Maillard Reaction Kinetics in Model Preservation Systems in the Vicinity of the Glass Transition: Experiment and Theory

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Rates of reactant consumption for the Maillard reaction between lysine and glucose were measured for a noncrystallizing trehalose−sucrose−water matrix in the glass transition region. At temperatures above the glass transition temperature (T_g), the consumption rates showed Arrhenius temperature dependence with activation energies of 135 and 140 kJ mol^{-1} for lysine and glucose, respectively. Finite reaction rates were observed for glassy samples that were faster than that of one of the nonglassy samples. A comparison of experimental results with predicted diffusion-controlled reaction rate constants indicated that the reaction was reaction-controlled at temperatures above T_g and approached the diffusion-influenced regime in the glassy state. The needs for further research on reactant diffusivity, the theory of the orientation dependence of reactivity, and a detailed understanding of the reaction mechanism and kinetics were identified.

Keywords: *Glass transition; Maillard reaction; reaction control; diffusion control; kinetics*

INTRODUCTION

The ability to control rates of deteriorative chemical reactions is important to the preservation of foods, pharmaceuticals, and other biological materials (*1*−*3*). The physical, "molecular mobility" approach to the chemical stability of foods (*4*) is based on the reasoning that, if the molecular motions involved in a particular chemical reaction can be slowed sufficiently, then the reaction will be slowed and a degree of preservation achieved. This idea has been discussed in the literature on low-water-content foods for many years (*4*) and, more recently, has gained prominence in the work of Slade and Levine (*5*, *6*), who identified the glass transition temperature (T_g) as the temperature that controls the rate of diffusion-controlled processes. The high viscosity of glassy systems was associated with limited molecular mobility and the arrest of chemical reaction. There are several aspects to the proposed relationship between the glass transition, molecular mobility, and chemical reaction rates (*7*, *8*), i.e., diffusion control of chemical reactions in the vicinity of the glass transition, Williams−Landel−Ferry (WLF) (*9*) temperature dependence in the temperature interval from T_g to $T_g + 100$ °C, and arrest of chemical reaction in the glassy state. The rate constant, k, of a chemical reaction is said to obey Williams−Landel−Ferry kinetics if its temperature dependence can be described by

$$\log k = \log k_g + \frac{C_1(T - T_g)}{C_2 + (T - T_g)}$$

where k_g is the rate constant at T_g and C_1 and C_2 are constants. Diffusion control and WLF kinetics are well-founded in the physical chemistry literature, a good example being Dainton et al. (*10*), a comprehensive photochemical study of diffusion-controlled energy transfer kinetics in a glass-forming solvent at temperatures above its glass transition temperature. The question is whether the deteriorative reactions of interest to food, pharmaceutical, and biological scientists are retarded by the relatively limited molecular mobility found in highly viscous systems. Interest in this approach has led to a number of studies of chemical kinetics in glassy and near-glassy systems (*8*, *11*−*16*) and also, more generally, studies of molecular dynamics in low-water-content amorphous food materials or, more commonly, their models (*17*−*22*).

In the present study, the Maillard reaction in amorphous mixtures in the vicinity of the glass transition is examined using both experiment and theory. The Maillard reaction occurs between reducing sugars and amino groups of amino acids and proteins in intermediate- and low-water-content foods, resulting in browning and changes in nutritional value (*23*, *24*). Its rate has often been studied in relation to the water activity associated with the foods' vapor pressure (*25*). More recently, browning in dehydrated vegetables (*8*) and whey powders (*26*) have been investigated in relation to their glass transition behavior. There is now a body of data showing that the Maillard reaction proceeds in the glassy state (e.g., Schebor et al. (*27*) and references therein). In investigations of the molecular mobility approach to Maillard reaction kinetics, the reaction has commonly been characterized in terms of the variation in the absorbance of its brown products (*8*, *11*) because of the complex nature of the consecutive and competing reactions (*28*). Here, to obtain a closer link with theory, absolute measurements of the disappearance of the primary reactants, glucose and lysine, are made. The relationship between molecular mobility and chemical kinetics is described explicitly using physicochemical theory (*29*−*32*). The aim is to clarify the factors that affect chemical reactions in systems close to the glass

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10.1021/jf0100752 CCC: $20.00 © 2001 American Chemical Society
Published on Web 09/14/2001

transition, with particular attention to the question of whether the Maillard reaction is diffusion-controlled.

MATERIALS AND METHODS

Materials. D-Trehalose dihydrate (reduced metal ion content), sucrose (SigmaUltra), and L-lysine monohydrochloride (>98%) were all purchased from Sigma Chemical Co. (Poole, Dorset, U.K.); sodium hydroxide (HPLC grade) and phosphorus pentoxide from Fisher Scientific U.K. (Loughborough, Leics., U.K.); and D-glucose (AnalaR) from BDH (Poole, Dorset, U.K.). Water was from a water purification system (Elgastat Maxima, Elga, High Wycombe, U.K.) and had a conductivity of <1 μS m^{-1}. Saturated solutions of lithium chloride (Sigma, ACS grade) and potassium acetate (SigmaUltra) were used to create constant relative humidity environments.

