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Vanillin Phosphorescence as a Probe of Molecular Mobility in Amorphous Sucrose

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Abstract Changes in molecular mobility are important in defining the stability and quality of amorphous solid foods, pharmaceuticals, and other solid biomaterials. Predictions of stability must consider matrix mobility below and above T_g (the glass transition temperature); measurement of molecular mobility in amorphous solids over time scales ranging from $<10^{-9}$ s to $>10^{8}$ s requires specialized methods. This research investigated how the steady-state and time-resolved emission and intensity of phosphorescence from vanillin (4-hydroxy-3-methoxy benzaldehyde), a common flavor compound, can be used to probe molecular mobility when dispersed within amorphous pure sucrose films. Phosphorescence emission spectra and time-resolved intensity decays, measured in sucrose as a function of temperature in the absence of oxygen, were strongly modulated by matrix molecular mobility. Temperature had a significant effect on vanillin phosphorescence peak frequency and bandwidth, intensity, and lifetime both in the glass and in the melt. Time-resolved phosphorescence intensity decays from vanillin were multiexponential both below and above the glass transition temperature, indicating that the pure (single component) amorphous matrix was dynamically heterogeneous on the molecular level. These data show that vanillin is a promising intrinsic probe of molecular mobility and dynamic heterogeneity in amorphous solid foods and perhaps pharmaceuticals.

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Introduction

In amorphous solid foods and pharmaceuticals, shelf-life is influenced by modes of molecular mobility that modulate the kinetics of physical and chemical processes. Molecular mobility is strongly affected by temperature and water content [1–3]. Changes in molecular mobility are thus important in defining the shelf-life and quality of amorphous solid foods, pharmaceuticals, and other biomaterials. Molecular motions have being shown to occur in glass even 50 °C below T_g [4] and in partially dried seeds at cryogenic temperatures [5]. Predictions of stability must thus consider matrix mobility both below and above the glass transition temperature (T_g). Monitoring molecular mobility in amorphous solids over time scales ranging from $<10^{-9}$ ns to $>10^{8}$ s at a wide range of temperatures requires specialized methods. In glass below T_g, motions are mainly local, including localized vibrational modes involving groups of atoms [6, 7] and in the rubber/melt above T_g these motions involve large scale, whole molecule rotational and translational motions [7–9]. These motions are of intense concern as they affect rates of molecular diffusion, and thus chemical reaction rate in amorphous solid foods, as well as the rates of other physical processes such as crystallization. In glass to liquid transitions relaxation time of material is considered to be similar to experimental time scale and changes are observed, but below T_g these relaxation times are very long and demand special consideration [8].

Phosphorescence spectroscopy is a sensitive, site-specific method that can be used to detect molecular

mobility in the local environment of a probe embedded within an amorphous matrix [10]. Phosphorescence is more sensitive than fluorescence to deactivation by collisions with solvent molecules, quenchers and energy transfer processes and hence is most intense in rigid media (e.g., glass) where diffusional quenching is largely absent [10–12]. Due to the natural lifetime of the excited triplet state, phosphorescence spectroscopy responds to molecular events in the ms to s time scale [11, 13] and has been used to monitor molecular mobility in a range of amorphous biomaterials near room temperature [14–19].

Strong phosphorescence of organic compounds has been observed in the gas phase, in rigid media, and at cryogenic temperatures [20, 21]. Solid-surface room-temperature phosphorescence has been observed from a wide variety of conjugated organic compounds, including vanillin, when adsorbed on a suitable solid surface. The rigid matrix prevents the non-radiative decay of the triplet state by collisional energy transfer (internal conversion processes) and by inhibiting quenching by ground state triplet oxygen; solid surfaces such as paper provide excellent supports for observing room temperature phosphorescence of organic compounds [22].

Vanillin (4-hydroxy-3-methoxy benzaldehyde) has being shown to exhibit phosphorescence at cryogenic temperatures in rigid glass and at room temperature when adsorbed on a substrate such as filter paper [20, 23]. Vanillin has long been an expensive and sought after flavoring for foods. This research focuses on exploring the potential of using vanillin as a triplet state probe to monitor molecular mobility in amorphous biomaterials.

This research has investigated how the phosphorescence emission and intensity of vanillin can be used to probe molecular mobility within amorphous pure sucrose films. Steady state and time-resolved emission, collected over the temperature range from −20 to 100 °C, was found to be sensitive to modes of molecular motion in both the glass below and the melt above the sucrose T_g.

