Reactivities of D-glucose and D-fructose during Glycation of Bovine Serum Albumin

Faustinus K. Yeboah, Inteaz Alli,* and Varoujan A. Yaylayan

Depertment of Food Science and Agricultural Chemistry, Macdonald Campus of McGill University, 21,111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, Canada H9X 3V9

Glycation of bovine serum albumin by D-glucose and D-fructose under dry-heating conditions was studied. The reactivities of D-glucose and D-fructose, with respect to their ability to utilize primary amino groups of proteins, to cross-link proteins, to develop Maillard fluorescence, and to reduce protein solubility in the presence and absence of air (molecular oxygen) were investigated. D-Glucose showed a higher initial rate of utilization of primary amino groups than D-fructose, both in the presence and in the absence of oxygen. Subsequent reactions of the Amadori and Heyns rearrangement products, cross-linking, development of Maillard fluorescence, oxidation, and fragmentation, indicated that the α-hydroxy carbonyl group of Amadori products is more reactive than the *aldehydo* group of Heyns products. D-Fructose showed a greater sensitivity than D-glucose toward the presence of oxygen at the initial stages of the Maillard reaction. The presence or absence of oxygen in the glycation mixture did not seem to have an influence on the nature of products generated in the glycation mixtures during the advanced stages of the Maillard reaction.

Keywords: *Glycation; reactivitiy; fluorescence; BSA; D-glucose; D-fructose; cross-linking*

INTRODUCTION

The interactions between proteins and carbohydrates in foods have attracted considerable attention during the past decade. This may be attributed to the ubiquitous nature of protein glycation reactions and the modification of some functional properties of proteins after their conjugation with carbohydrates (Mat Easa et al., 1996; Kato et al., 1990, 1991,1992; Nakamura et al., 1992a,b, 1991). In addition, the application of glycated food proteins in the food industry presents fewer safety issues when compared with chemically modified food proteins (Kato et al., 1996). Nonetheless, there are concerns relating to nutritional and toxicological aspects of glycated food proteins. These include the loss of available lysine (Naranjo et al., 1998) and other essential amino acids such as tryptophan (Moreaux and Birlouez-Aragon, 1997), reduction of protein digestibility (Hurrel, 1990; Umetsu and Van Chuyen, 1998), and formation of toxic advanced glycation end products (AGEs) (Koschinsky et al., 1997). Protein glycation is initiated by a condensation reaction between the terminal α-amino group or the ϵ-amino group of lysine residues of proteins and the α-hydroxy carbonyl group of a reducing sugar or saccharide. Either Amadori or Heyns intermediate rearrangement products may be formed from the initial condensation reaction depending on whether the reducing sugar is an aldose or a ketose (Yaylayan, 1997). A complex series of reactions follows, from the formation of the initial intermediates, leading to (a) the formation of highly reactive compounds that may decompose into small volatile flavor compounds or polymerize to form high molecular weight brown pigments called mellanoidins and (b) the formation of fluorescent compounds that can be measured to monitor the Maillard reaction in food or model systems (Morales et al., 1996; Moreaux and Birlouez-Aragon, 1997).

The major factors that influence the rate and extent of protein glycation are temperature and water activity (Labuza and Saltmarch, 1981; Davies et al., 1998) and the nature and amount of the reducing sugar. The initial kinetics of glycation is dependent on the proportion of the reducing sugar existing in the acyclic or active form under the reaction conditions (Yaylayan et al., 1993; Labuza, and Baisier, 1992) and on the electrophilicity of the sugar carbonyl group (Bunn and Higgins, 1981). The reactivity of reducing sugars was reported to decrease in the following order: aldopentoses > aldohexoses > aldoketoses > disaccharides (Spark, 1969). Kato et al. (1990) also reported that a terminal pyranose group at the C-4 position of the reducing end of disaccharides retarded further reactions of protein−disaccharide adducts. Although it is accepted that, in general, aldoses are intrinsically more reactive than ketoses, there have been conflicting reports on the reactivities of glucose and fructose. Several researchers (Suarez et al., 1995; Walton et al., 1989; Mauron, 1981; Kato et al., 1969) have reported that fructose is more reactive than glucose. Other researchers (Naranjo et al., 1998; Baxter, 1995; Spark, 1969) have reported glucose to be more reactive. It has also been reported that fructose is more effective in causing protein cross-linking and in generating protein-bound Maillard fluorescence than glucose (Sakai et al., 1990; Suarez et al., 1991; Walton et al., 1989). The discrepancies in the literature may be related to differences in the conditions under which the Maillard reactions were conducted and the methods used to monitor the reaction. Our examination of the literature suggests that studies conducted in buffered solutions give variable results when compared with studies conducted under dry heating. Re-

* Author to whom correspondence should be addressed [telephone (514) 398 7898; fax (514) 398 7977; e-mail alli@agradam.lan.mcgill.ca].