Preparation of Amorphous Reaction Mixtures. The reaction between glucose and lysine was studied in a high-viscosity amorphous trehalose-based solvent. Trehalose was chosen because it is both a glass former and a nonreducing carbohydrate. During initial experiments using solvents with high trehalose contents (>90% w/w), it was found that, under certain conditions, the mixture crystallized, and so, a proportion of sucrose was introduced into the mixture to inhibit crystallization. The solids composition of the high-viscosity reaction mixture was trehalose dihydrate, 60% w/w; sucrose, 25% w/w; lysine monohydrochloride, 10% w/w; and glucose, 5% w/w. The concentrations of lysine and glucose are within the range of those used in previous studies (*11*, *14*), and under the conditions of the present study, they reacted over an experimentally convenient time scale. The mixture was dried into a glassy state by freeze-drying (Dura-Top MP bulk tray dryer with Dura-Dry MP condenser module, FTS Systems, Inc., Stony Ridge, NY), starting from an aqueous solution at 10% w/v solids at pH 5.4, and employing the following temperature program: −40 °C for 8 h, −32 °C for 30 h, −20 °C for 8 h, −5 °C for 8 h, and 25 °C for 36 h. The chamber pressure dropped from 20.0 Pa at the start of the secondary drying to 4.5 Pa on completion of drying. On removal from the freeze-dryer, the mixtures were further dried by vacuum desiccation over phosphorus pentoxide for 1 week at room temperature.

Adjustment of the Water Content of the High-Viscosity Mixtures. To obtain different water contents, the high-viscosity reaction mixtures were humidified for 1 week in desiccators over saturated salt solutions at 20 °C. To obtain the lower water contents required to study glassy-state reactivity, samples of the freeze-dried mixture were further dried in an evacuated desiccator over phosphorus pentoxide at 20 and 30 °C for 1 week. Samples were subsequently stored in screw-topped bottles, and their exposure to ambient humidity was minimized. Water contents were determined by automated Karl Fischer titration (model AF5, Baird and Tatlock) (*33*) with Hydranal Composite 5 Karl Fischer reagent (Riedel-de Haen, Gillingham, U.K.). The titrator was calibrated using water and a sodium tartrate standard (Fluka, Gillingham, U.K.). Prior to titration, a sample of each mixture (~100 mg) was weighed into a screw-capped vial (2 mL) and completely dissolved in a 2:1 methanol/formamide mixture (1 mL) (methanol, Riedel-de Haen, analytical grade; formamide, SigmaUltra). The water contents are reported in Table 1.

Differential Scanning Calorimetry. A Perkin-Elmer DSC7 differential scanning calorimeter (DSC) (Perkin-Elmer, Beaconsfield, U.K.) was used to determine the T_g of the high-viscosity reaction mixtures. Powdered samples were weighed (Mettler ME30) into 40-μL aluminum pans (Perkin-Elmer), hermetically sealed, and then reweighed. In the DSC, each sample was heated at 10 °C min^{-1} from approximately 40 °C below its T_g to about 40 °C above its T_g before quenching and rescanning. The T_g was taken as the temperature at which the heat capacity was midway between that in the liquid and glassy states of the rescanned sample (*34*). To determine this value, the heat capacities of the liquid and glass were extrapolated into the glass transition region from above and below the glass transition region, respectively, and the heat capacity midway between them was determined.

Table 1. Details of Reaction Kinetics Study Using Trehalose−Sucrose−Glucose−Lysine Mixtures

initial molar concentration (mol dm^{-3})		water content	T_g	temp	initial rate of reactant consumption (mol dm^{-3} day^{-1})	
glucose	lysine	(% w/w)	(°C)	(°C)	lysine	glucose
0.45	0.85	1.1	82	65	0.019	0.022
0.45	0.84	1.8	76	65	0.021	0.019
0.44	0.82	3.3	44	53	0.026	0.037
0.44	0.82	3.3	44	62	0.14	0.12
0.44	0.82	3.3	44	70	0.37	0.77
0.44	0.82	3.3	44	80	2.25	2.99
0.44	0.82	3.3	44	85	6.01	6.47
0.42	0.79	5.3	33	43	0.0045	0.011
0.42	0.79	5.3	33	50	0.043	0.026
0.42	0.79	5.3	33	57	0.14	0.11
0.42	0.79	5.3	33	65	0.43	0.39
0.42	0.79	5.3	33	73	1.61	0.93

Reaction Kinetics in High-Viscosity Mixtures. Samples (150 mg) of the amorphous, humidified mixtures were sealed in GC headspace vials (22 mL) with butyl rubber seals and aluminum caps (Perkin-Elmer). The vials were then held at constant temperature, with 10 sets of samples (at least eight samples in each set) at a range of temperatures above their T_g's for times ranging from 4 h to approximately 1 month to investigate the temperature dependence of reaction rate above T_g and two sets of lower water content mixtures (1.1 and 1.8% w/w water content) at 65 °C to investigate reaction in the glassy state. After storage at elevated temperatures, the samples were frozen at −30 °C and stored prior to bulk analysis by HPLC.