Materials and methods

Materials Water was deionized and then glass distilled. Sucrose (99.5% pure) was from Sigma Chemical Co. (St. Louis, MO) and vanillin (3-methoxy-4-hydroxy benzaldehyde) was from Sigma-Aldrich (Milwaukee, WI). Sucrose, purified of luminescence impurities using activated charcoal as described in Pravinata et al. (2005), was prepared at a final concentration of 65–67 wt % in water, confirmed using a refractometer (NSG Precision Cells, Farmingdale, NY). The sucrose solution was filtered through a 0.2 μm membrane to remove particulates prior to film formation.

A 66 mM stock solution of vanillin was prepared in distilled deionized water. This concentration was selected to simplify the addition of the probe to the sucrose solution. For absorbance, fluorescence and phosphorescence measurements in solution the 66 mM stock was diluted to 50 μM in distilled deionized water. Vanillin was added to the sucrose solution at a mole ratio of $1{:}10^3$ (dye: sucrose). Other ratios, $3{:}10^4$, $5{:}10^4$ and $2{:}10^3$, were also tested and vanillin lifetimes were found to be unaffected by concentration over this range; this is consistent with lack of probe aggregation in the sugar, suggesting that the vanillin probe is present as individual molecules monitoring the molecular mobility of the sucrose.

Sucrose films To produce glassy sucrose films, 20 μL of a sucrose solution containing vanillin were spread on a quartz slide (30×13.5×0.6 mm; custom made by NSG Precision Cells (Farmingdale, NY). After spreading, the slides were dried under a heat gun (Vidal Sassoon) for 5 min; the final film thickness was ~0.05 mm. The slides were stored at room temperature against P_2O_5 and DrieRite, protected from light to prevent any photobleaching, for at least 7 days before any phosphorescence measurements were made. The desiccant was refreshed as needed to maintain a relative humidity close to 0%.

Instrumentation Absorption measurements were recorded on a Cary 50 spectrophotometer and fluorescence and phosphorescence measurements were made on a Cary Eclipse fluorescence spectrophotometer (Varian Instruments, Walnut Creek, CA). The solution or quartz slides were placed in a standard 1 cm×1 cm×3 cm quartz fluorescence cuvette, which was capped with a lid having inlet and outlet ports for gas lines. For phosphorescence measurements, the cuvette was flushed with a gentle stream of nitrogen for 15 min to eliminate oxygen. An oxygen-free nitrogen stream was generated by passage of high purity nitrogen through a Supelco (Bellefonte, PA) gas purifier. The temperature was controlled by using a TLC-50 thermoelectric heating/cooling system (Quantum Northwest, Spokane, WA). The TLC-50 sample compartment was fitted with a jacketed cover and the temperature of the cuvette was monitored using a thermocouple in the cuvette holder. The film was equilibrated for 15 min at each temperature before collecting data.

Spectroscopic measurements Absorbance spectra of vanillin (50 μM) were collected in aqueous solution from 250 nm to 400 nm at 20 °C. Luminescence emission spectra (fluorescence and phosphorescence) of vanillin in aqueous solution were collected at 20 °C with excitation at 320 nm. Phosphorescence of vanillin in amorphous sucrose were collected from 400 nm to 800 nm (10 nm bandwidth)

using excitation at 320 nm (20 nm bandwidth) over the temperature range from −20 °C to 100 °C. Each data point was collected from a single flash with 0.2 ms delay, 100 ms gate time, and 0.12 s total decay time. Transient phosphorescence decay measurements were made as a function of temperature. The samples were excited at 320 nm (20 nm bandwidth) and emission transients collected at 490 nm (20 nm bandwidth) at temperatures ranging from −20 °C to 100 °C. Each decay was the average of 50 cycles, and for each cycle data were collected from a single flash with a delay of 0.2 ms; windows for gate time and total decay time were varied at each temperature. All measurements were made in quadruplicate.

Emission energy as a function of temperature Emission spectra were fit using the program Igor (Wavemetrics, Inc., Lake Oswego, OR) to a log-normal function over the temperature range −20 °C to 100 °C.

$$I(\nu) = I_0 \exp\left\{ -\ln(2)\left(\frac{\ln\left[1 + 2b\left(\nu - \nu_p\right)/\Delta\right]}{b}\right)^2\right\} \quad (1)$$

In this equation I_0 is the maximum intensity of the emission spectra, ν_P is the frequency (in cm^{-1}) of the emission maximum, Δ is a line width parameter, and b is an asymmetry parameter. The bandwidth of the emission, the full width at half maximum (Γ), is related to b and Δ.

$$\Gamma = \Delta\left(\frac{\sinh(b)}{b}\right) \quad (2)$$

Activation energies for quenching of phosphorescence intensity were calculated from the slopes of linear fits to plots of ln(1/I) versus 1/T over the temperature range from −10 °C to 30 °C and 60 °C to 100 °C; break-point temperatures were determined from the intersection of these trendlines.

