Bioorganic Chemistry **27,** 443–450 (1999)
Article ID bioo.1999.1148, available online at http://www.idealibrary.com on IDEAL®

Formation of Advanced Glycation Endproducts from Low Molecular Weight Model Compounds

Mariana D. Argirova, Irina I. Kerina,* and Ognian K. Argirov†

*Department of Chemistry and Biochemistry, Higher Medical Institute, 15a Vassil Apprilov Street, 4000 Plovdiv, Bulgaria; *Department of Organic Chemistry, University of Plovdiv, 24 Tzar Assen Street, 4000 Plovdiv, Bulgaria; and †Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ United Kingdom*

Received January 25, 1999

The process of glycation was studied in 12 model systems containing carbohydrates (Glc, Fru) and peptides (Gly-Gly, Gly-Phe, Phe-Gly, Gly-Lys) or acetylated amino acids (Ac-Lys, Ac-Arg) in order to clarify the role of different structural elements of the reacting components. The course of reaction was followed by the changes of the UV spectra of the reaction systems. The results show that the reactivity of the NH_2 group correlates with its pK_a value. The presence of benzene ring in the amino component accelerates glycation. Strong correlation between the intensity of the fluorescence and the absorption at 325 nm was found for all reaction systems.

INTRODUCTION

Protein glycation is a complex set of chemical reactions taking place within the living systems. It is considered to be related to different clinical complications, including diabetic cataract development (*1*). The formation of advanced glycation end-products (AGEs) is the most significant result of glycation from a biological point of view.

Research work on the chemical course of glycation is complicated because of the polyfunctional nature of the protein molecules. At least three different structural elements are proved to be reactive toward carbohydrates: N-terminal amino groups of the polypeptide chains (*2*); the ε-amino groups of lysine residues (*3*); guanidino groups in arginine residue (*4*). In the present work we compare the process of glycation in 12 model systems, containing carbohydrates and peptides or acetylated amino acids in order to clarify the role of different structure elements of the reacting amino component and carbohydrate.

The complex nature of glycation and the fact that AGEs are formed as a mixture of different (and not completely identified) structures makes difficult the direct implementation of the equations of formal kinetics for characterization of this process. Usually the course of glycation is followed by integral quantities like change of the

0045-2068/99 $30.00

absorption in the ultraviolet and visible (uv/vis) region (*5*), appearance of nontryptophan fluorescence (*6*), immunological measurements (*7*), and change of the amino-acids composition of proteins (*8*).

For the present study we have chosen the first two methods mentioned above, in as much as they are appropriate to the studied systems and are widely used for the characterization of glycation *in vitro* and *in vivo*. This is why, when we compare the "reactivity" of the different compounds in the text below, we mean their ability to form chromophores and fluorophores. Although some of the AGEs like carboxymethyllysine (*9*) do not possess specific chromophores, a number of the known AGEs, like pentosidine (*10*), pyrraline (*11*), and glyoxal-lysine dimer GOLD (*12*) are conjugated systems, absorbing in the uv region. Some are also fluorescent. As long as the formation of different AGEs goes on in a parallel way, the changes of the uv spectra and fluorescence of the reaction systems can be considered as a reliable measure of the general course of glycation.

The role of glycation *in vivo* is partly limited because of the turnover of the proteins in biological systems. Its importance is greater in organs where long-living proteins exist. An example of such a system is the eye lens, which can contain quite old proteins, depending on the age of the subject. In the present work the reaction conditions and especially pH were chosen in order to correspond to those of the eye lens (*13*).

MATERIALS AND METHODS

Carbohydrates were purchased from Merck (Darmstadt, Germany) and peptides were from Sigma (St. Louis, MO).

Uv/vis spectra were recorded by spectrophotometer Cary-1 (Varian, Australia), and the fluorescence was measured by a Perkin–Elmer LS-3B fluorescence spectrometer.

Glycation has been studied in systems containing 50 mM carbohydrate (D-glucose or D-fructose), 50 mM amino component (Phe-Gly, Gly-Phe, Gly-Gly, Gly-Lys, Ac-Lys, Ac-Arg), at pH 6.9, in 200 mM phosphate buffer with 0.05% NaN_3 and 1 mM EDTA. The samples with a total volume of 1.5 ml were incubated at 37°C. Aliquots of 150 μl were taken at 5, 10, 15, and 20 days, diluted to 2.5 ml with distilled water, and their electron spectra were scanned in the region of 200–500 nm in cells with optical path 1 cm. Then 150 μl of the reaction systems obtained at the end of the incubation period was diluted to 3.5 ml with distilled water and their fluorescence spectra were recorded.

RESULTS

Changes of the Electron Spectra of the Reaction Systems

The starting reaction mixtures do not practically absorb in the region of 280–500 nm (absorbance less than 0.01). The compounds containing benzene ring (Gly-Phe and Phe-Gly) show its typical multiplet at 256 nm and all amide groups show intensive absorbance at 210 nm.