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ports reviewed by Baynes et al. (1989) show that different buffer systems can affect the rate of glycation and the specificity of the different amino groups of proteins. Wu et al. (1990) also reported that glycation specificity can be influenced by water activity. The objective of this study is to investigate the reactivities of glucose and fructose, the nature of their Maillard reaction products under dry-heating conditions with bovine serum albumin (BSA), and the influence of oxygen on glycation.

MATERIALS AND METHODS

Materials. BSA (>96% purity) and D-glucose and D-fructose were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents were of analytical grade from Fisher Scientific (Nepean, ON, Canada). Ultrapure water from a Nanopure water purification system (Barnstead, Thermolyne, Dubuque, IA) was used throughout the study.

Sample Preparation. BSA (15 g) and D-glucose or D-fructose (2.5 g) were dissolved in water (50 mL) to give a protein to sugar molar ratio of 1:61 (~1:1 ratio of sugar carbonyl to lysine residues). The solutions were adjusted to pH 7 with dilute HCl or NaOH, freeze-dried, and stored at −20 °C until further analysis. The freeze-dried protein−sugar mixtures (1 g) were placed in glass vials, covered with perforated aluminum foil, and incubated at 50 °C in sealed glass desiccators maintained at a relative humidity of ~65% (Greenespan, 1977) with saturated aqueous KI solution, throughout the incubation period. The samples were incubated for 1, 2, 5, 10, and 14 days in separate desiccators, to prevent repeated opening and closing of the sealed desiccators. After incubation, samples were stored at −20 °C until they were analyzed. To determine the effect of molecular oxygen on glycation, another experiment was conducted, in which the desiccators were evacuated and the air was replaced with ultrapure nitrogen prior to incubation. The water used to prepare the saturated aqueous KI solution for this experiment was previously degassed and saturated with ultrapure nitrogen.

Fluorescamine Assay. A modification of the fluorescamine assay (Yaylayan et al., 1992) was used to determine unreacted free ϵ-amino groups in the incubated samples. A quantity (25 mg ± 5 mg) of the incubated samples was suspended in water (10 mL), vortexed, and sonicated for 30 min. The resulting solution was filtered through a 0.45 μm syringe filter. The filtrate was diluted (10×), and 200 μL of the diluted solution was pipetted into a centrifuge tube (10 mL) containing 4 mL of borate buffer (0.2 M potassium borate, pH 8.5). An aliquot (1 mL) of fluorescamine reagent (15 mg in 100 mL of acetone) was added rapidly during vortexing. A borate buffer (4.2 mL) containing no protein was used as a blank. Fluorescence was measured on a Kontron spectrofluorometer (Kontron Instruments SFM 25 spectrofluorometer, Zurich, Switzerland), at excitation and emission wavelengths of 390 and 475 nm, respectively. The fluorescence readings were normalized by the soluble protein content of the respective solutions.

Protein Solubility. Aliquot (500 μL) of the diluted filtrate (above) was used to determine the water soluble protein content of the incubated sample, using the Lowry method (Lowry et al., 1951).

Size Exclusion Chromatography and Water Soluble Maillard Fluorescence. A Superose 12-HR column (molecular mass range 300−3000 Da, Pharmacia Biotech Inc., Baie d'Urfe, PQ, Canada) was used to determine the molecular weight profile of the water soluble fraction of the incubated BSA/fructose or BSA/glucose samples. A Beckman Gold HPLC system fitted with a UV diode array detector (Beckman Instruments, Inc., Fullerton, CA) and a Shimadzu spectrofluorometer (RF-551, Shimadzu Corp. Kyoto, Japan) were used for detection. The UV detector was set at 214 nm, and the fluorometer was set to measure total Maillard fluorescence at 350 and 440 nm for excitation and emission, respectively. Separation was achieved by isocratic elution using a phosphate buffer (50 mM sodium phosphate, 0.1 M NaCl, pH 7). Aliquots (100 μL) of the diluted filtrates (above) were injected for the chromatographic analysis. The relative total Maillard fluorescence of the aqueous solutions of the glycated samples were determined as a ratio of the total fluorescence area counts to the total UV absorbance area counts as obtained from the chromatograms. Protein-bound fluorescence was determined from the fluorescence area count of the peaks that eluted within the void volume and the retention volume of BSA; these were considered as cross-linked BSA and non-cross-linked BSA (modified or unmodified with sugars). Non-protein-bound fluorescence was determined on peaks that eluted after the retention volume of BSA; these were considered to be fragmentation products of the Maillard reaction arising from the interaction between BSA and the reducing sugars. All experiments described were performed in duplicate.