Photophysical scheme The phosphorescence intensity decays were fitted using a multi-exponential function:

$$\mathrm{I(t)} = \mathrm{I(0)} \sum_{\mathrm{i}=1}^{\mathrm{n}} \alpha_{\mathrm{i}} \exp(-\mathrm{t}/\tau_{\mathrm{i}}) \quad (3)$$

Where I(0) is the amplitude at time zero, τ_i are the lifetimes and α_i are the fractional amplitudes of each component, and n is the number of lifetimes. Phosphorescence lifetimes were analyzed using the statistical program Igor (Wavemetrics, Inc., Lake Oswego, OR). Fits were judged satisfactory if the R^2 values were in the range of 0.995–1.0 and the modified residuals ((data–fit)/data$^{1/2}$) varied randomly about zero. The average lifetime was calculated as follows.

$$\tau_{\mathrm{avg}} = \sum_{\mathrm{i}=1}^{\mathrm{n}} \alpha_{\mathrm{i}} \tau_{\mathrm{i}}^2 / \sum_{\mathrm{i}=1}^{\mathrm{n}} \alpha_{\mathrm{i}} \tau_{\mathrm{i}} \quad (4)$$

The phosphorescence lifetimes were used to calculate the rate constants associated with the various processes that depopulate the excited triplet state. The lifetime τ is related to the rate constants for de-excitation of the triplet excited state of the probe according to the following (Papp and Vanderkooi 1989).

$$1/\tau = \mathrm{k_P} = \mathrm{k_{RP}} + \mathrm{k_{TSO}(T)} + \mathrm{k_Q[O_2]} \quad (5)$$

Where k_P is the total decay rate, k_{RP} is the rate of radiative decay to the ground state (2.69 s^{-1}) [25], and k_{TS0} is the rate of non-radiative decay to the ground singlet state S_0. The term $k_Q[O_2]$ represents the collisional quenching between the excited chromophore and triplet (ground state) oxygen; under the anoxic conditions of this study $k_Q[O_2] \approx 0$.

Activation energies were calculated from the slopes of linear fits to Arrhenius plots of ln(k_{TS0}) versus 1/T for each lifetime component and for the average lifetime at low temperature (−20 to 20 °C) and high temperature (50 to 80 °C). (Data for individual or average lifetimes were not fit above 80 °C because only two lifetimes were used to fit decays at these temperatures rather than the four components used at lower temperature.) The R^2 values were 0.985, 0.991, 0.935, and 0.859 (low temperature) and 0.986, 0.999, 0.998, and 0.999 (high temperature) for linear fits to Arrhenius plots of non-radiative rate constants calculated from individual lifetimes (long to short, respectively), and 0.987 (low temperature) and 0.990 (high temperature) for linear fits to the Arrhenius plot of the average non-radiative rate constant calculated from the average lifetime.

Results

Luminescence emission spectra Vanillin exhibits room temperature phosphorescence (RTP) when embedded in amorphous sucrose films. The phosphorescence excitation spectrum of vanillin (at 20 °C) in amorphous sucrose at mole ratio of 1:10^3 (probe:sucrose) is plotted in Fig. 1 along with emission spectra collected from −20 °C to 100 °C. The excitation spectrum has maxima at 315 nm and 280 nm and is identical, except for a small change in the relative intensity of the two peaks, to the absorbance spectrum in sucrose (data not shown). Vanillin phosphorescence emission has a maximum at ~490 nm and the intensity was quite sensitive to temperature, decreasing nearly to zero at 100 °C. Emission spectra were fit to a log-normal function (Eq. 1) to determine

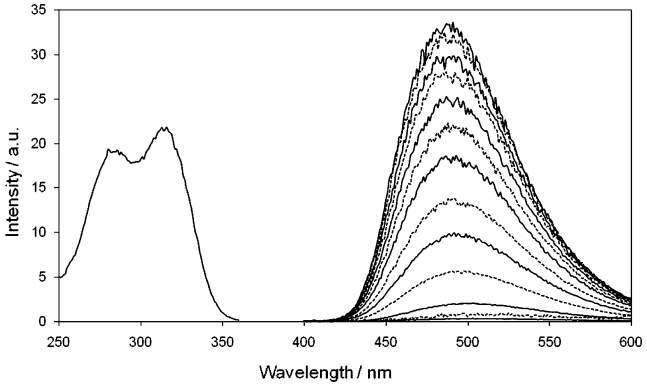

Fig. 1 Excitation and emission spectra of vanillin dispersed in amorphous sucrose film. Excitation spectrum (*single solid curve at left*) at 20 °C. Emission spectra (*multiple spectra at right*) collected as a function of temperature from −20 °C (top curve) to 100 °C (*bottom curve*)

the peak intensity (I_P), peak frequency (ν_P), and bandwidth (Γ). The emission energy (peak frequency) decreased gradually at low temperature and more steeply at temperatures above ~50 °C, slightly below the glass transition temperature of sucrose ($T_g \approx 65$ °C; calculated from the average of the values reported in Roe and Labuza [26]). A decrease in emission energy indicates an increase in the average extent of matrix dipolar relaxation around the excited triplet state [27]. The phosphorescence bandwidth increased gradually at low temperature and more steeply at temperatures above ~60 °C. The bandwidth reflects the extent of inhomogeneous broadening of the spectrum due to an increase in the distribution of distinct energetic interactions between the vanillin probe and the local sucrose matrix (Fig. 2).

