Sugar-derived Glasses Support Thermal and Photo-initiated Electron Transfer Processes over Macroscopic Distances*

Mahantesh S. Navati and Joel M. Friedman
From the Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461

Trehalose-derived glasses are shown to support long range electron transfer reactions between spatially well separated donors and protein acceptors. The results indicate that these matrices can be used not only to greatly stabilize protein structures but also to facilitate both thermal and photo-initiated heme protein reduction over large macroscopic distances. To date the promise of exciting new protein-based technologies that can harness the exceptional tunability of protein functionality has been significantly thwarted by both intrinsic instability and stringent solvent/environment requirements for the expression of functional properties. The presented results raise the prospect of overcoming these limitations with respect to incorporating redox active proteins into solid state devices such as tunable batteries, switches, and solar cells. The findings also have implications for formulations intended to enhance long term storage of biomaterials, new protein-based synthetic strategies, and biophysical studies of functional intermediates trapped under nonequilibrium conditions. In addition, the study shows that certain sugars such as glucose or tagatose, when added to redox-inactive glassy matrices, can be used as a source of thermal electrons that can be harvested by suitable redox active proteins, raising the prospect of using common sugars as an electron source in solid state thermal fuel cells.

Many plants and animals express large amounts of sugars when osmotically stressed (1). The ability of sugars as such trehalose to form glassy matrices under conditions of drying is generally thought to be responsible for anhydrobiosis (2, 3), whereby the cellular machinery is essentially frozen in the glass allowing for long term survival of the organism under conditions of extreme dryness. This phenomenon is the basis for substantial efforts directed toward the creation of suitable carbohydrate-based glassy matrices for the purpose of long term preservation of pharmaceuticals and food items (4–7). Biophysical studies show that glassy matrices can significantly damp protein motions (8–12), which results in dramatic conformational stabilization with respect to thermal denaturation and degradation. Both the damping of macromolecular dynamics and the dramatic decrease in the mobility of water within sugar-derived glasses are viewed as the foundation for designing stable sugar-based matrices for long-term maintenance of proteins and other biomolecules under relatively severe conditions. Under such conditions it is assumed that the matrix would be chemically inert. In the present study it is demonstrated that dry glassy matrices can support very long range electron transfer initiated by generating either thermal or photo electrons.

Most proteins when incorporated into glassy matrices can no longer function due to the extreme damping of many functionally important motions. Redox reactions, although influenced by protein dynamics, can still occur even when the proteins are immobilized. In a preliminary study (13) it was shown that doping trehalose glasses containing either methemoglobin or metmyoglobin with glucose (a reducing sugar) resulted in samples that undergo facile thermal reduction. In the present work we demonstrate not only that such processes are likely to be general but that these glass-facilitated redox reactions can occur over surprisingly large macroscopic distances.

EXPERIMENTAL PROCEDURES

Materials—Reagents and proteins were all obtained from Sigma with the exception of tagatose (generous gift from Spherix Inc.), deazaflavin (a generous gift of Dr. Bruce Palfey, University of Michigan), myoglobin mutants (generous gifts from Dr. John Olson, Rice University), and oxygenated human adult hemoglobin (a generous gift from Dr. Seetharama Acharya).

Methods—Thin (~1 mm or less) glassy matrices were prepared from stock solutions of either (a) 80:20 mg/ml of trehalose:sucrose or (b) 60:20:20 mg/ml trehalose:sucrose:glucose (or either fructose or tagatose, D-lyxohexulose, a stereoisomer of fructose) in deionized water. The use of the combined trehalose/sucrose protocol over one with just trehalose eliminated the occasional formation of crystals during drying (14, 15). The glucose, fructose, and tagatose were introduced as potential sources of thermally generated electrons. The above solutions were mixed with aliquots of stock solution of proteins to achieve concentrations of 0.25 mM in protein. Small aliquots of the resulting solutions were layered on a glass plate, dried in a desiccator for several days, and then warmed at 40 °C for 40 min. The cooled samples were stored in a sealed container at room temperature. For the two-layer sandwich experiments, the protein-containing first layer was prepared by the first method (a). For the thermal-mediated reduction experiments, protocol b without protein was used to generate the second layer. For light-mediated reduction, 10 μl of a deazaflavin solution (0.5 mg/ml in deionized water) was added to protocol b.
without the addition of protein. Deazaflavin is an effective source of photo-generated electrons (16). The two extensively dried and preheated glassy layers were then sandwiched together and either heated or illuminated (390-nm light). Subsequent to either the heating or the illumination protocols, the two sandwiched layers were separated. The visible absorption spectrum was then generated from the protein-containing layer and in some cases from a protein solution derived from redissolving the protein-containing glassy layer.