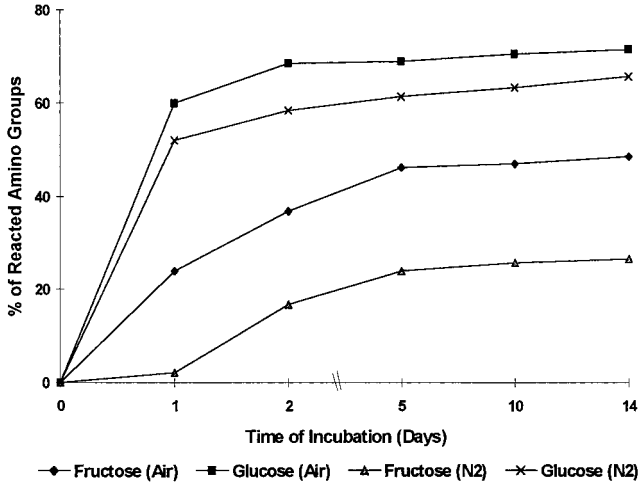

Figure 1. Percentage of ϵ-amino groups of lysine residue of BSA that had reacted with the carbonyl groups of D-glucose and D-fructose in the glycation mixtures during the 14 day incubation period, in air and in nitrogen.

RESULTS AND DISCUSSION

Fluorescamine Assay. Figure 1 shows the results of the percentage of free ϵ-amino groups of lysyl residues of BSA that had reacted with D-glucose and D-fructose after 1, 2, 5, 10, and 14 days of incubation in the presence and absence of air. The results show that glucose had a faster initial rate of utilization of ϵ-amino groups than fructose, both in the presence and in the absence of oxygen. This result conforms with the findings of other investigators (Naranjo et al., 1998; Baxter, 1995; Spark, 1969). The BSA/D-glucose system reacted with 35% more ϵ-amino groups of BSA in the presence of air and 50% more in the absence of air, when compared with BSA/D-fructose system at 1 day of incubation. After the first day of incubation, however, the rates of utilization of ϵ-amino groups in the BSA/D-glucose and in the BSA/D-fructose systems were quite similar, both in the presence and in the absence of air. The results suggest that molecular oxygen (in air) plays a significant role in the initial stages of the Maillard reaction. The exact role of molecular oxygen is not well understood; however, it is believed that it catalyzes the autoxidation of the α-hydroxy carbonyl function of reducing sugars and Amadori rearrangement products (Yaylayan and Huyghues-Despointes, 1994; Thornalley et al., 1984) into more reactive α-dicarbonyls, which can also react with free amino groups of proteins, as well as participate in other side reactions, such as protein cross-linking and degradation.

The initial rate of ϵ-amino group utilization in the BSA/D-fructose system in the absence of air was very low when compared with that in the presence of air. By the end of the first day of incubation only 2% of the ϵ-amino groups of BSA had been utilized in the absence of oxygen, compared with 24% in the presence of oxygen. Over the 14 day incubation period, the BSA/D-fructose system incubated in the presence of air utilized an average of 20% more ϵ-amino groups than the BSA/D-fructose system incubated in the absence of air. The BSA/D-glucose system, on the other hand, showed similar extents of utilization of ϵ-amino groups, both in the presence and in the absence of air. There was an average of 8% more utilization of ϵ-amino groups in the presence of air over the 14 day incubation period. A comparison of the extent of utilization of ϵ-amino groups after 1 day of incubation in the BSA/D-glucose system with the BSA/D-fructose system shows that the BSA/D-glucose system utilized 35 and 50% more ϵ-amino groups of BSA than the BSA/D-fructose system in the presence and absence of air, respectively. In the following discussion we designate incubation in nitrogen as the absence of oxygen and incubation in air as the presence of oxygen.

The higher initial rate of utilization of amino groups, both in the presence and in the absence of oxygen in the BSA/D-glucose system when compared with the BSA/D-fructose system, may be explained by the fact that the *aldehydo* group of the acyclic form of glucose is more electrophilic than the *keto* group of the acyclic form of fructose, so that glucose will condense more quickly with the ϵ-amino groups of BSA before molecular oxygen in air has time to catalyze the oxidation of its α-hydroxy carbonyl function into the more reactive α-dicarbonyl form. In this regard, there will be little difference in the utilization of ϵ-amino groups by glucose in the presence and absence of oxygen. The lower reactivity of the acyclic form of intact fructose, on the other hand, will allow time for molecular oxygen to catalyze the oxidation of significant amounts of the α-hydroxy carbonyl function of fructose into more reactive dicarbonyl form, which can also react with ϵ-amino groups of BSA. This will result in a significant increase in the initial rate of utilization of ϵ-amino groups in the BSA/D-fructose system in the presence of oxygen when compared with that in the absence of oxygen. Over the 14 day incubation period, the BSA/D-glucose system utilized an average of 40 and 25% more free ϵ-amino groups than the BSA/D-fructose system in the absence and in the presence of oxygen, respectively. The results indicate that intact D-fructose is more sensitive to the presence of oxygen than intact D-glucose. The utilization of free ϵ-amino groups by glucose was generally higher than by fructose, both in the presence and in the absence of oxygen.