Determination of Glucose and Lysine Contents of Reaction Mixtures. Reactant concentrations were determined by anion-exchange HPLC with a refractive index detector (model 131, Gilson, Anachem Ltd., Luton, Beds, U.K.). Baseline separation was achieved using a Dionex CarboPac PA1 column with PA1 guard (Dionex U.K. Ltd., Camberley, Surrey, U.K.), a 52 mM NaOH mobile phase at a flow rate of 1 mL min^{-1}, and a reaction mixture concentration of 10−20 mg mL^{-1} diluted in distilled water. The injection volume was 25 μL. The molar concentrations of the reactants were calculated by assuming volume additivity of the partial specific volumes, which were estimated from the crystal densities (*35*). The partial specific volumes of trehalose, sucrose, lysine monohydrochloride, glucose, and water are 0.633, 0.633, 0.714, 0.640, and 1.000 cm^3 g^{-1}, respectively. The initial rates of consumption of lysine and glucose were estimated by fitting the concentration−time data to an exponential function with a finite long limit, i.e., $[A] = [A]_\infty + a \exp(-bt)$, where [A] denotes the concentration of species A, t denotes the time, and a and b are constants. The initial rate of consumption, $d[A]/dt|_{t=0}$, is obtained by differentiating the above function and is given by $d[A]/dt|_{t=0} = -ab$.

X-ray Diffraction. The physical state of the high-viscosity mixture was characterized using X-ray diffraction (XRD) (Philips Scientific PW1710 vertical goniometer with Anton-Paar TTK camera, Anton-Paar GmbH, Graz, Austria) on finely ground samples. Samples from reaction experiments were quenched to −30 °C and stored overnight prior to examination. Measurements were performed both prior to the beginning and at the end of the reaction kinetics experiments.

Theory. The simplest theoretical model that combines molecular mobility with chemical reaction is that of Collins and Kimball (*29*), a model for an irreversible bimolecular reaction with a finite reactivity. The molecular mobility of the reactants is characterized by their translational diffusion coefficients, D_i, and their reactivity constants, k_{act}, which recognizes that only a fraction of encounters of the reactants leads to reaction, as it is an activated process. The reactants, species A and B, combine irreversibly to form a product (AB). The rate of reaction is characterized by the second-order rate

law

$$\frac{d[(AB)]}{dt} = k[A][B]$$

where [A] and [B] are the concentrations of reactants A and B, [(AB)] is the concentration of the product, and k is a second-order rate constant. At steady state, the Collins and Kendall theory shows that

$$k = \frac{4\pi r_c D k_{act}}{4\pi r_c D + k_{act}}$$

where r_c is a collision diameter and D is the relative diffusivity of the reactants (equal to the sum of the individual diffusion coefficients). As $k_{act} \rightarrow \infty$, $k \rightarrow k_D = 4\pi r_c D$, the diffusion-controlled rate. The above theory is for molecules with a reactivity that is independent of their orientations; a more realistic model incorporating the effects of orientation by specifying circular reactive patches on the surface of spherically symmetric particles has been described by Solc and Stockmayer (*30*, *31*). In this model, the reaction rate depends on the size of the reactive patches and both the translational and rotational diffusion coefficients of the reactants.

To proceed further with the Collins–Kimball theory, a common approach is to assume the validity of the Stokes–Einstein relation (*36*), $D_i = kT/6\pi\eta r_h$, which relates the reactant diffusivity D_i to the viscosity of the medium η and the hydrodynamic radius r_h of the reactant (k is the Boltzmann constant, and T is the absolute temperature). Substituting this equation into the expression for the diffusion-controlled rate, k_D, and equating the hydrodynamic and collision radii yields $k_D = 8RT/3\eta$, where R is the ideal gas constant. This expression, based on Smoluchowski theory, is sometimes called the Smoluchowski–Stokes–Einstein rate coefficient (*37*). In the absence of either reactant diffusivity data or viscosity data for the high-viscosity reaction mixtures, we assume that the reactant diffusivities obey the Stokes–Einstein relation and that the viscosity can be calculated using the WLF equation (*9*)

$$\log \eta = \log \eta_g + \frac{C_1(T - T_g)}{C_2 + (T - T_g)}$$

where η_g is the viscosity at T_g [$= 10^{12}$ Pa s (*38*)] and C_1 and C_2 take their "universal" values of -17.44 °C^{-1} and 51.6 °C, respectively.

RESULTS

Effect of Temperature and Water Content on Reaction in the High-Viscosity Mixture. Extensive use of XRD established that all samples of this mixture remained amorphous throughout the reaction kinetics study. Figure 1 shows the effect of temperature on the time course of the lysine and glucose concentrations in the mixture with 3.3% w/w water. These reaction mixtures are highly viscous being between 9 and 36 °C above T_g. Over the duration of the experiment, extensive consumption of both lysine and glucose occurs. The initial rates of consumption are tabulated in Table 1. Some differences in the consumption rates of lysine and glucose are observed, e.g., at 3.3% w/w water and 70 °C the rate of lysine consumption is less than half that of glucose, an effect that might be due to amine recycling (*28*). By considering only the initial rates of consumption of the reactants, the complicating effect of water produced by the reaction is avoided. Arrhenius plots summarizing the initial rate data for lysine and glucose are shown in Figure 2. For measurements made over this temperature interval the Arrhenius plots are linear,