For the long distance photo-electron transfer measurement, glassy matrices were prepared using three different stock solutions as follows; (a) 80 mg of trehalose and 20 mg of sucrose added to and dissolved in 1 ml of a stock solution of 0.1 mM FMN and 0.2 mM NADPH in deionized water; the combination of FMN and NADPH has been shown to be effective in the photo-reduction of hemeproteins in solution (17); (b) 80:20 mg/ml trehalose:sucrose dissolved in 1 ml of deionized water; (c) 80:20 mg/ml trehalose:sucrose dissolved in 1 ml of a 0.2 mM cytochrome c (Fe(III)) solution.

A small aliquot of solution from a and then c were placed separately on the same glass slide separated by ~4 cm. These two separated “drops” were dried extensively, yielding well separated uniform glassy matrices. Then the gap between the separated glasses was bridged with a thin fast drying strip of the viscous protocol b solution. In one case this glassy linker actually made contact with the two initially prepared glasses, and in the other case silver paste was used to bridge a small residual gap between the ends of glassy linker and the two initially prepared glasses. The drying time of the linker was sufficiently fast to preclude any obvious dissolving of the initially prepared glasses. The FMN/NADPH-containing portion of the sample was selectively illuminated (420 nm).

For thermally initiated long range electron transfer measurements, samples were prepared as in the above photo-initiated measurements except that the protein used was met human adult hemoglobin (HbA), and instead of the FMN/NADPH-doped glass, a tagatose-doped glass (as in method b) was used. The sample was then subjected to heating cycles, and the absorption spectrum of the Hb was recorded after each cycle. The control utilized a tagatose-free glass. In the following sections we use the designations Glass 1, 2, and 3 to indicate that the control utilized a tagatose-free glass. In the following sections we use the designations Glass 1, 2, and 3 to indicate that the control utilized a tagatose-free glass.

**RESULTS**

*Heating in the Absence and Presence of Added Glucose (Glasses 1 and 2)—Fig. 1 compares Glass 1 and Glass 2 with respect to the spectral changes occurring when heating glass-embedded oxidized derivatives of horse myoglobin (Mb), human adult hemoglobin (Hb), and cytochrome c (Cc) embedded in a thin glassy layer from a trehalose:sucrose mixture. The panels on the left, labeled Mb, Hb, and Cc, show the progressive changes in the absorbance spectrum of the Mb, Hb, and Cc as a function of heating.*

The three panels on the left show the heating-induced changes for samples embedded in glassy matrices without (Mb, Hb, and Cc) and with (Mb*, Hb*, and Cc*)-added glucose, respectively. The heating protocols were as follows: Mb, Mb*, before heating (a), 67 °C, 4 h (b), 65 °C, 3 days (c); Hb, Hb*, before heating (a), 75 °C, 2 h (b), 85 °C, 45 min (c); Cc, Cc*, before heating (a), 40 °C, 45 min (b), 60 °C, 45 min (c). The inset in the top two panels on the right shows the appearance with heating of Band III, which is exclusively associated with reduced forms of Hb and Mb.

The spectra after the heating cycle for the aquomet Mb and Hb samples in the absence of added glucose show no indication of reduction. Although the spectra show no indication of reduction, they do demonstrate the progressive formation of the oxidized six-coordinate derivative of the two proteins known as the hemichrome (18). Hemichrome formation, often associated with significant osmotic stress (13, 19), is the result of the imidazole side chain of the distal histidine replacing water as the sixth ligand. Dissolving the glass in aqueous buffer resulted in the full recovery of the standard spectra of the initial aquomet derivatives. The corresponding heat cycling for the glucose-free Cc samples showed no substantive changes in the initial spectrum attributed to the initial oxidized Cc derivative.

