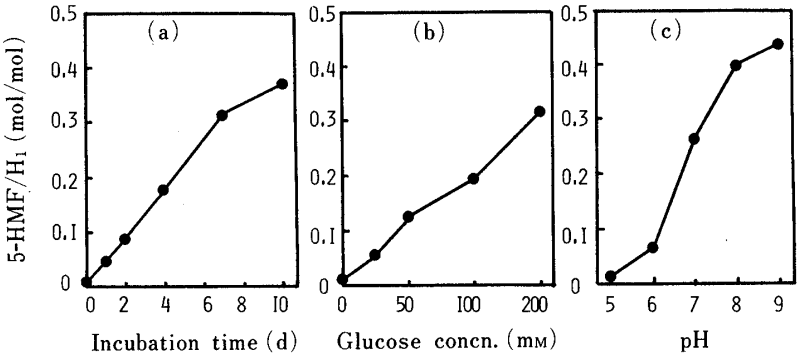

Fig. 1. Nonenzymatic Glycation of H1-Histone

H1 solutions (5 mg/ml) were incubated at 37 °C; (a) incubation periods: 1, 2, 4, 7 and 10 d (150 mM glucose, pH 7.4); (b) glucose concentration: 0, 25, 50, 100 and 150 mM (7 d, pH 7.4); (c) pH 5, 6, 7, 8 and 9 (150 mM glucose, 7 d). Extent of glycation is shown as 5-HMF/H1 (mol/mol).

the incubation period and glucose concentration. A sigmoidal curve of glycation was obtained against increasing pH. The glycation occurs by rapid formation of a Schiff base, followed by slower Amadori rearrangement to the stable ketoamine linkage. The pH dependency of glycation reflects the pK_a of amino groups, because protonated amino groups can not form the Schiff base with glucose. The apparent pK_a value of the glycated amino acid residue was estimated to be 6.9. Since the N-terminal amino group in Hl is acetylated, the glycated amino acid residue is restricted to lysine. The content of lysine residues is 25.5 mol% in Hl.[11] The extent of glycation for 7 d at 150 mM glucose and pH 7.4 was about 0.3 (5-HMF/Hl(mol/mol)). The extent of glycation per lysine residue in Hl molecule was 2.4 times larger than the value for human serum albumin,[12] thus indicating that Hl is more likely to undergo glycation than human serum albumin. The ε-amino group of lysine in an aqueous medium has a pK_a value of 10.3, in contrast to the apparent pK_a of 6.9 of the glycated lysine. Therefore, the lysine residue which can be glycated seems to be located in a restricted environment.

The Hl molecule contains one tyrosine and one phenylalanine residue, each having an absorption band at about 280 nm. Figure 2 shows the absorption spectra of Hl for various periods of incubation. The absorption bands at 280 nm and in the region of 300—350 nm increased with incubation time. These spectral features were essentially the same as those noted for glycated poly-lysine *in vitro*.[14] To examine environmental change in the tyrosine residue, the fluorescence of tyrosine (Ex. 275 nm, Em. 303 nm) was assayed for various incubation periods. Glycation had no significant effect on the fluorescence intensity or spectra, though incubation for 10 d caused a slight decrease in intensity. The increase in absorbance at 280 nm may thus be considered to originate from a chromophore produced by glycation. We previously reported new fluorescence in glycated human serum albumin and glycated poly-lysine, derived from a new chromophore observed at 285 nm and in the wavelength region of 300—350 nm.[14] Glycated Hl showed almost the same fluorescence. The relationship between extent of glycation and induced fluorescence or absorption spectral change is illustrated in Fig. 3. Induced fluorescence in Hl at 420 nm during excitation at 340 nm was closely correlated to the extent of glycation ($r=0.9690$). Increase in absorbance at 285 nm ($r=0.8541$) and at 330 nm (0.9433) also showed good correlation with the degree of glycation. The present data confirm that the extent of glycation can be determined sensitively by measuring new fluorescence. For example, 1 mg of glycated Hl (incubated for 7 d with 150 mM glucose) gave an optical density of only 0.04 at 443 nm after the thiobarbituric acid reaction, but 0.5 mg/5 ml of the same glycated Hl gave a sufficient fluorescence intensity at 420 nm. Fluorescence at 420 nm is characteristic of extent of glycation.

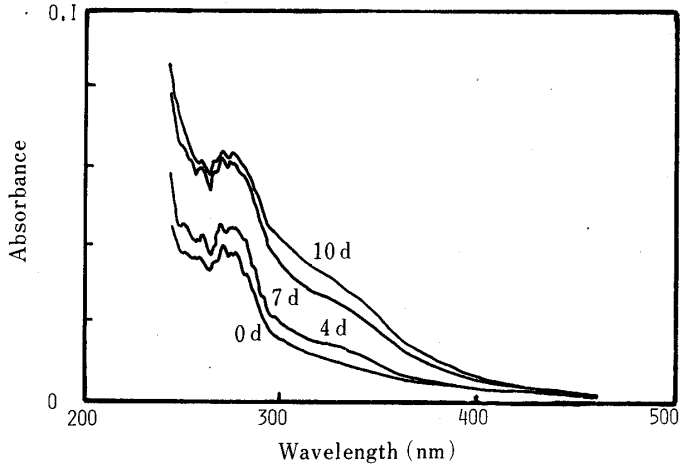

Fig. 2. Absorption Spectra of H1-Histone during Glycation

Incubation conditions were the same as in Fig. 1(a).

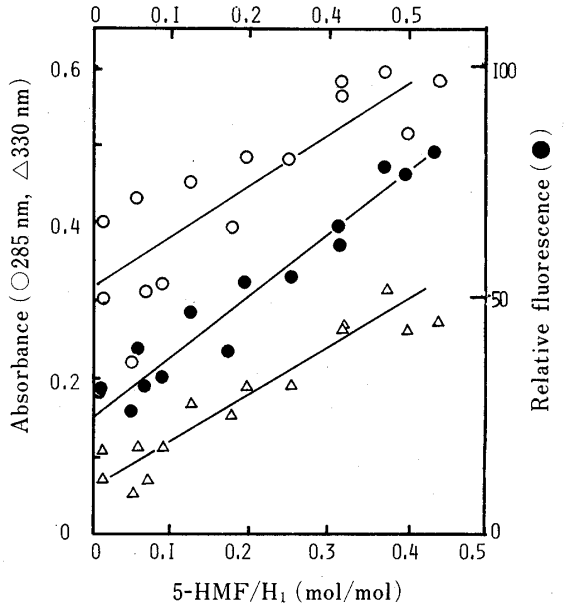

Fig. 3. Relationship between Extent of Glycation and Induced Fluorescence or Increased UV Absorption

Fluorescence: (●) Em. 420 nm (Ex. 340 nm), $r=0.9690$. UV absorption: 285 nm (○), $r=0.8541$ and 330 nm (△), $r=0.9433$. All solutions of glycated H1 shown in Fig. 1 were analyzed.

Recently, Cerami *et al.* isolated a fluorescent chromophore from glycated poly-lysine and identified it as 2-(2-furoyl)-4(5)-(2-furanyl)-1*H*-imidazole.[15] They also found the collagen-linked fluorescence to increase with severity of retinopathy, nephropathy, and arterial and joint stiffness.[16] Fluorescence may increase without glucose if fluorescence products are derived from a further reaction of glycated lysine with free lysine residue, as described by Elbe *et al.*[17] More detailed studies on the metabolism and disposition of glycated proteins, and on further reactions of glycation and the inhibition of these reactions seem desirable.

References and Notes

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