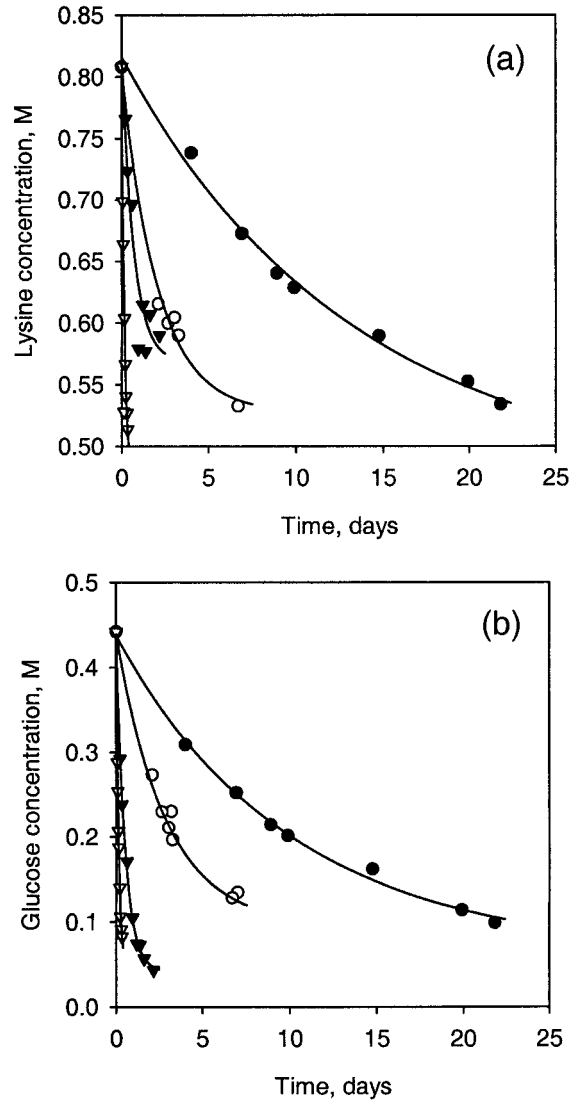

Figure 1. Effect of temperature on (a) lysine and (b) glucose consumption in a high-viscosity reaction mixture containing 3.3% w/w water (T_g, 44 °C). Temperature: ●, 53 °C; ○, 62 °C; ▼, 70 °C; ▽, 80 °C.

i.e., Arrhenius-type behavior with activation energies of 135 and 140 kJ mol^{-1} for lysine and glucose, respectively. Results for two reaction mixtures in the glassy state (1.1 and 1.8% w/w water) are also shown in Figure 2. The mixtures, which are 17 and 11 °C below their glass transition temperatures, respectively, react with a finite rate, reacting faster than one of the nonglassy samples (5.3% w/w water at 43 °C).

Predictions of Collins–Kimball and Solc–Stockmayer Theories. Some general features of the Collins–Kimball theory (*29*) are that, (i) at constant reactivity (k_{act}), there is a crossover between reaction control ($k = k_{act}$) and diffusion control ($k = k_D = 4\pi r_c D$) as the diffusive mobility is reduced, as shown in Figure 3. The term "diffusion-influenced" is used to describe reactions with finite reactivities that are affected by the diffusive mobility of the reactants, and (ii) only reactions with high reactivities compared to the diffusion-controlled rate ($k_{act} \gg 4\pi r_c D$) are influenced by the diffusive mobility of the reactants. To incorporate orientational effects into the theoretical predictions (Solc–Stockmayer theory), data on the size of reactive patches and both translational and rotational diffusivities would be required. Calculations on the effect of the reactive patch

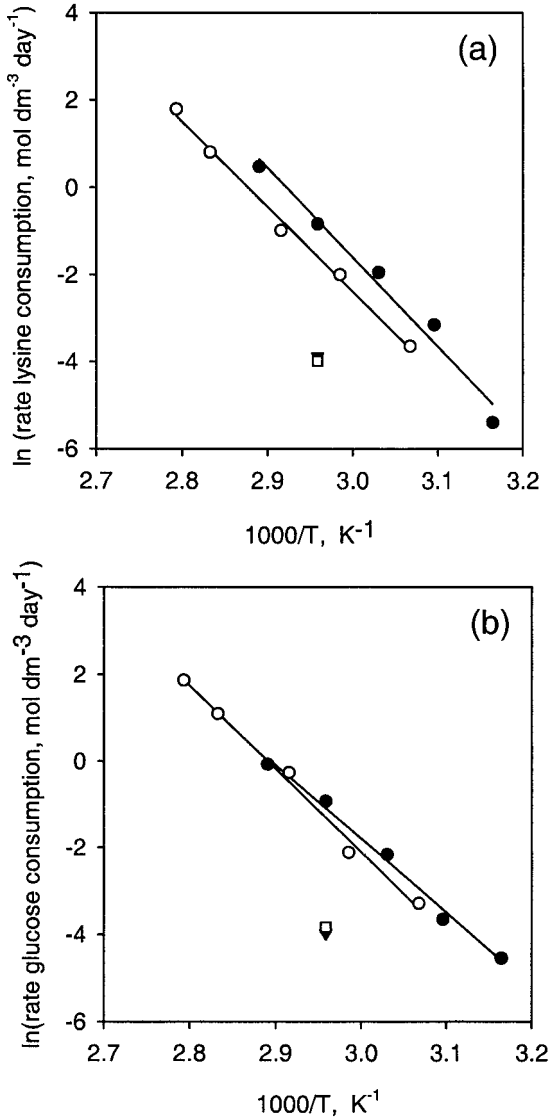

Figure 2. Arrhenius plot of the effect of temperature on the initial rate of (a) lysine and (b) glucose consumption in a high-viscosity reaction mixture. Water content: □, 1.1% w/w (glass); ▼, 1.8% w/w (glass); ○, 3.3% w/w; ●, 5.3% w/w. See Table 1 for details.

