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Preliminary Communication

Binding of Maillard products to proteins: formation of pyrrole carbimines

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During the past years the reactions of reducing sugars with amino acids, proteins, and simple amines have been intensively investigated. In spite of these efforts important reaction sequences are still unknown. A great variety of low molecular weight, volatile substances, several of which are aroma constituents of heated foods, have been identified, but they represent only a small portion of the totality of Maillard reaction products. Far less is known about the formation of hydrophilic and high molecular weight substances. Recent investigations established the importance of glucose-protein reactions in medical biochemistry [1,2].

As a result of several investigations it has been postulated that glycation of proteins and the subsequent chemical transformation of these adducts may be responsible for long-term complications caused by diabetes. Furthermore, reactions of this kind are thought to be associated with arteriosclerosis and the aging process in the human body. It has been shown that not only glucose itself but also glucose degradation products may bind to proteins [1–3], and that the well documented protein–protein crosslinking is induced by the reaction with sugars, but the mechanism of this interaction is still unknown. Sell and Monnier isolated a fluorescent compound (pentosidine) which is detectable in several proteins and which is increased in response to hyperglycemia in diabetes [4]. In this substance arginine and lysine are linked by a sugar derived C_5 residue.

Among the sugar–amine reaction products pyrrole carbaldehydes with hydroxyalkyl side chains are of special importance [5–7]. Compounds having structures **1**, **2**, and **3** have been isolated from pentose- and hexose-amine reaction mixtures [5,6,8,9]. The protein bound derivative called pyrraline (**1**, R = C-chain of a lysine residue) is formed when proteins are incubated with glucose [10]. This compound has been detected in glycated proteins after hydrolysis [9,11,12].

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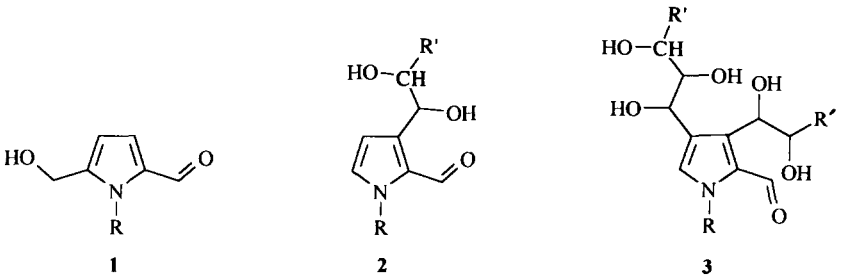