Phosphorescence intensity decays Time-resolved phosphorescence intensity decays of vanillin in amorphous sucrose films were measured over the temperature range from −20 °C to 100 °C. All intensity decays exhibited complex, non-exponential decay kinetics; a representative decay at 20 °C is plotted in Fig. 3a. Efforts to fit these decays with a sum of two or even three exponentials were statistically unsatisfactory, giving R^2 values significantly greater than 1 and modified residuals plots that varied substantially, systematically, and non-randomly about zero (Fig. 3b). Statistically satisfactory fits were obtained, however, using a four exponential function; such a fit is also plotted in Fig. 3a and the modified residuals for this fit are plotted in Fig. 3b. All intensity decays over the temperature interval from −20 °C to 80 °C were well-fit using a four exponential function while those at 90 °C and 100 °C, due to low intensity, were well-fit using only a two exponential function. The fit lifetimes (τ_i) at each temperature varied over a broad range (nearly two orders of magnitude); at 20 °C, for example, the fit lifetimes were 184 ms, 76.7 ms, 18.2 ms and 2.7 ms.

The phosphorescence lifetimes decreased with increasing temperature; the results of these analyses are plotted in Fig. 4a. The decrease in lifetime as a function of temperature was dramatic both below and especially above the sucrose T_g. The fractional amplitudes (α_i) of each lifetime component also varied as a function of temperature (Fig. 4b). Interestingly, the amplitudes of the longer lifetime components (α_1 and α_2) decreased with temperature while the amplitudes of the shorter lifetime components (α_3 and α_4) increased with temperature. The average lifetime (calculated using Eq. 4), also plotted in Fig. 4a, varied ~200-fold from 192 ms at −20 °C to 0.85 ms at 100 °C. Lifetimes from samples with probe:sucrose ratios of $3{:}10^4$, $5{:}10^4$, $1{:}10^3$ and $2{:}10^3$ were identical within error, indicating that there was no effect of probe concentration.

The decrease in vanillin lifetime as a function of temperature corresponds to an increase in the non-radiative decay rate k_{TS0} (Eq. 5, Materials and methods) which reflects vibrational relaxation of the excited state [28,

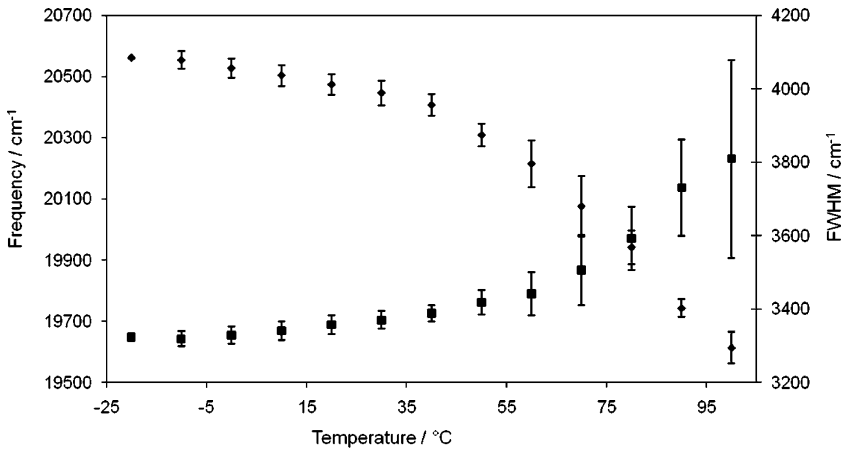

Fig. 2 Effect of temperature on the phosphorescence peak emission energy (♦) and bandwidth (■) of vanillin dispersed in amorphous sucrose