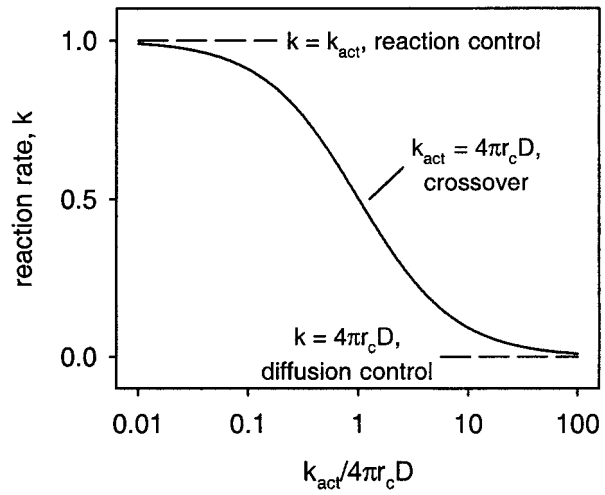

Figure 3. Effect of diffusivity on the rate of a diffusion-influenced bimolecular reaction. With decreasing diffusivity (D), there is a crossover from reaction control to diffusion control.

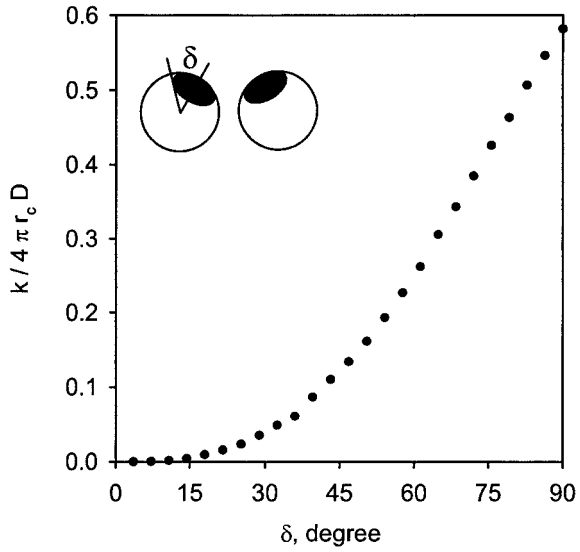

Figure 4. Effect of reactive patch size on the rate of a bimolecular diffusion-controlled reaction. Data from Zhou (*32*).

size on the diffusion-controlled reaction rate between two equal-sized spherical particles have been reported by Zhou (*32*) (Figure 4). The patch size is quantified by the angle δ, the angle between a radius to the center of the patch and a radius to the edge of the patch. For small reactive patches ($\delta < 30°$), $k = 1.46 \times 10^{-6}\ 4\pi r_c D\delta^3$. Thus, for $\delta = 19.0°$, the diffusion-controlled rate is reduced by a factor of 10^2, and for $\delta = 8.8°$, by a factor of 10^3 compared with the orientation-independent rate ($= 4\pi r_c D$). As it is not clear how to estimate the reactive patch sizes from molecular structure, this computationally more demanding approach will not be used.

Applying Theory to Experiment. The Collins–Kimball theory describes a single step, bimolecular reaction, whereas the overall Maillard reaction is a multistep reaction. We apply the theory to the first step of the Maillard reaction, namely, the formation of the Schiff base (*28*), and assume that, in the high-viscosity reaction mixture, this step is irreversible and rate-determining. Figure 5 shows a comparison of the rate constants calculated from the rates of consumption of lysine and glucose in the high-viscosity reaction mixtures and the theoretical diffusion-controlled rate constants plotted in terms of $T - T_g$, the interval by which the temperature exceeds the glass transition temperature. The theoretical calculations are for a temperature of 65 °C, the mean temperature of the experimental dataset. The experimentally observed reaction rates are lower than the theoretical predictions throughout, ranging from a factor greater than 10^6 at $T - T_g = 40$ °C to about 10 at $T \leq T_g$. On the basis of these calculations, the conclusions are that the reaction is reaction-controlled at temperatures above T_g but is approaching the diffusion-influenced regime at T_g and below. The prediction is easily verified by calculating the half-life ($t_{1/2} = 1/k[A]_0$) of a reaction obeying the Smoluchowski–Stokes–Einstein expression at T_g ($\eta \approx 10^{12}$ Pa s), $[A]_0 \approx 0.6$ mol dm^{-3}, say, then $t_{1/2} \approx 2.2 \times 10^5$ s ≈ 2.6 days. For this reactant concentration at T_g, the reaction is predicted to be close to the crossover, diffusion-influenced regime for practical preservation time scales.