Solubility. Figure 2 shows the effect of glycation of BSA with D-fructose and D-glucose, in the presence and absence of oxygen, on the solubility of BSA. The results represent the soluble protein content of the glycation mixture as a percentage of the total protein used at the initial stage of the glycation reaction. In the presence of oxygen, there was a gradual loss of BSA solubility in both BSA/D-glucose and BSA/D-fructose systems during the first 2 days of incubation. This was followed by a sharp decrease in protein solubility between the 2nd and 10th days of incubation. By the 14th day, BSA/D-glucose and BSA/D-fructose systems had lost 97 and 92% of BSA

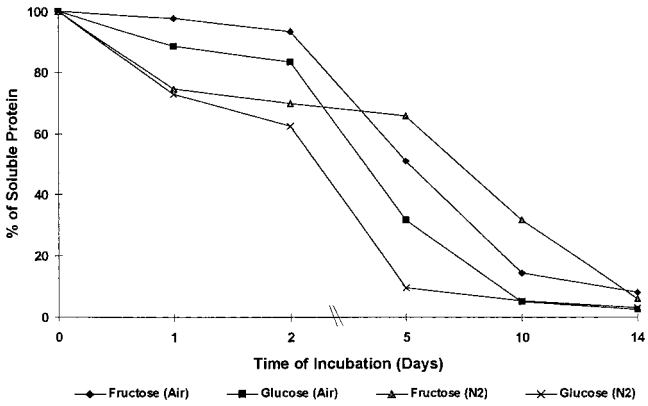

Figure 2. Percentage of water soluble BSA remaining, as a percentage of the initial amount of BSA in the glycation mixture after incubation with D-glucose and D-fructose, in air and under nitrogen over a 14 day incubation period.

solubility, respectively. The patterns of loss of BSA solubility over the incubation period were similar in both BSA/D-glucose and BSA/D-fructose systems, but the average loss in BSA solubility was ~10% higher for the BSA/D-glucose throughout the incubation period. In the absence of oxygen, both systems showed a sharp decrease in BSA solubility after only 1 day of incubation. Between the 1st and 5th days of incubation, the BSA/D-glucose system showed a higher rate of reduction in BSA solubility than the BSA/D-fructose system, resulting in a 60% decrease in BSA solubility in the BSA/D-glucose system compared with a 10% decrease in the BSA/D-fructose system. After the 5th day of incubation, the BSA/D-fructose system also experienced a high rate of protein solubility loss, resulting in a 60% decrease in BSA solubility by the 14th day of incubation. The observed decrease in BSA solubility when incubated with D-glucose and D-fructose may be attributed to extensive cross-linking reactions between intact BSA, Amadori or Heyns rearrangement products, and the reducing sugars or their reactive intermediates such as dicarbonyl derivatives to form large insoluble covalent complexes (Dills, 1993; Sakai et al., 1990; Suarez et al., 1991). The results indicate that D-glucose is more effective in causing protein cross-linking than D-fructose. This observation is contrary to several reports of glycation experiments conducted in solution (Sakai et al., 1990; Suarez et al., 1989, 1991, 1995). A possible explanation for this discrepancy could be the effect of different buffer systems on the rate and site specificity of glycation, when glycation is carried out in solution (Baynes et al., 1989). It could also be that when protein-reducing sugar systems are incubated in solution, insoluble complexes formed as a result of extensive protein cross-linking precipitate out of solution and are not observed during subsequent analysis of the solution. This can obscure the interpretation of results derived from analysis of the incubated solutions. In a comparison of the reactivities, and subsequent cross-linking, fragmentation, and oxidation reactions of Amadori and Heyns products, it is important to note that the open chain form of Amadori products exists only in the *keto* form with a α-hydroxy carbonyl function, whereas Heyns products exist mainly in the *aldehydo* form, without an α-hydroxy carbonyl functional group. Reactions of Amadori or Heyns products will be influenced by the reactivities of their carbonyl functional groups. Through enolization and autoxidation, the α-hydroxy carbonyl functional groups of Amadori products can be

converted into their corresponding deoxyosones more readily, compared with Heyns products. These osones are more reactive and can also generate oxygen free radicals in the reaction system (Ortwerth et al., 1998), thereby increasing the oxidative potential of the BSA/D-glucose system. The α-hydroxy carbonyl group of Amadori products can also migrate to C3, C4, or C5 of the sugar moiety (Yaylayan and Huyghues-Despointes, 1994), thus eliminating the problem of steric hindrance and promoting further condensation of the carbonyl group with another primary amino group.