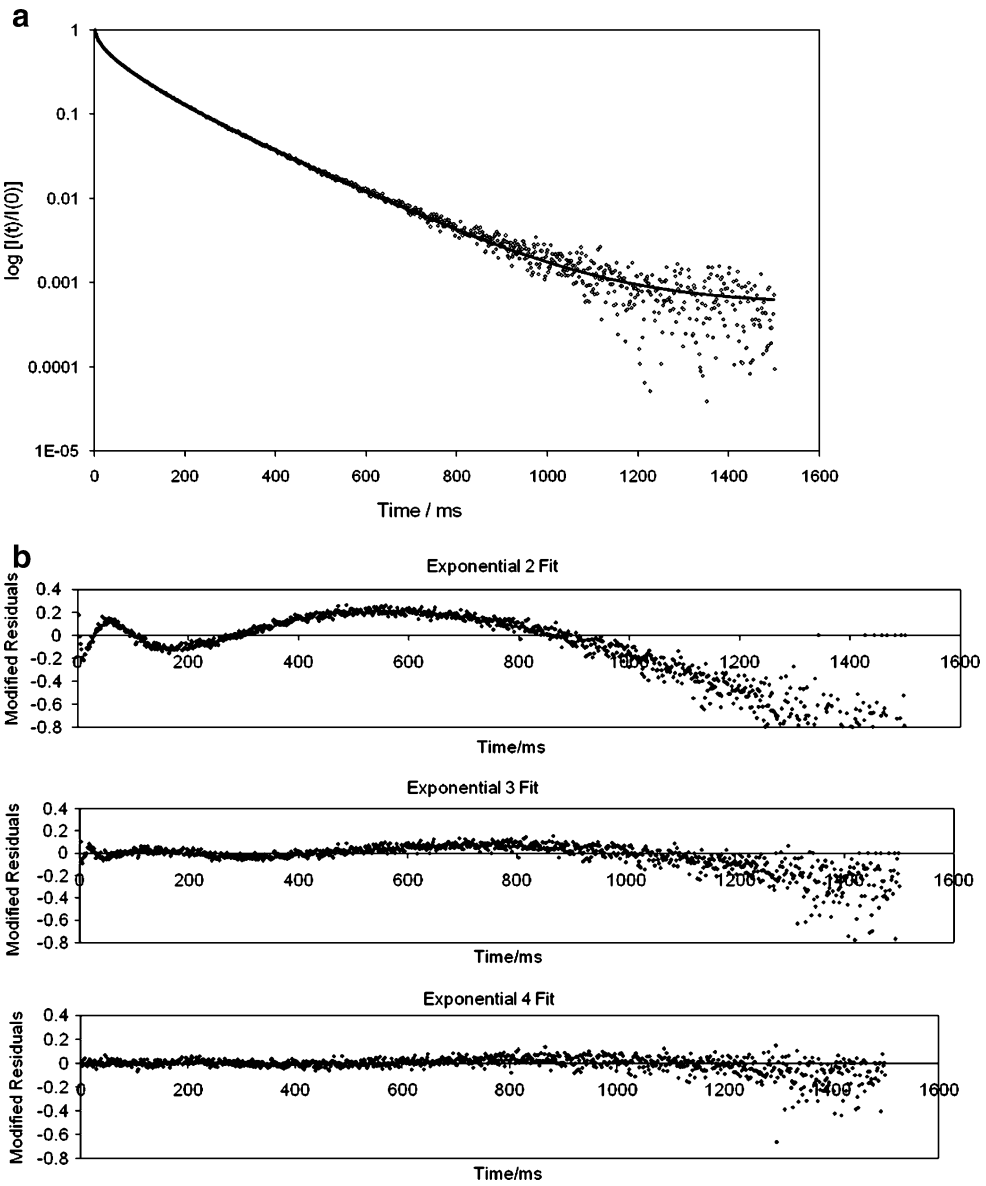

Fig. 3 **a** Time resolved intensity decay of vanillin dispersed in amorphous sucrose at 20 °C; intensity decay data (*points*) and fit using a four exponential decay function (*solid line*). **b** Modified residuals ((data–fit)/data^2) for two, three and four exponential fits to data at 20 °C

29]. The efficiency of vibrational dissipation is related to the overall mobility of the matrix; the magnitude of k_{TS0} thus provides a measure of molecular mobility [10]. The k_{TS0} values for each lifetime component and for the average lifetime were calculated by subtracting the radiative decay rate k_{RP} from the total decay rate k_P. The value of k_{RP} (=2.69 s^{-1}) was determined from the lifetime, 372 ms, of vanillin in glycerol/water at 77 K [25], conditions under which it is assumed that non-radiative quenching pathways are negligible. (Interestingly, the lifetime, 272 ms, of vanillin in sucrose glass under the same conditions was significantly lower than the value in glycerol/water, indicating that local mobility in sucrose at 77 K may quench the vanillin triplet state.)