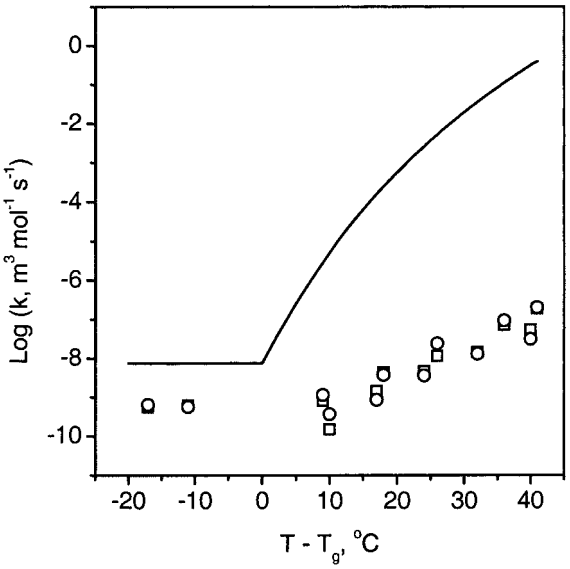

Figure 5. Comparison of second-order rate constants for consumption of lysine (○) and glucose (□) with predicted diffusion-controlled rates (solid line) for reaction in the glass transition region. Rates in the glass calculated assuming $\eta = 10^{12}$ Pa s.

DISCUSSION

Assessment of Assumptions Made in Theoretical Predictions. The assumptions that were made to make the theoretical calculations need to be assessed.

(1) Validity of the Stokes–Einstein Relation. For atomic, ionic, and small molecular species, it is well-known that the Stokes–Einstein relation underestimates diffusive mobility, i.e., molecules experience a viscosity that is smaller than the macroscopic viscosity (*17*, *22*, *36*). This means that k_D should be larger than calculated and would favor the argument that the reaction is reaction-controlled. In our system, the reactants, glucose and lysine, are about the same size as the molecules of the glass-forming solvent, and so, this effect is expected to be relatively small.

(2) Orientation-Independent Reactivity. Introducing orientational constraints into the calculation of the reaction rate has the opposite effect on k_D as the Stokes–Einstein breakdown, as shown in Figure 4. As the relative orientations of the molecules in an encounter become increasingly important to achieve reaction (i.e., as the reactive patch sizes decrease), then k_D decreases. Zhou's calculations (*32*) show that small reactive patches can have a significant effect on the diffusion-controlled rate, i.e., a δ of 8.8° giving a reduction of 10^3 and a δ of 0.88° giving a reduction of 10^6. Although it is clear that orientational constraints should be incorporated into the calculation, thus reducing estimates of k_D, a method for estimating the precise magnitude is currently not clear.

(3) Rate-Determining Irreversible Bimolecular Reaction. Alternative kinetic schemes for the reaction could be considered; however, in general, these do not alter the conclusion that the reaction is reaction-controlled. At large extents of reaction, recycling of the amine (*28*) has been proposed, but as our measurements are for initial rates and the conclusion can be based solely on the glucose consumption data, the conclusion that the reaction is reaction-controlled does not need to be revised.

Alternative Experimental Approaches. A common approach in investigating the diffusion influence in a chemical reaction is to use solvents of different viscosities. The approach is based on the assumption that the viscosity determines the diffusive mobility of the reactants, as in the Stokes–Einstein relation. In the present study, the viscosity variation is estimated to be between 2×10^4 Pa s ($T - T_g = 41$ °C) and 10^{12} Pa s (T_g and below). A wider viscosity variation could be achieved using a wider range of solvent compositions [e.g., water with $\eta \approx 1$ mPa s or glycerol with $\eta \approx 1.4$ Pa s at 20 °C (*39*)]. However, larger changes of solvent composition mean that changes in reaction chemistry or, more generally, "medium effects" (*40*) could occur. In our particular case, the formation of a Schiff base is a condensation reaction occurring with the elimination of water. If this reaction were reversible, then variations of the concentration of water could affect the overall reaction rate. A more general effect associated with our low-water-content carbohydrate-based solvents is that their dielectric constants [$\epsilon_S \approx 30$–40 (*41*)] are lower than that of water, which results in longer-ranged ionic interactions and greater ionic association (*42*). This could also affect pH, which is known to have a strong influence on the Maillard reaction in aqueous solution (*43*) and low-water-content poly(vinyl pyrrolidone) glasses (*13*). Comparisons between more widely different solvents are probably better made in the context of more systematic studies of medium effects on reaction kinetics.

Assessment of the Molecular Mobility Approach to Chemical Reaction Kinetics. Quantitative rate data and theoretical modeling aid the understanding of the molecular mobility approach to food stability. Figure 5 illustrates the conditions under which reactant mobility is predicted to affect overall reaction rates. The conditions are more limited than might have previously been expected, with the reaction only approaching diffusion influence in the glassy state and not above. Above T_g, a reaction-controlled reaction would commonly be expected to exhibit Arrhenius temperature dependence (*44*), as is observed in Figure 2, rather than "WLF kinetics". Further research is needed to reduce the number of assumptions made, e.g., reactant diffusivity data, theoretical approaches for predicting orientation dependence of reactivity, and detailed mechanistic understanding of reactions and their kinetics are all required. The present theory is for reactions in which the rate-determining step is a bimolecular diffusional encounter; alternative approaches are required for the effect of glass formation on reactions that do not involve diffusional encounters, e.g., intramolecular rearrangements (*45*).

CONCLUSIONS

Practically, it can be concluded that, although drying products into the glassy state retards the Maillard reaction, it does not stop it, so that additional steps such as storage at reduced temperature, reduction of the concentrations of reactants, optimization of the pH, and addition of chemical preservatives should also be considered to achieve the required level of preservation.