The results also show that the loss of protein solubility, which results from protein cross-linking, occurred more rapidly in the absence of oxygen than in the presence of oxygen during the first day of incubation in both BSA/D-glucose and BSA/D-fructose systems. This may be due to differences in the rates of competing reaction pathways that lead to protein cross-linking, fragmentation, and oxidation. It appears that, when oxygen is absent at the initial stages of the Maillard reaction, cross-linking of intact Amadori or Heyns products is the dominant reation pathway. As the reaction proceeds, substantial amounts of deoxyosones can be formed through enolization of the sugar moiety of the Amadori or Heyns products, which in turn generate superoxide anions into the reaction system (Ortwerth et al., 1998). At this stage, degradative reactions leading to extensive protein cross-linking, fragmentation, and oxidative damage become more important. The absence of molecular oxygen at the initial stages of the Maillard reaction seems to delay the onset of fragmentation and oxidative reactions. When molecular oxygen is present at the initial stages of the Maillard reaction, however, it catalyzes oxidative reactions that lead to the formation of reactive dicarbonyls, free radicals, and fragmentation. These oxidative reactions compete with cross-linking reactions, so that protein cross-linking, and hence loss of protein solubility, is not as extensive at the initail stages of the Maillard reaction when molecular oxygen is present as when it is absent. The results indicate that molecular oxygen influences the initial rates of the myriad of reactions that constitute the Maillard reaction and that, in the absence of molecular oxygen, protein-reducing sugar reaction systems can generate other oxidative species, such as low molecular weight dicarbonyls and oxygen free radicals.

SEC and Water Soluble Maillard Fluorescence. Figure 3 shows the relative Maillard fluorescence in the BSA/D-glucose and BSA/D-fructose systems over the incubation period. The results show that during the first 2 days of incubation, the rates of development of Maillard fluorescence were quite similar in both the BSA/D-glucose and BSA/D-fructose systems. After the 5th day of incubation, the Maillard fluorescence in the BSA/D-glucose system increased sharply to 6486 units, compared with 812 units by the 14th day of incubation. The rates of development of Maillard fluorescence were also similar for both BSA/D-glucose and BSA/D-fructose systems incubated in air and in nitrogen. The sharp increase in Maillard fluorescence in the BSA/D-glucose system after the 5th day of incubation was due to the production of low molecular weight fluorescent compounds (Figures 4 and 5).

Table 1 shows the results of the total Maillard fluorescence developed in the glycation mixtures and the percentages of the total fluorescence that are intact

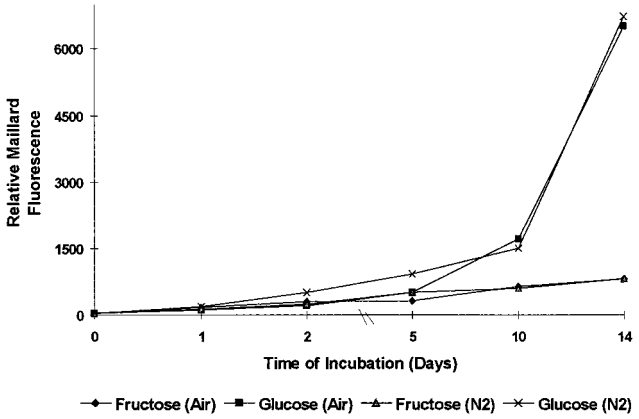

Figure 3. Total Maillard fluorescence in the water soluble fraction of the glycation mixtures (BSA/D-glucose and BSA/D-fructose mixture incubated in air and under nitrogen over a 14 day period).

protein-bound and non-protein-bound. The results show that the Maillard fluorescence in the BSA/D-fructose systems, both in the presence and in the absence of oxygen, was mainly protein-bound fluorescence throughout the incubation period. The BSA/D-glucose system, however, started developing non-protein-bound fluorescence by the 5th day of incubation. The production of non-protein-bound fluorescence increased sharply thereafter, accounting for >90% of the total Maillard fluorescence of the BSA/D-glucose systems by the 14th day of incubation, both in the presence and in the absence of oxygen. The development of highly fluorescent, low molecular mass compounds (MW < 66 kDa) in the BSA/D-glucose systems occurred after substantial amounts of insoluble cross-linked proteins had been formed. This suggests that the low MW fluorescent compounds may be protein-bound fluorescent moieties that had been cleaved from the high MW insoluble cross-linked protein complexes. The absence of fluorescent fragments in the BSA/D-fructose system also suggests that the nature of cross-linked protein complexes formed in the BSA/D-fructose system could be different from that formed in the BSA/D-glucose system. It may also be that the BSA/D-glucose system generated more oxidative byproducts, such as oxygen-centered free radicals which facilitated oxidative fragmentation of the cross-linked protein complexes. The highly fluorescent low MW compounds could also be the product of polymerization of highly reactive oxidation and fragmentation products, produced during the cause of the Maillard reaction. The molecular masses of the fragments ranged from 20 to 3 kDa. The BSA/D-fructose system did not produce detectable quantities of the fluorescent fragments within the 14 days of incubation. Further work is ongoing to characterize the nature of the low molecular weight fluorescent compounds.