Arrhenius plots of $\ln(k_{TS0})$ for the individual lifetime components (Fig. 5) were biphasic, with k_{TS0} increasing gradually at low and much more steeply at higher temperature, exhibiting substantial curvature around the sucrose glass transition temperature. The Arrhenius plots were linear at low (−20 to 20 °C) and high (50 to 80 °C) temperature (Fig. 5) with R^2 values of 0.99 for most curves (see Materials and methods for details.) The activation energies for the molecular motions that quenched the vanillin triplet state ranged from 4 to 14 kJ mol^{-1} at low and from 54 to 68 kJ mol^{-1} at high temperature (summarized in Table 1). The average non-radiative decay rate, calculated from the average lifetime, exhibited Arrhenius behavior similar to that of the individual life-

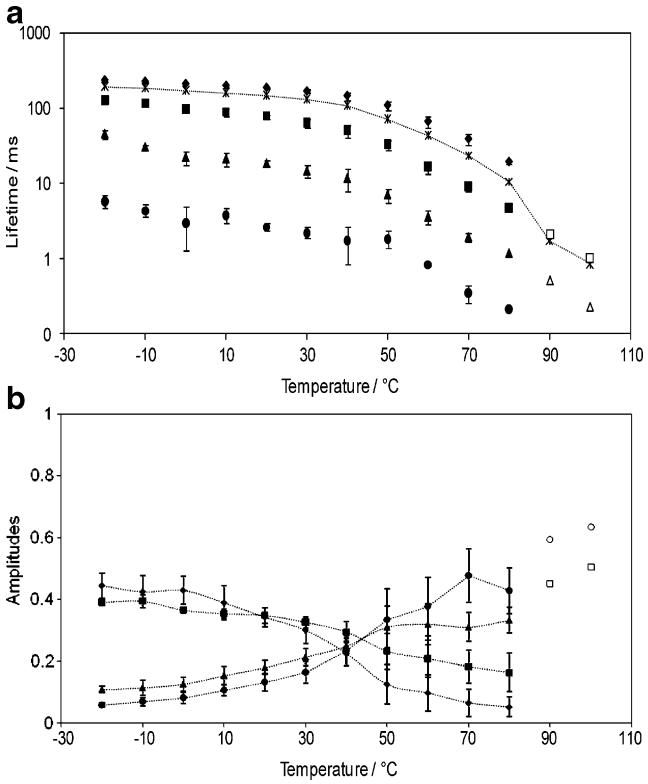

Fig. 4 Effect of temperature on the fit lifetimes (**a**) and amplitudes (**b**) for phosphorescence of vanillin dispersed in amorphous sucrose. In (**a**), the average lifetime (*dotted line*) is also plotted

times with activation energies of 7.63 kJ mol^{-1} at low and 66.8 kJ mol^{-1} at high temperature.

Discussion

The phosphorescence properties of vanillin in amorphous solid sucrose reflect specific responses of the probe to temperature-dependent changes in the physical state of the matrix. Phosphorescence emission spectrum, intensity, and lifetime in general are modulated by matrix molecular mobility in distinct ways. The peak emission energy is sensitive to the rate of dipolar relaxation of solvent molecules around the polar excited triplet state that stabilize and thus lower the energy of this state [27]. The intensity and lifetime reflect the rate of non-radiative quenching of the triplet state mediated by interactions with matrix molecules. This quenching reflects both internal factors related to the rate of intersystem crossing from the lowest vibronic level of the excited triplet state to highly excited vibrations of the ground singlet state, and external factors related to the rate at which the probe's highly excited ground state vibrations can couple with vibrations of the amorphous sugar to dissipate energy into the matrix [24, 28].

The vanillin phosphorescence emission intensity, emission peak energy, and lifetime all decreased significantly over the temperature range from −20 °C to 100 °C. Each parameter decreased gradually at low and more dramatically at high temperature. Vanillin phosphorescence is thus quite sensitive to matrix relaxations in both the glass below and the melt above the sucrose T_g of 65 °C. This is in contrast to, for example, the phosphorescence probe

Table 1 Activation energy (E_A) calculated from non-radiative decay rates (k_{TS0}) for individual lifetime components

Lifetime component	E_A/(kJ/mol)	
	Low temperature	High temperature
τ_1 (long)	3.61	53.9
τ_2	7.76	60.5
τ_3	13.8	53.7
τ_4 (short)	11.8	68.2
τ_{avg}	7.63	66.8