Uv/vis absorbances of the samples increase as a result of glycation. The spectra of reaction systems, containing glucose or fructose after 20 days of incubation are given in Figs. 1a and 1b, correspondingly. No clearly defined new maximum can be

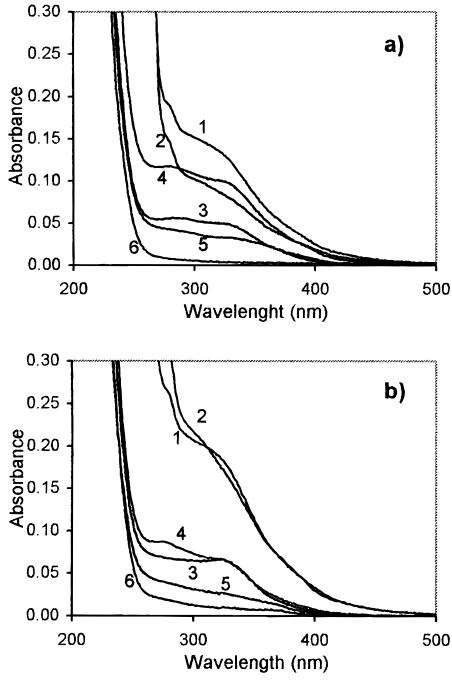

FIG. 1. Spectra of reaction systems, containing glucose (a) or fructose (b) after 20 days of incubation. Amino components: **1**, Phe-Gly; **2**, Gly-Phe; **3**, Gly-Gly; **4**, Gly-Lys; **5**, Ac-Lys; **6**, Ac-Arg.

seen but a shoulder appears at about 325 nm and the absorbance gradually falls in the visible region. Data for change of the absorbance at 325 nm are given in Figs. 2a and 2b and are used for comparison of the reactivity of the different participants in glycation.

Appearance of Nontriptophane Fluorescence of the Reaction Mixtures

Reaction mixtures were studied for appearance of fluorescence in the 290- to 400-nm and 390- to 500-nm regions for excitation and emission correspondingly. Formation of fluorophores was found in all systems. Data for the excitation and emission maximums and their relative intensities are given in Table 1. In most cases one clearly defined maximum of excitation and a maximum of emission can be seen. In the system Ac-Arg + Fru there are two excitation maximums: at 312 and 370 nm.

DISCUSSION

Relative Reactivity of the Amino Groups in Glycation

Although each polypeptide chain contains only one N-terminal amino group and often more than one lysine residue, a number of proteins are predominantly glycated with participation of their N-terminal amino groups (hemoglobin (*2*), γ-II-crystallin (*14*), insulin (*15*)). Two alternative explanations for this fact can be considered: (i) N-terminal amino groups are chemically more reactive than those in the side chains

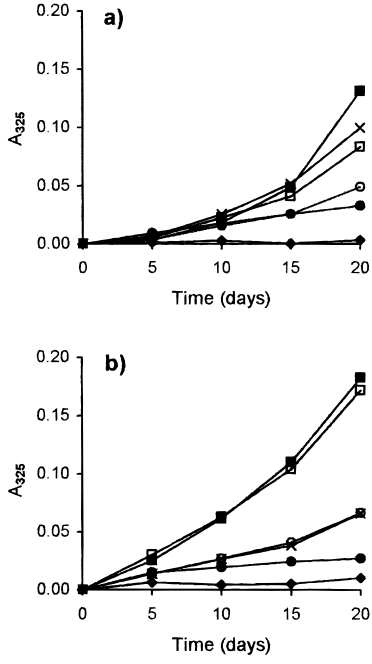

FIG. 2. Change of the absorbance at 325 nm of reaction systems containing glucose (a) or foructose (b). Amino components: ■ Phe-Gly; □ Gly-Phe; ○ Gly-Gly; × Gly-Lys; ● Ac-Lys; ◆ Ac- Arg.

TABLE 1

Maximal Fluorescence of the Reaction Mixtures after 20 Days of Incubation

Reaction System	Excitation λ_{max}(nm)	Emission λ_{max}(nm)	Fluorescence (arbitrary units)
Phe-Gly + Glc	335	414	66.7
Phe-Gly + Fru	343	412	89.1
Gly-Phe + Glc	339	416	44.5
Gly-Phe + Fru	340	419	88.7
Gly-Lys + Glc	332	412	57.0
Gly-Lys + Fru	329	403	43.6
Gly-Gly + Glc	334	407	22.4
Gly-Gly + Fru	334	406	35.4
Ac-Lys + Glc	343	423	7.0
Ac-Lys + Fru	343	426	6.3
Ac-Arg + Glc	371	427	1.3
Ac-Arg + Fru	312	426	4.8

of lysine and arginine; (ii) the tertiary structure of the polypeptides diminishes the reaction activity of lysine and arginine residues by electron interactions or steric hindrance which results in predominant glycation of the N-terminal amino groups.