Chromatograms of the water soluble fraction of the incubated samples are presented in Figures 4 and 5. Table 2 shows the relative amounts of cross-linked BSA, non-cross-linked BSA, and fragmentation products in the water soluble fraction of the glycation mixtures, presented as a percentage of total soluble fraction. The results show that the initial rate of accumulation of soluble cross-linked BSA in both BSA/D-glucose and BSA/D-fructose samples incubated in the absence of oxygen was higher than that of the samples incubated in the presence of oxygen. This is consistent with the higher initial rate of decrease in protein solubility

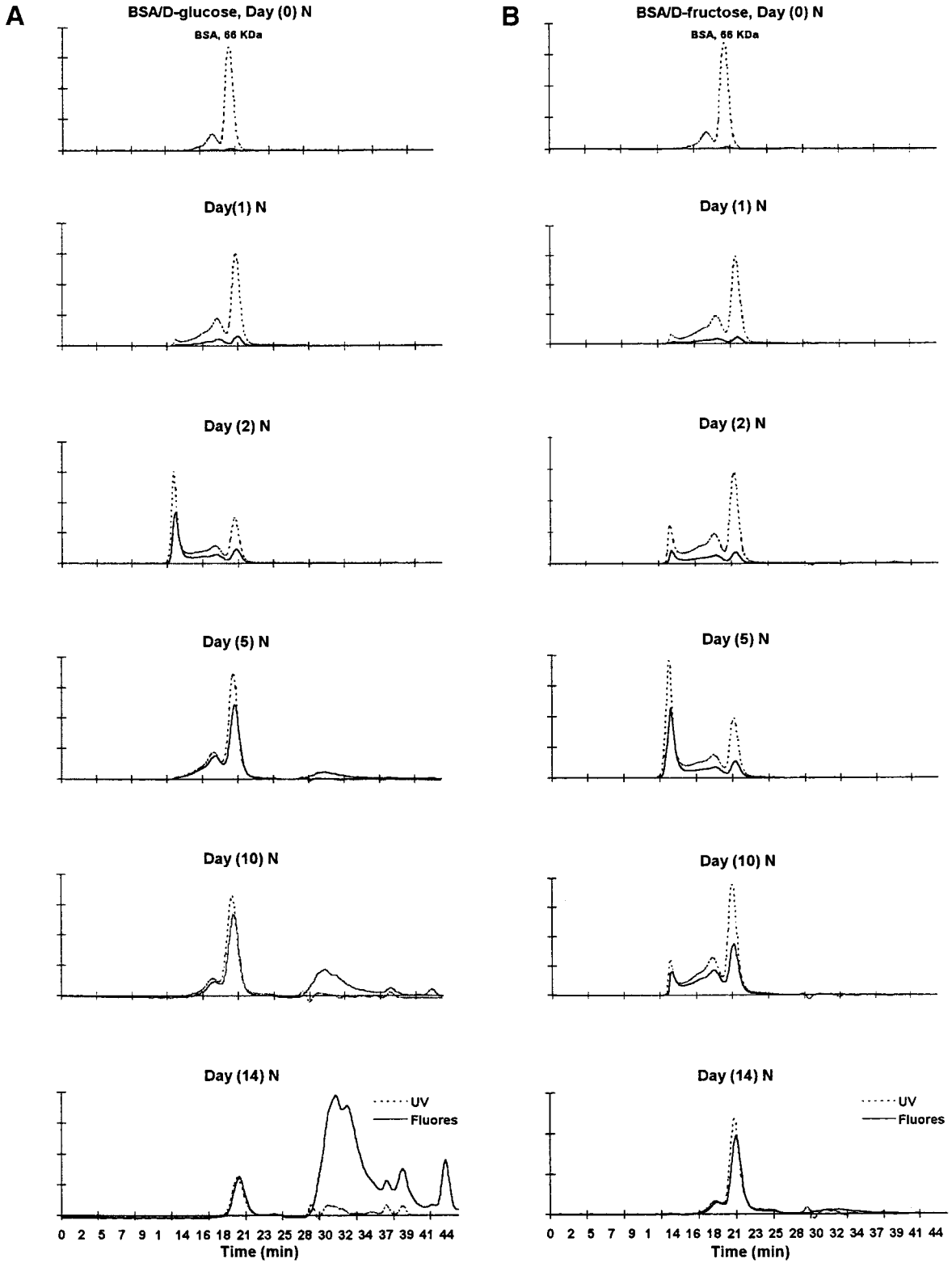

Figure 4. Size exclusion chromatograms of the water soluble fraction of BSA/D-glucose (A) and BSA/D-fructose (B) mixtures incubated in nitrogen over a 14 day period. Dotted line chromatogram represents UV absorbance, and full line chromatogram represents fluorescence.

observed for samples incubated in the absence of oxygen when compared with those incubated in the presence of oxygen (Figure 2). The results also confirm an earlier assertion that among the myriad reactions that occur after the formation of Amadori and Heyns products, protein cross-linking seems to be the dominant reaction in the absence of oxygen. The molecular masses of the soluble cross-linked proteins were in excess of 300 kDa (exclusion limit of of the Superose 12 column). Cross-linked proteins in the water soluble fraction of the BSA/D-glucose samples incubated in the absence of oxygen increased sharply and peaked after only 2 days of incubation at 53% of the total soluble proteins, compared with 30% for the BSA/D-fructose samples incu-