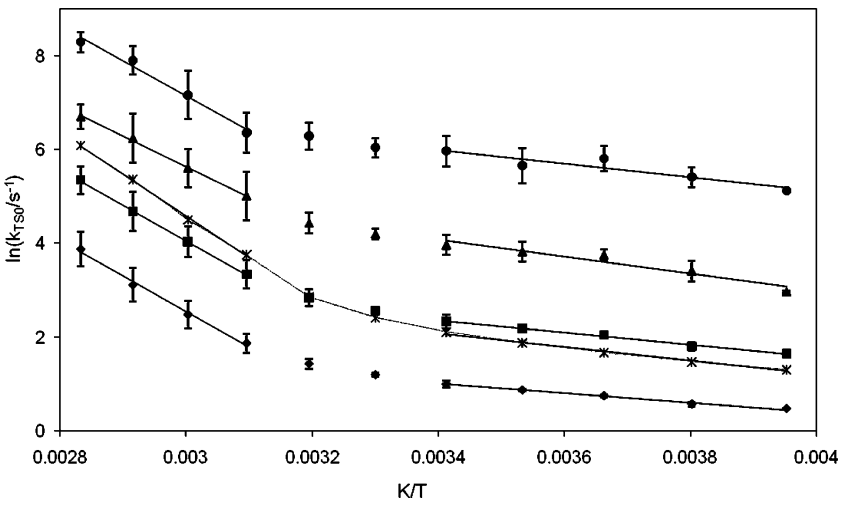

Fig. 5 Arrhenius plot of the non-radiative decay rate k_{TS0} for each individual lifetime component and for the average lifetime (*dotted line*) for phosphorescence of vanillin dispersed in amorphous sucrose. The solid lines are linear fits to the data at low (−20 to 20 °C) and high (50 to 80 °C) temperature. See text for additional details

erythrosin B which primarily senses only the onset of α-relaxations near T_g [10]. Since the radiative lifetime is significantly longer in vanillin (~370 ms, this work) than in erythrosin B (24.3 ms, [30]), the additional sensitivity of vanillin to molecular mobility at lower temperatures may reflect the longer time window over which the molecule can sense relaxation processes; alternatively, vanillin vibrations may couple more effectively to matrix vibrations than those of erythrosin B and thus quench more readily.

Similar increases in bandwidth to those of vanillin have been seen in erythrosin B dispersed in amorphous sucrose [10], in maltose and maltitol [31] and in lactose and lactitol [18] around their glass transition temperatures. Amorphous solids are dynamically heterogeneous and exhibit a range of structural features due to local differences in packing density [32, 33]. Vanillin, as does Erythrosin B [10], thus appears to respond to increases in this heterogeneity through increases in the emission bandwidth.

Vanillin phosphorescence was moderately sensitive to matrix molecular mobility in the glassy region over the temperature range from −20 °C to 40 °C: the peak frequency decreased 155±36 cm^{-1} (from 20,561±1 cm^{-1} to 20,406±35 cm^{-1}), the intensity decreased ~45%, and the average lifetime decreased approximately two-fold from 192 to 108 ms. Since the peak frequency reflects the extent of dipolar relaxation that occurs during the excited state lifetime prior to emission [13], a small decrease in frequency with a two-fold decrease in average lifetime indicates that the rate of dipolar relaxation increased significantly over this temperature range. The activation energy for quenching the triplet state in this glassy region, based on analysis of the average lifetime, was 7.6 kJ mol^{-1}. This is considerably less than the energy of a typical H···OH hydrogen bond [34]. A similar activation energy for quenching the phosphorescence intensity of vanillin on filter paper over the temperature interval from ~250 K to 373 K, 10.3 kJ mol^{-1}, led Nishigaki et al. [35] to conclude that quenching in filter paper involved breaking of a hydrogen bond. A subsequent study of vanillin phosphorescence at cryogenic temperatures [23] found an activation energy of 6.2 kJ mol^{-1} for quenching vanillin intensity over the temperature range from 95 K to 115 K in glassy ethanol; this activation energy was interpreted as the energy required for formation of solvent cages.

Mobility in amorphous sucrose well below T_g is due to localized molecular motions, so-called β-relaxations, within the glassy matrix. The specific physical origin of these localized relaxations in sugars is uncertain; proposals have included segmental rotations of the linear chain of monosaccharides [36], contributions of both intra- and intermolecular interactions [37], and rotations of the hydroxymethyl groups attached to the sugar ring [38]. Recent work has identified two β-relaxations in simple sugars, with the slow β relaxation (also called the Johari-Goldstein relaxation) thought to correspond to localized motion of the entire molecule and the fast β relaxation (referred to as the γ relaxation by these authors) to local motions involving hydrogen bonded groups [39]. Fast γ and slow β processes have also be identified in sucrose [40, 41] with activation energies of 52 and 98 kJ mol^{-1}, respectively. Champion et al. [37] measured an activation energy of 57–62 kJ mol^{-1} for the β relaxation in sucrose. These values are considerably larger than the activation energy for quenching vanillin phosphorescence in the sucrose glass.