The widespread empirical development of glassy-state stabilization indicates that, in many cases, effective stabilization can be achieved by this route. However, the present study suggests that, even at the high viscosities and low diffusive mobilities that occur at the

glass transition, there is sufficient mobility for slow deteriorative reactions to be either reaction-controlled or close to the crossover from reaction control to diffusion control. A mechanistic understanding of glassy-state stabilization is not yet complete. An integrated physical (molecular mobility) and chemical approach is required to achieve this. Applications to real foods will also have to tackle the added complexity involved in multiphase heterogeneous systems.

LITERATURE CITED

(1) Brake, N. C.; Fennema, O. R. Lipolysis and lipid oxidation in frozen minced mackerel as related to T_g, molecular diffusion, and presence of gelatin. *J. Food Sci.* **1999**, *64*, 25−32.

(2) Lai, M. C.; Topp, E. M. Solid-state chemical stability of proteins and peptides. *J. Pharm. Sci.* **1999**, *88*, 489−500.

(3) Buitink, J.; Leprince, O.; Hemminga, M. A.; Hoekstra, F. A. Molecular mobility in the cytoplasm: An approach to describe and predict lifespan of dry germplasm. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2385−2390.

(4) Fennema, O. R. Water and ice. In *Food Chemistry*; Fennema, O. R., Ed.; Marcel Dekker: New York, 1996.

(5) Levine, H.; Slade, L. A polymer physicochemical approach to the study of commercial starch hydrolysis products (SHPs). *Carbohydr. Polym.* **1986**, *6*, 213−244.

(6) Slade, L.; Levine, H. Glass transitions and water−food structure interactions. *Adv. Food Nutr. Res.* **1995**, *38*, 103−269.

(7) Karel, M.; Saguy, I. Effects of water on diffusion in food systems. In *Water Relationships in Foods*; Levine, H., Slade, L., Eds.; Plenum Press: New York, 1991.

(8) Karmas, R.; Buera, M. P.; Karel, M. Effect of glass transition on rates of nonenzymatic browning in food systems. *J. Agric. Food Chem.* **1992**, *40*, 873−879.

(9) Williams, M. L.; Landel, R. F.; Ferry, J. F. The temperature dependence of relaxation mechanisms in amorphous polymers and other glass-forming liquids. *J. Am. Chem. Soc.* **1955**, *77*, 3701−3707.

(10) Dainton, F. S.; Henry, M. S.; Pilling, M. J.; Spencer, P. C. Viscosity dependence of diffusion-controlled triplet energy transfer in 2-methylpentan 2-,4-diol. *J. Chem. Soc., Faraday Trans.* **1977**, *73*, 243−249.

(11) Roos, Y. H.; Himberg, M.-J. Nonenzymatic browning behaviour, as related to glass transition, of a food model at chilling temperatures. *J. Agric. Food Chem.* **1994**, *42*, 893−898.

(12) Streefland, L.; Auffret, A. D.; Franks, F. Bond cleavage reactions in solid aqueous carbohydrate solutions. *Pharm. Res.* **1998**, *15*, 843−849.

(13) Bell, L. N.; White, K. L.; Chen, Y. Maillard reaction in glassy low-moisture solids as affected by buffer type and concentration. *J. Food Sci.* **1998**, *63*, 785−788.

(14) Lievonen, S. M.; Laaksonen, T. J.; Roos, Y. Glass transition and reaction rates: Nonenzymatic browning in glassy and liquid systems. *J. Agric. Food Chem.* **1998**, *46*, 2778−2784.

(15) Lai, M. C.; Hageman, M. J.; Schowen, R. L.; Borchardt, R. T.; Topp, E. M. Chemical stability of peptides in polymers. 1. Effect of water on peptide deamidation in poly(vinyl alcohol) and poly(vinyl pyrrolidone) matrixes. *J. Pharm. Sci.* **1999**, *88*, 1073−1080.

(16) Lai, M. C.; Hageman, M. J.; Schowen, R. L.; Borchardt, R. T.; Laird, B. B.; Topp, E. M. Chemical stability of peptides in polymers. 2. Discriminating between solvent and plasticizing effects of water on peptide deamidation in poly(vinylpyrrolidone). *J. Pharm. Sci.* **1999**, *88*, 1081−1089.

(17) Parker, R.; Ring, S. G. Diffusion in maltose−water mixtures at temperatures close to the glass transition. *Carbohydr. Res.* **1995**, *273*, 147−155.

(18) Champion, D.; Hervet, H.; Blond, G.; LeMeste, M.; Simatos, D. Translational diffusion in sucrose solutions in the vicinity of their glass transition temperature. *J. Phys. Chem. B* **1997**, *101*, 10674−10679.

(19) van Dusschoten, D.; Tracht, U.; Heuer, A.; Speiss, H. W. Site specific rotational mobility of anhydrous glucose near the glass transition as studied by 2D echo decay ^{13}C NMR. *J. Phys. Chem. A* **1999**, *103*, 8359−8364.

(20) van den Dries, I. J.; van Dusschoten, D.; Hemminga, M. A.; van der Linden, E. Effects of water content and molecular weight on spin probe and water mobility in malto-oligomer glasses. *J. Phys. Chem. B* **2000**, *104*, 10126−10132.