In marked contrast to the results obtained for the glucose-free samples, the glucose-containing samples show clear evidence of reduction upon heating. The panels labeled Mb* and Hb* both show that the initial heating cycle results in the loss of the thermal-induced changes for glucose-doped glass (Glass 2). In all cases discussed in this work, the absorption spectra are recorded after the heated sample has cooled back down to ambient temperatures. The initial glassy sample before heating typically manifests the absorption spectrum of the corresponding solution phase sample unless otherwise noted. For Mb and Hb these spectra correspond to the oxidized derivative in which water is the sixth ligand of a high spin ferric heme iron. The spectrum from the ferric Cc sample is characteristic of a six-coordinate low spin ferric heme. In contrast to Mb and Hb, the heme-iron for Cc has a permanent intrinsic sixth ligand derived from a methionine side chain.
the aquomet spectra and the appearance of five coordinate ferrous heme spectra both in the visible region and near IR regions. Continued heating produces a contribution to the spectra from the six-coordinate reduced species known as the hemochrome (18), again the result of osmotic stress. Dissolving these hemochrome samples in aerated buffer yields the standard spectra associated with the fully oxygenated derivatives of either Hb or Mb. The Cc* panels show the conversion with heating of the oxidized Cc spectrum to that of the fully reduced Cc spectrum. The reduction of oxidized Cc is observed to occur at substantially lower temperatures than for either Mb or Hb.

**Thermal-mediated Protein Reduction; Sugar Dependence**—For a given protein and preparative protocol, the thermal reduction profile is highly sensitive to the specific sugar added to the trehalose/sucrose glass. Fig. 2 shows that the degree of reduction for a glass-embedded sample of aquomet HbA heated at 65 °C for 45 min is a function of added sugar to the trehalose/sucrose glass. The three added sugars, all monosaccharides, are glucose, fructose, and tagatose. The extent of reduction as reflected in the appearance of deoxy spectral features increases in going from glucose to fructose to tagatose. When tagatose was added to a ferric Cc-containing glass (Glass 3), extensive reduction was fully apparent upon drying, even at ambient temperatures without any heat cycling (not shown).

**Sugar-mediated Thermal Reduction of Mb Does Not Require Either a Distal Histidine or a Sixth Ligand**—Fig. 3 shows that glucose-mediated thermal reduction occurs for two Mb mutants that have distal histidine (His-64) replacements. In Fig. 3, panel A, the thermal reduction of Mb(H64L) is shown. The H64L mutation resulted in the introduction of a nonpolar side chain in lieu of the glucose from fructose to tagatose. When tagatose was added to a ferric Cc-containing glass (Glass 3), extensive reduction was fully apparent upon drying, even at ambient temperatures without any heat cycling (not shown).

**Glass Composition Dependence of the Thermal Reduction Process**—Fig. 4 shows the influence of doping an HbA-containing Glass 2 with 5% by volume glycerol (no preheating). In contrast to the glycerol-free Glass 2 sample, which manifests the unaltered aquomet HbA spectrum, the initial spectrum of the glycerol-doped samples at ambient temperature is characteristic of the hemichrome form of HbA. Subsequent to a 45-min 75 °C heating cycle, the glycerol-doped sample had undergone nearly complete reduction, but the resulting species was the hemochrome derivative, in contrast to the five-coordinate deoxy derivative that was generated using a comparable glycerol-free Glass 2 sample subjected to a similar heating cycle. Preliminary results with chitosan-doped trehalose/sucrose glasses (made from 12.5 mg of chitosan dissolved in 1 ml of the trehalose/sucrose starting solution) show a similar enhancement in the formation of the hemichrome; however, the chitosan also reduces the threshold for thermal reduction, i.e. reduction occurs at lower temperatures when compared with the corresponding chitosan-free sample. The addition of polyvinyl alco-
hol to the glass samples enhanced the flexibility of the glass but eliminated the thermal reduction capabilities of the resulting glass. We also fully bathed a thin aquomet Hb-containing tetramethoxysilane-derived porous sol-gel sample in an excess of the Glass 2 starting solution and found that after allowing the sample to sit for several days then pouring off the excess solution and allowing the sample to thoroughly dry, we obtained a visually glassy sample that still manifested the starting aquomet HbA spectrum. Repeated heating cycles similar to those described above did not result in any observed sample reduction; however, with heating the spectrum of the sample rapidly converted to and remained that of the hemichrome. Soaking the sample in an excess of N₂-purged buffer did not reverse the hemichrome spectrum; however, subsequent addition of reducing agent (dithionite) and CO to the buffer resulted in a ferrous CO Hb spectrum for the encapsulated HbA.