Over the temperature range from 50 °C to 100 °C vanillin phosphorescence was extremely sensitive to matrix motions occurring in the melt: the peak frequency decreased 695±62 cm^{-1} (from 20,308±36 cm^{-1} to 19,613 ±50 cm^{-1}), the intensity decreased over 98%, and the average lifetime decreased ~80-fold from 71.2 to 0.85 ms. The activation energy for quenching the triplet state in this region, based on analysis of the average lifetime, was 67 kJ mol^{-1}. This is about the energy of a typical H···OH hydrogen bond [34]. Although the α-relaxation in sucrose (and other sugars) exhibits WLF rather than Arrhenius temperature-dependence at the glass transition and above [40, 41] these activation energies for vanillin quenching are considerably smaller than what would be expected for α-relaxations, 281 to 395 kJ mol^{-1} [37].

The significant differences between the activation energies for vanillin quenching and the activation energies for matrix mobility in both the glass and the melt suggest that the probe is only weakly coupled to the matrix; vanillin photophysics thus only indirectly tracks changes in the mobility of the sucrose matrix. Weak interactions with the matrix allow the probe to undergo independent internal motions that quench the excited state. The structure of vanillin provides only three sites for hydrogen bonds that might couple vibrational motions of the probe to matrix motions: –CHO and $–OCH_3$ which are only hydrogen bond acceptor sites and –OH which is both a donor and an acceptor site; conformational calculations suggest weak internal hydrogen bonding between the hydroxyl hydrogen and the methoxyl oxygen [23]. The specific vibrational mode(s) of vanillin that could couple with the matrix motions through these potential hydrogen bond sites to dissipate the excited triplet state energy are uncertain. The only other detailed investigation of vanillin phosphorescence [23] used conformational analysis of the influence of molecular geometry on state energies to implicate rotational motions of the $–OCH_3$ group about the ring C–O bond as important for both modulating the triplet state energy and quenching the excited triplet state. We thus hypothesize that the dominant probe vibration involved in dissipating energy into the matrix involves methoxyl group rotation about the C–O bond, that matrix mobility modulates this dissipation

by affecting the rate and extent of this rotational motion, and that weak interactions between the probe and the matrix uncouple these rotations from directly following matrix motions.

This speculation is consistent with free volume theory (FVT) which posits that temperature dependence of viscosity is related to changes in free volume [42]. Free volume in an amorphous system is geometrically interpreted as a continuous network of lakes and channels which permits the diffusive motion of molecules [43]. The FVT is based on changes in volume expansion coefficient at glass transitions and assumes that molecular motions depend on the presence of holes or voids [44] that allow molecular movement to take place. The free volume based WLF theory has been shown to be useful in predicting temperature dependent mechanical properties of amorphous materials [45–47]. Our unpublished studies of the temperature-dependence of phosphorescence from different analogs of vanillin with different sized groups attached to the vanillin ring [25] are consistent with this interpretation.

Time-resolved phosphorescence intensity decays from vanillin were multi-exponential both below and above the glass transition temperature. We have used a four exponential decay function to model these decays from −20 to 80 °C. Such a complex decay model is consistent with current views that the pure (single component) amorphous matrix is dynamically heterogeneous on the molecular level [33, 48, 49]. The different lifetime components correspond to populations of probes with different values of the non-radiative quenching constant k_{TS0}, that is, to populations of probes in environments that differ in terms of matrix molecular mobility [9, 10, 50]. The fit lifetimes varied from 184 to 2.7 ms at 20 °C, indicating the presence of local environments whose mobility differs ~70 fold; similar ranges in lifetime were seen at other temperatures. The systematic temperature-dependence of both lifetimes and amplitudes suggests that matrix environments not only become more mobile with increase in temperature but also that the distribution of matrix environments changed systematically from predominately more rigid to predominately less rigid with increase in temperature.

Lifetime complexity reflective of dynamic matrix heterogeneity in amorphous sucrose has also been seen in phosphorescence studies of tryptophan and its halogenated analogs [51], of N-acetyltryptophanamide (NATA) [52], and of erythrosin B (Ery B) [10, 52]. Tryptophan and its analogs showed three phosphorescence lifetimes ranging from ~10 ms to over 1 s in glassy sucrose at 20 °C and phosphorescence intensity decays from NATA and Ery B were multi-exponential both above and below the sucrose glass transition temperature.

In conclusion, vanillin phosphorescence peak energy, bandwidth, and lifetime are sensitive to changes in the molecular mobility in both the glass below and the melt above the sucrose T_g. The decay of vanillin phosphorescence is strictly multi-exponential indicating that the probe is also sensitive to the known site heterogeneity in the molecular dynamics of the matrix. Vanillin phosphorescence thus provides a novel and sensitive probe of the μs-ms dynamics of amorphous solids in the glass and in the melt. Since vanillin is a common flavor molecule widely and safely used in foods for centuries, this raises the possibility of using the photophysical behavior of a constituent of a solid food or even pharmaceutical as an intrinsic probe of the physical state of the amorphous matrix.

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