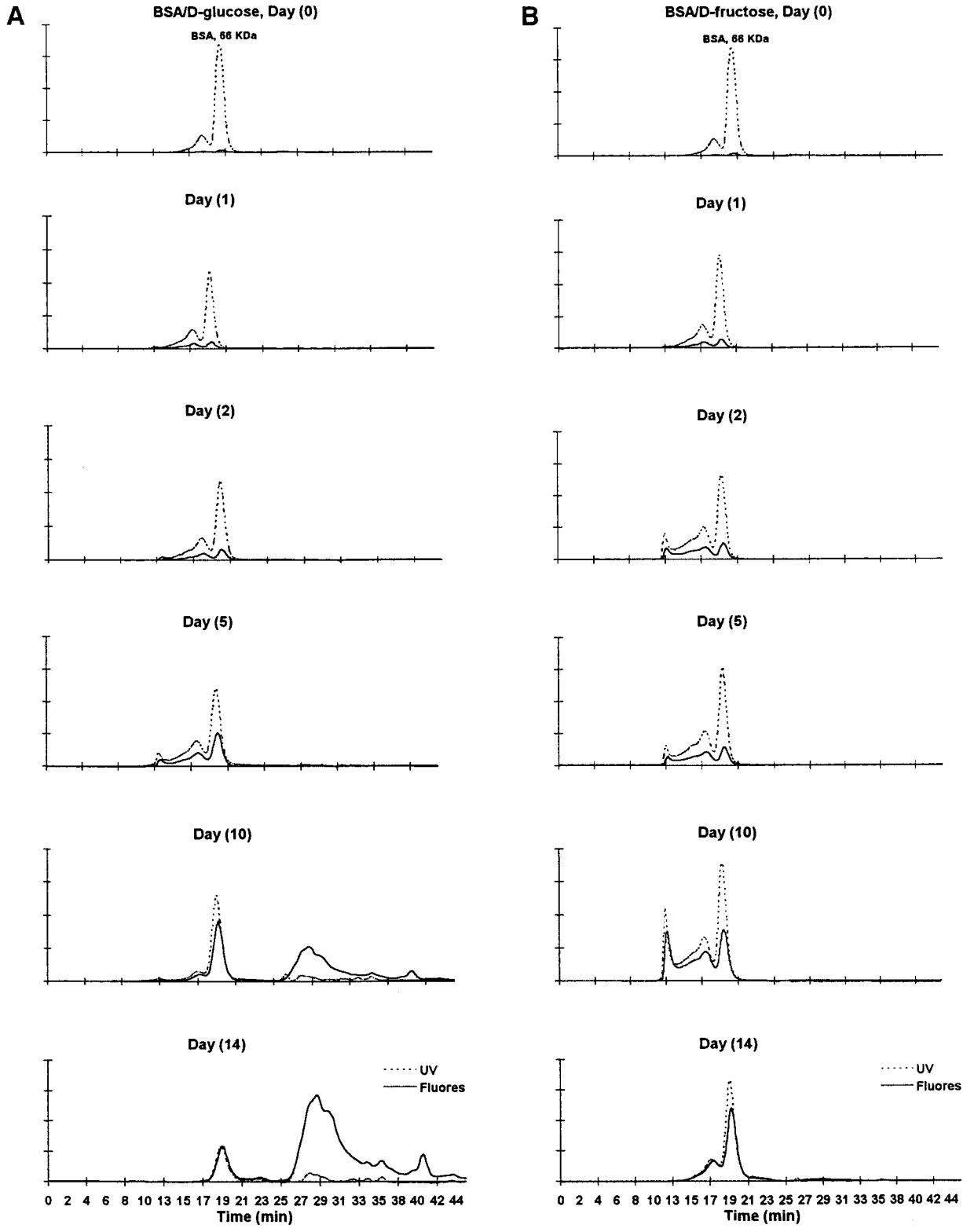

Figure 5. Size exclusion chromatograms of the water soluble fraction of BSA/D-glucose (A) and BSA/D-fructose (B) mixtures incubated in air over a 14 day period. Dotted line chromatogram represents UV absorbance, and full line chromatogram represents fluorescence.

bated for the same length of time. Cross-linked proteins in the water soluble fraction of the BSA/D-fructose system incubated in the absence of oxygen also increased fairly quickly, peaking after 5 days of incubation at 56%. The higher initial rate of protein cross-linking in the BSA/D-glucose system indicates that in the absence of oxygen, glucose is more effective in cross-linking proteins than fructose. The abrupt loss of cross-linked proteins in the water soluble fraction of the BSA/D-glucose system (after the 2nd day of incubation) and in the BSA/D-fructose system (after the 5th day of incubation) corresponds to the sharp decrease in soluble protein at corresponding times (Figure 2). This confirms previous observations that the loss of protein solubility during glycation is due to extensive cross-linking of proteins to form insoluble covalent complexes.