**Trehalose/Sucrose Glass Supports Photo-initiated Hemeprotein Reduction at Ambient Temperatures**—The above results all show that the monosaccharide-doped trehalose/sucrose glasses support thermally initiated hemeprotein reduction. Fig. 5 shows that photo-generated electrons within the glass at ambient temperatures are also effective in reducing embedded hemeproteins. The shown spectra are from aquomet HbA embedded in a deazaflavin-doped Glass 3. Similar results were obtained with a deazaflavin-doped Glass 1, but the extent of reduction as a function of illumination time was more extensive for the Glass 3 sample. The figure shows the initial met Hb spectrum being converted to the deoxy Hb spectrum with continued illumination. Without deazaflavin there is no reduction under these illumination conditions (excitation at ~390 nm); however, illumination in the absence of the deazaflavin with 280–290-nm light does result in progressive reduction of the met Hb sample, which is likely the result of the direct tryptophan excitation (21).

**Electron Transfer in Two-layer Glassy Sandwiches**—The above results clearly show that sugar-derived glasses can support both thermal and photo-initiated redox processes. In those single film experiments it is not clear to what extent the electron donor and acceptor are physically separated. In the two-layer sandwich experiments the donor and acceptor are in separate dry films. These two-layer experiments also address the question of whether the monosaccharide-dependent differences in thermal reduction efficiency arise from differences in the physical properties of the different monosaccharide doped glasses as a function of temperature or from differences in the ability of these sugars to generate thermal electrons.

Fig. 6 shows both a schematic for the two-layer protocol and results from two different sandwich experiments. In both these and all the other two-layer experiments, the two layers were separated subsequent to the heating cycle before the absorption measurements. In all cases there was no visual evidence of mixing of the two dry glasses as reflected in the absence of any heme-associated red coloring in the protein-free layer.

The Fig. 6 top panel below the schematic shows results from a two-layer thermal reduction experiment. An aquomet HbA-containing trehalose/sucrose layer was sandwiched with a second protein-free glassy layer containing tagatose as a dopant (60:20:20 mg/ml trehalose:tagatose in deionized water). The sandwich was heated at 75 °C for 45 min, and then the two layers were separated once the sample cooled to ambient temperature. The figure shows that with heating of the sandwich, the initial aquomet HbA spectrum (black curve) converted to the spectrum (red curve) associated with the reduced high spin form of HbA (often referred to as deoxy-HbA). The
appearance as well as the near infrared absorption band at ~760 nm, associated only with the reduced deoxy derivative, is also shown in the inset. Dissolving the Hb-containing sample under atmospheric conditions yields a sample that manifests the spectrum associated with the standard oxygenated ferrous derivative of HbA. As previously reported for the single layer trehalose experiments (13), we observe that when the protein-free layer is derived exclusively from the trehalose/sucrose mixture, the only heat-induced spectral changes are those associated with the formation of the hemichrome.

The bottom panel of Fig. 6 shows an analogous sandwich experiment but with photo electrons instead of thermal electrons being the source of the protein reduction. In this case the protein-free layer was lightly doped with deazaflavin (10 µl of 0.5 mg/ml stock of deazaflavin in deionized water added to the 1 ml of solution containing trehalose and sucrose), and the sandwich was illuminated with spectrally isolated (using a filter) 390-nm light at ambient temperature for approximately 2 h. The figure shows that after the illumination cycle the protein-containing layer underwent changes, indicating significant reduction of the initial aquomet HbA sample. Similar results were obtained for ferric cytochrome c and aquomet Mb.