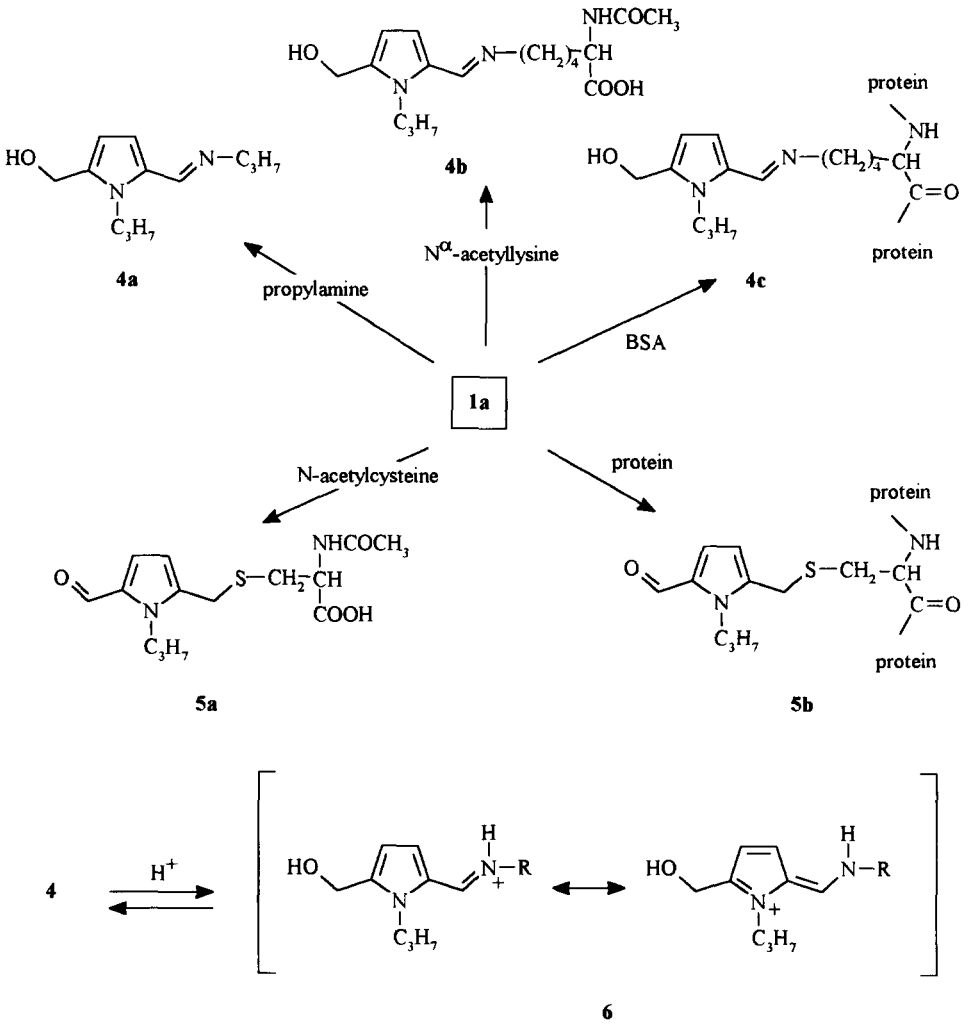
1: R = alkyl
1a: R = propyl

R′= H or CH_2OH

Structure 1.

Pyrrole carbaldehydes are reactive intermediates, and it can be expected that substances of this kind may bind to proteins and may be responsible for cross linking. Previously we have shown that hydroxyalkylpyrroles react under physiological conditions with *N*-acetylcysteine or glutathione to give substitution products of type **5** [13]. It is reasonable to expect that Maillard compounds having structure **5b** can be formed in proteins with available SH-groups. According to our results an analogous substitution proceeds with amino groups only under drastic conditions. On the other hand, pyrrole aldehydes react readily with primary amines to give Schiff bases (e.g. **1a** → **4a**). In this paper we present evidence that Maillard products with the pyrrole carbaldeyde structure interact with lysine side chains of proteins.

When glucose is heated with propylamine in neutral or slightly acidic aqueous solution the pyrrole aldehyde **1a** is formed as one of the main products. In concentrated solution, besides **1a** the Schiff base **4a** is obtained. The yields of **1a** and **4a** depend on the molar ratio of sugar to amine, the water content, and the reaction temperature. The condensation products **4a** and **4b** are formed as well by reaction of the aldehyde **1a** with propylamine or *Nα*-acetyllysine (Scheme 1). Compound **4a** is obtained practically pure by heating equimolar amounts of **1a** and propylamine in ethanol, ^{1}H NMR (400 MHz, CD_3OD): δ 0.89–97 (m, 6 H, $CH_3(CH_2)_2N^1$ and $CH_3(CH_2)_2N=CH$), 1.64–1.77 (m, 4 H, $CH_2CH_2N^1$ and $CH_2CH_2N=CH$), 3.45 (t, 2 H, *J* 6.6 Hz, $CH_2N=CH$), 4.33–4.37 (m, 2 H, CH_2N^1), 4.61 (s, 2 H, CH_2OH), 6.11 (d, 1 H, *J* 3.67 Hz, pyrrole H-4), and 6.42 (d, 1 H, *J* 3.67 Hz, pyrrole H-3). In methanol (pH 7) **4a** is characterized by a UV maximum at 292 nm (log ϵ 4.736). In the same way **4b** is synthesized from **1a** and the potassium salt of *Nα*-acetyllysine and purified by preparative HPLC (column packed with Lichrosorb Diol, eluent of 2:1 ethyl acetate–methanol), ^{1}H NMR (400 MHz, CD_3OD): δ 0.94 (t, 3 H, *J* 7.34 Hz, CH_3CH_2), 1.41 (m, 2 H, Lys CH_2-4), 1.63–1.75 (m, 5 H, CH_3CH_2, Lys CH_2-5, and Lys CH-3a), 1.85–1.90 (m, 1 H, Lys CH-3b), 1.94 (s, 3 H, CH_3CO), 3.50 (t, 2 H, *J* 7.34 Hz, Lys CH_2-6), 4.20–4.28 (m, 3 H, CH_2CH_2N and Lys CH-2), 4.56 (s, 2 H, CH_2OH), 6.09 (d, 1 H, *J* 3.67 Hz, pyrrole H-4), and 6.40 (d, 1 H, *J* 3.67 Hz, pyrrole H-3). In methanol (pH 7) **4b**, like **4a**, exhibits a UV maximum at 292 nm (log ϵ 4.09). The Schiff bases have considerable



