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Model Studies of the Maillard Reaction of Arg-Lys with D-Ribose

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Abstract: *The condensation of the guanidine and the ε-amino functions of an Arg-Lys dipeptide with D-ribose produces a cyclic pentosidine-crosslink.*

Advanced glycosylation endproducts (AGEs) have been linked to the development of many of the long-term complications of diabetes, renal insufficiency, and normal aging.[1-3] Although the structures of the most abundant AGEs which occur *in vivo* are unknown, Monnier, *et al.* recently isolated the fluorescent crosslink pentosidine from human dura collagen.[4] Pentosidine appears to form as the condensation product of lysine, arginine, and a reducing sugar precursor. In *vitro*, pentosidine may be readily produced upon incubation of Nα-protected derivativess of arginine, lysine, and sugars such as ribose, glucose, fructose, ascorbate, or dehydroascorbate.[5]

Measurements of pentosidine content in a variety of biological specimens have revealed that this bi-functional condensation product accounts for only a small percentage (<1%) of potential glucose-derived crosslinks.[2] Furthermore, when bovine serum albumin (BSA), which contains 59 lysine and 23 arginine residues, is incubated with D-glucose in phosphate buffer, pentosidine forms in a yield of only 1 mmol/mol protein. It also has been noted that while many proteins such as ovalbumin and BSA can undergo a high degree of modification or "impairment" of lysine and arginine residues during advanced glycosylation, protein oligomerization rarely ensues. These observations prompted us to examine the contribution of *intra*molecular crosslinking to the formation of pentosidine in model systems *in vitro*. First, we noted that the amino acid sequence of BSA contains an arg-lys (RK) sequence at positions 411-412 which, on the basis of kinetic considerations, might serve as a highly reactive site for intramolecular pentosidine formation. Second, a molecular modeling and energy minimization study suggested that the 16-membered ring resulting from an RK dipeptide intramolecular crosslink would not be markedly constrained as shown in Fig. 1.

We heated the RK dipeptide at 70 °C with two equivalent of D-ribose, D-glucose or D-fructose, in phosphate buffer for 48 h. The incubations then were analyzed by HPLC with UV (λ_{max} 320 nm) detection. In each case, a major peak with an identical elution time and the characteristic UV spectrum (λ_{max} 320 nm) of pentosidine was evident. The MS spectra (m/z 361) and ^{1}H-NMR spectra confirmed the structure of a pentosidine-RK condensation product formed by intramolecular crosslinking of the lysine ε-amino group with the guanidino group of arginine. Other peaks also were identified to be present in each incubation. These occurred in very low concentrations and were assigned the expected structure of a pentosidine moiety linking two molecules of RK (*inter*molecular crosslinks).

In a second model study, the α-amino group of RK was protected in order to study the

reactivity of the R and K side chains in a domain more closely resembling that of a native protein. The incubation of N^{α}-CBZ-RK with two equivalents of D-ribose in 0.2 M phosphate buffer (pH 7.4) for three weeks at 37 °C yielded pentosidine as a major fluorescent compound in 4.5% yield. Separation and purification of this compound was carried out by reversed phase HPLC.[4] The ^{1}H-NMR spectrum of the compound revealed, beside the protons of the starting material, three protons which resonate as an ABX system at δ = 7.85 (d, J = 6.4 Hz), 7.67 (d, J = 7.4 Hz), and 7.15 (bdd, J = 7.2, 6.9 Hz), and which are consistent with the pentosidine structure.[4,5] The electron spray (ES) mass spectrum showed a molecular ion at 495 (M) which is consistent with the molecular formular $C_{25}H_{31}N_6O_5$ (hrFAB 495.2482, calc. 495.2356).

These model studies support the high reactivity of dibasic amino acid systems toward Maillard reactants and suggest a potentially active competition between intra- and intermolecular crosslink formation *in vivo*. These data also may account for the low frequency of intermolecular crosslink and pentosidine formation in certain protein substrates.

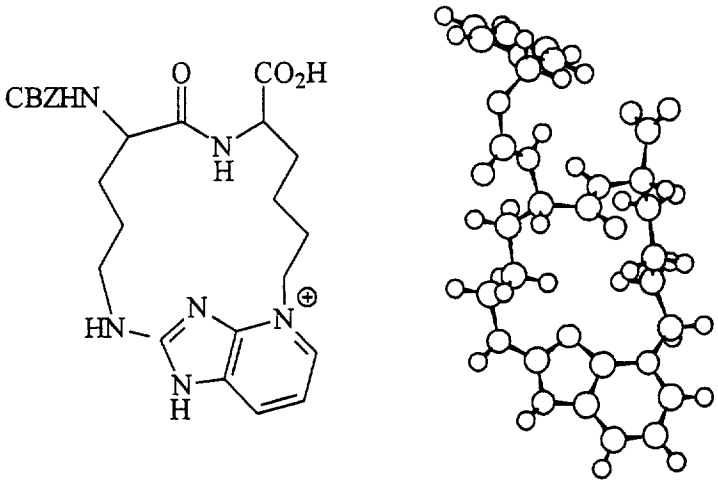

Fig. 1. Energy minimized, molecular model of a cyclic pentosidine-RK dipeptide (*InsightII* and *Discover* software, Biosym, Inc). Forcefield potentials and charges were assigned automatically using parameters from the CVFF forcefield file. Energy minimization proceeded through the steepest descent method until the rms derivative fell below 0.01 kcal $mol^{-1}A^{-1}$.

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