Control single or double layer samples that were without deazaflavin did not show any changes in the spectrum of the initial ferric derivatives after illumination at 390 nm. FMN/NADPH-doped glasses were not as effective as deazaflavin with respect to complete reduction of Hb or Mb samples.

Fig. 7 shows the resulting spectra for four met HbA two-layer sandwich samples that have been subjected to the identical heating cycle (70 °C for 1 h). In each case the Hb-containing layer is a Glass 1 sample, and the protein-free layer is a trehalose/sucrose glass that is varied with respect to added monosaccharides (control with only trehalose/sucrose, glucose, fructose, tagatose). The extent of reduction follows the same pattern as seen for the single layer samples. The extent of reduction again increases in the progression control (trehalose/sucrose with no other added sugar) < glucose < fructose < tagatose.

Electron Transfer between Macroscopically Separated Proteins—The single and double layer experiments show electron transfer to proteins from either sugars or flavins. These results indicate that the glass matrices should also support interprotein redox reactions. Fig. 8 shows the spectra of the individual layers comprising a two-layer sandwich consisting of a deoxy-HbA Glass 1 layer and an Fe(III) Cc Glass 1 layer both before and after heating (50 °C for 45 min) the two-layer sandwich. Before heating, the spectra are consistent with the HbA and Cc samples being in the reduced and oxidized states, respectively. The redox status of the samples reverses subsequent to the heating cycle. The Cc spectrum is now clearly that of the reduced derivative, and that of the HbA sample resembles that of the hydroxyl met derivative. The results are consistent with thermal-mediated electron transfer from the HbA sample to the Cc sample as would be anticipated based on the latter having the known higher redox potential.
Very Long Range Electron Transfer within Glassy Matrices—A more dramatic illustration of the capacity of the glassy matrices to support electron transfer was obtained using a protocol where a glass containing the electron source and a second well separated (~40 mm) glass containing the protein electron acceptor were physically linked by a strip of glass that was free of either the addition of added sugars or protein (Glass 1). Fig. 9 shows a schematic of the physical arrangement and the results obtained for a heat cycling protocol utilizing met HbA and tagatose as the physically separated but linked electron acceptor and thermal electron source, respectively. It can be seen that with continued heating the HbA spectrum showed at first the transition from the aquomet to the hemichrome species followed by reduction to the deoxy derivative and finally with continued heating to the hemochrome species. No reduction was observed if a dopant-free protocol a glass was used instead of the tagatose-containing glass. Fig. 9 illustrates that the glass also supported long distance electron transfer at ambient temperatures by using photoelectrons as the electron source. In this case localized illumination at ambient temperatures of an electron-source glass containing a combination of FMN and NADPH was used to initiate the electron transfer process. Upon initiating the illumination protocol, there was an almost immediate visual change in the color of the protein-containing segment. The absorption spectrum confirmed the occurrence of the reduction process. Two protocols were tested with comparable results. In one case the glass linker made actual contact with the two initially prepared separated matrices. In the other case the glass linker was allowed to form without directly contacting the Cc and FMN-containing matrices. Once the linker glass formed (after drying), the links to the Cc- and FMN-containing glasses were then created with silver paint. In both cases illumination of the FMN-containing matrix resulted in the well separated Cc-containing matrix immediately turning red on the time scale of visual observation. The figure illustrates the result for Cc where complete reduction occurs. The use of met HbA as an electron acceptor resulted in partial reduction and eventual reversal of the spectrum back to the met derivative. In single-composite-layer or two-layer sandwich experiments, the use of deazaflavin as a source of photoelectrons proved much more effective in photo-reducing all three proteins compared with the FMN/NADPH mixture. Limited supplies of deazaflavin precluded its use in the long distance experiments.

DISCUSSION

The above results show that glassy matrices derived from sugars can support long distance electron transfer reactions between redox active proteins and either thermal or photo electron sources. There are several possible mechanisms for the long range electron transfer. One possibility is that transport is through electron hopping via the extended proton-oxygen hydrogen bonding network that is characteristic of sugar-derived glasses. We envision that the glass, with respect to electron diffusion, has a potential energy surface comprised of numerous shallow potential minima associated with each hopping site within the network. The absence of any deep potential

FIGURE 8. The absorption spectra of separated glassy layers of aquomet HbA and oxidized Cc before and after the two layers are sandwiched together, heated (50 °C for 45 min), and then re-separated. Both proteins are embedded in a trehalose/sucrose glass with any additional sugars or additives.