(21) van den Dries, I. J. Molecular mobility in sugar glasses. Ph.D. thesis, Wageningen University, Gelderland, The Netherlands, 2000.

(22) Gunning, Y. M.; Parker, R.; Ring, S. G. Diffusion of short chain alcohols from amorphous maltose−water mixtures above and below their glass transition temperature. *Carbohydr. Res.* **2000**, *329*, 377−385.

(23) Lea, C. H. Chemical changes in the preparation and storage of dehydrated foods. In *Fundamental Aspects of the Dehydration of Foodstuffs*; Society of Chemical Industry: London, 1958; pp 178−194.

(24) Eichner, K. The influence of water content on nonenzymic browning reactions in dehydrated foods and model systems and the inhibition of fat oxidation by browning intermediates. In *Water Relations of Foods*; Duckworth, R. B., Ed.; Academic Press: London, 1975; pp 417−434.

(25) Labuza, T. P.; Tannenbaum, S. R.; Karel, M. Water content and stability of low-moisture and intermediate-moisture foods. *Food Technol.* **1970**, *24*, 543−550.

(26) Burin, L.; Jouppila, K.; Roos, Y.; Kansikas, J.; Buera, M. P. Color formation in dehydrated modified whey powder system as affected by compression and T_g. *J. Agric. Food Chem.* **2000**, *48*, 5263−5268.

(27) Schebor, C.; Buera, M. P.; Karel, M.; Chirife, J. Color formation due to nonenzymatic browning in amorphous, glassy, anhydrous, model systems. *Food Chem.* **2000**, *65*, 427−432.

(28) Yaylayan, V. A.; Huyghues-Despointes, A. Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions, and spectroscopic properties. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 321−369.

(29) Collins, F. C.; Kimball, G. E. Diffusion-controlled reaction rates. *J. Colloid Sci.* **1949**, *4*, 425−437.

(30) Solc, K.; Stockmayer, W. H. Kinetics of diffusion-controlled reaction between chemically asymmetric molecules. I. General theory. *J. Chem. Phys.* **1971**, *54*, 2981−2988.

(31) Solc, K.; Stockmayer, W. H. Kinetics of diffusion-controlled reaction between chemically asymmetric molecules. II. Approximate steady-state solution. *Int. J. Chem. Kinet.* **1973**, *5*, 733−752.

(32) Zhou, H. X. Brownian dynamics study of the influences of electrostatic interaction and diffusion on protein−protein association kinetics. *Biophys. J.* **1993**, *64*, 1711−1726.

(33) Bassett, J., Denney, R. C., Jeffrey, G. H., Mendham, J., Eds. Determination of water content with Karl Fischer reagent. In *Vogel's Textbook of Quantitative Inorganic Analysis*, 4th ed.; Longman: Harlow, U.K., 1978.

(34) Wunderlich, B. *Thermal Analysis*, Academic Press: New York, 1990.

(35) Lide, D. R., Ed. *CRC Handbook of Chemistry and Physics*, 75th ed.; CRC Press: Boca Raton, FL, 1994.

(36) Tyrrell, H. J. V.; Harris, K. R. *Diffusion in Liquids*; Butterworth: London, 1984.

(37) Rice, S. A. *Diffusion-Controlled Reactions*, Elsevier: Amsterdam, 1985; p 28.

(38) Angell, C. A.; Bressel, R. D.; Green, J. L.; Kanno, H.; Oguni, M.; Sare, E. J. Liquid fragility and the glass

transition in water and aqueous solutions. *J. Food Eng.* **1994**, *22*, 115−142.

(39) Segur, J. B.; Oberstar, H. E. Viscosity of glycerol and its aqueous solutions. *Ind. Eng. Chem.* **1951**, *43*, 2117−2120.

(40) Alder, R. W.; Baker, R.; Brown, J. M. *Mechanism in Organic Chemistry*, Wiley: London, 1971.

(41) Chan, R. K.; Pathmanathan, K.; Johari, G. P. Dielectric relaxations in the liquid and glassy states of glucose and its water mixtures. *J. Phys. Chem.* **1986**, *90*, 6358−6362.

(42) Robinson, R. A.; Stokes, R. H. *Electrolyte Solutions*, 2nd ed.; Butterworth: London, 1959.

(43) Ajandouz, E. H.; Puigserver, A. Nonenzymatic browning reaction of essential amino acids: Effect of pH on caramelization and Maillard reaction kinetics. *J. Agric. Food Chem.* **1999**, *47*, 1786−1793.

(44) Atkins, P. W. *Physical Chemistry*; Oxford University Press: Oxford, U.K., 1978; pp 864−866.

(45) Hagen, S. J.; Hofrichter, H. J.; Bunn, H. F.; Eaton, W. A. Comments on the physics and chemistry of trehalose as a storage medium for hemoglobin-based blood substitutes: "From Kramers theory to the battlefield". *Transfusion Clin. Biol.* **1995**, *2*, 423−426.

Received for review January 18, 2001. Revised manuscript received July 19, 2001. Accepted July 30, 2001. The study has been carried out with the financial support from the Central Strategic Grant from the BBSRC and from the Commission of the European Communities. Agriculture and Fisheries (FAIR) Specific RTD Program, CT96-1085, Enhancement of Quality of Food and Related Systems by Control of Molecular Mobility. This work does not necessarily reflect the views of this program and in no way anticipates future policy in this area.

JF0100752