Scheme 1.

stability under neutral conditions, and **4a** and **4b** are detectable by analytical HPLC under reversed phase conditions. Prolonged standing in dilute aqueous solution, however, leads to the hydrolysis of **4a** and **4b** to give the pyrrole aldehyde.

The results discussed so far lead to the assumption that pyrrole aldehydes might react as well with lysine side chains of proteins. Condensation of the pyrrole aldehyde **1** with lysine side chains of bovine serum albumin (BSA) can be detected by UV spectroscopy. As shown above, imines of general structure **4** show an absorption maximum at about 292 nm. The detection of compounds of this type in BSA is however limited by the fact that the tryptophan residue gives rise to strong absorption at 276 nm. After the binding of a small amount of pyrrole aldehyde to the protein the tryptophan absorption curve

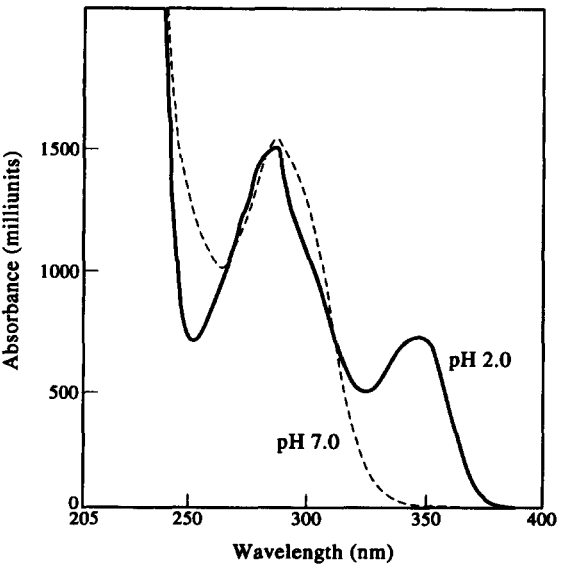


Fig. 1. UV spectrum of protein-bound pyrrole carbaldehyde **1** at acidic and neutral pH values.

shows only a shoulder at about 300 nm (Fig. 1). On the other hand, in slightly acidic solution the imine **4** is protonated to give the resonance-stabilized immonium salt **6**, which is characterized by a UV maximum at 343 nm. Under suitable conditions the hydrolytic cleavage of **4** proceeds only slowly. Thus the appearance of a further absorption maximum at 343 nm after acidification of the protein solution would show the presence of a protein bound Schiff base of type **4c**.

When BSA is allowed to react with the pyrrole aldehyde **1** in concentrated aqueous solution at room temperature for several hours or days a gelling or thickening of the solution is observed. After dilution with 75:25 methanol–water and addition of acid (about pH 2) the formation of an immonium salt of type **6** could be detected by UV spectroscopy (Fig. 1), showing that pyrrole aldehyde **1** can undergo condensation with available ϵ-amino groups of lysine side chains to give pyrrole carbimines derivatives. A reaction of this type could result in a cross-linking of proteins by sugar degradation products. It is likely that other Maillard compounds bearing carbonyl groups can be connected to proteins in a similar way.

Acknowledgements

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