Table 1. Total Maillard Fluorescence Developed in the Glycation Mixtures of BSA/D-Glucose and BSA/D-Fructose Samples during Incubation under Nitrogen and Air, Showing Protein- and Non-Protein-Bound Fluorescence[a]

	incubated under nitrogen			incubated in air (oxygen)		
	total fluorescence	% protein-bound	% non-protein-bound	total fluorescence	% protein-bound	% non-protein-bound
BSA/Fru (0)	30	100	0	30	100	0
BSA/Fru (1)	140	100	0	171	100	0
BSA/Fru (2)	235	100	0	232	100	0
BSA/Fru (5)	496	100	0	325	100	0
BSA/Fru (10)	598	100	0	633	100	0
BSA/Fru (14)	811	100	0	812	100	0
BSA/Glu (0)	30	100	0	30	100	0
BSA/Glu (1)	183	100	0	108	100	0
BSA/Glu (2)	512	100	0	207	100	0
BSA/Glu (5)	921	85	15	496	94	6
BSA/Glu (10)	1498	77	23	1704	35	65
BSA/Glu (14)	6722	8	92	6486	10	90

[a] Results presented are means of duplicate experiments.

Table 2. Distribution of Cross-Linked BSA, Non-Cross-Linked BSA (Modified by Sugar and Unmodified), and Fragmentation Products in the Water Soluble Fraction of the Glycation Mixtures, Presented as a Percentage of the Total Soluble Fraction of the Glycation Mixtures

	incubated under nitrogen			incubated in air (oxygen)		
	% cross-linked BSA	% non-cross-linked[a] BSA	% fragmentation products	% cross-linked BSA	% non-cross-linked[a] BSA	% fragmentation products
BSA/Fru (0)	0	100	0	0	100	0
BSA/Fru (1)	19	81	0	10	90	0
BSA/Fru (2)	30	70	0	20	80	0
BSA/Fru (5)	56	44	0	24	76	0
BSA/Fru (10)	11	90	0	34	66	0
BSA/Fru (14)	0	92	8	0	96	4
BSA/Glu (0)	0	100	0	0	100	0
BSA/Glu (1)	15	85	0	1	99	0
BSA/Glu (2)	53	47	0	12	87	1
BSA/Glu (5)	2	98	1	17	79	4
BSA/Glu (10)	0	93	7	2	81	17
BSA/Glu (14)	0	56	45	0	65	36

[a] Non-cross-linked BSA in the soluble fraction of the glucation mixtures may be unmodified or at various stages of modification.

The accumulation of soluble cross-linked proteins in the BSA/D-fructose samples incubated in the presence of oxygen (Table 2 and Figure 5) was much higher than that found in corresponding BSA/D-glucose samples. The solubility results (Figure 2), however, show that the loss of protein solubility in the BSA/D-glucose system incubated in the presence of oxygen was higher than that in the corresponding BSA/D-fructose system throughout incubation period. Because extensive cross-linking has been related to a decrease in protein solubility, it can be inferred that protein cross-linking in the BSA/D-glucose system occurs more quickly and more extensively to form insoluble aggregates, so that there was less accumulation of soluble cross-linked proteins in the BSA/D-glucose system when compared with the BSA/D-fructose system. This explanation is consistent with the observation that the formation of low molecular weight fragmentation occurred more quickly in the BSA/D-glucose systems.

CONCLUSION

This study has demonstrated that under dry-heating conditions, D-glucose is more reactive than D-fructose, suggesting that the electrophilicity of the carbonyl function of reducing sugars is a more important determinant of the initial rate of reaction when compared with the proportion of the reducing sugar that exists in the open chain form. After the formation of Amadori or Heyns rearrangement products, further reactions depend on, among other things, the reactivity of the carbonyl function of the Amadori or Heyns products. The presence of an α-hydroxy carbonyl function in Amadori products, compared with an *aldehydo* group in Heyns products, enables Amadori products to undergo a variety of reactions, such as enolization, migration of carbonyl group, oxidation of the carbonyl group, and retro-aldol cleavage of C−C bonds, more readily than Heyns products. For these reasons, further reactions of Amadori products are likely to be more extensive. These reactions include protein cross-linking, formation of low molecular weight fluorescent compounds that may result from protein fragmentation or polymerization of highly reactive fragments of the sugar moiety, and formation superoxide anions. The presence of molecular oxygen in the reaction system at the initial stages of the Maillard reaction seems to facilitate oxidative reactions such as the formation of deoxyosones, which compete with and slow condensation reactions between the carbonyl groups of reducing sugars or their derivatives and the amino groups of proteins, Amadori, or Heyns products, which lead to cross-linking. The results also indicate that D-fructose is more sensitive than D-glucose to the presence or absence of molecular oxygen in the reaction system at the initial stages of the Maillard reaction.

The higher glycation activity of glucose compared to fructose under dry or low-moisture conditions shows that the replacement of glucose with fructose in low-

moisture foods such as cereals and infant formulas will help reduce the extent of protein damage due to glycation during processing and storage. It will also help reduce the amount of sugar required to achieve the same sweetness in these foods because fructose has a higher sweetness threshold than glucose.

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