FIGURE 9. Changes in the visible absorption spectra of oxidized hemoproteins. In both the upper and lower panels, the hemoprotein-containing layer is linked via a dopant-free trehalose/sucrose glassy strip to an electron source (see the schematic on the top of the figure). In the upper panel the changes in the spectrum of oxidized cytochrome c are shown before (black) and after (blue) illumination of an FMN/NADPH-doped layer. The inset shows the corresponding changes in the FMN spectrum that indicate the light-induced change in redox status of the FMN. The bottom panel shows heat-induced changes in the absorption spectrum of aquomet HbA linked to a tagatose (tag)-doped glassy film. The first intermediate spectrum (blue) has a large contribution from the hemichrome, whereas the final spectrum is reflective of the hemochrome.
Long Distance Electron Transfer in Sugar Glasses

minima allows for extended hopping through the network. The embedded proteins contribute deep minima traps for the hopping electrons. Thus, the electron transfer process becomes an entropic search over a large energy landscape characterized by numerous shallow wells and an occasional deep trap. Mobile waters do not appear to be the vehicle for electron transfer since waters do not integrate into the relatively rigid hydrogen-bonding network. This hopping model would allow for electron or proton transport to occur not via the individual initially generated electrons/protons hopping from source to sink but by having the extended hydrogen-bonding network to propagate the successive uptake and release of the charges along the hydrogen-bonded network. An electron/proton that is transiently taken up at one end of a given long chain member of the hydrogen-bonding network could trigger an electron/proton release at the other end of the chain. This process is analogous to the Grothuss mechanism (30), which accounts for the much faster overall conduction of protons compared with other small ions in water. As in the water-wire model, the electron or proton can rapidly shuttle among the numerous linked hydrogen-bonded oxygens. As a result the transport of the electron/proton does not require the large amplitude displacement of molecular species for either the electron/proton or a mobile charge-carrying molecular species to diffuse from one site to another. It is, however, quite probable that the electron/proton hopping along the hydrogen-bonded network within the glass is activated via low frequency fluctuations of the vibration modes of the coupled network comprising the glass. These thermal fluctuations would facilitate the hopping from one shallow minimum to another.

The ability to support long range electron transfer is likely to be general with respect to polysaccharide-derived glasses based on preliminary results showing similar phenomena in glasses derived from chitosan- and glycerol-doped glasses. How and by what mechanism additives to the glass recipe impact electron transfer properties is very unclear. One possibility is that additives such as chitosan provide added long chain “wires” that facilitate the hopping process. The absence of thermal-mediated redox activity for the PVA-doped glass and the sol-gel sample may stem from an absence of the necessary extended hydrogen-bonding networks. Additives such as glycerol when present in the right amount could displace mobile waters and act as a hydrogen-bonding linker among discrete clusters of hydrogen-bonded sugars and, thus, extend the network.

Sugar and Protein Dependence—The heat cycling experiments show that sugar-derived glassy matrices support long distance electron transfer under conditions where sugars can function as a thermal source of electrons and suitable proteins can function as harvesters of the sugar-derived electrons. The single and two-layer experiments show that the efficacy of thermal generation of electrons is sugar-dependent, ranging from trehalose and sucrose with essentially no activity to increasing activity in the following progression: glucose, fructose, and tagatose. The observation of this progression in both the single and two-layer experiments support the hypothesis that it is the thermal-mediated electron donating properties of the glass-embedded sugars and not their impact on the glass properties per se that is responsible for the effect. The presented results also indicate that the electron-harvesting capacity of the embedded protein is also protein-specific. The limited data set suggests that this harvesting capacity scales with the redox potential. Oxidized Cc, which has a significantly higher redox potential than either met HbA or met Mb, undergoes thermal reduction at lower temperatures when compared with both of these other two proteins under identical conditions.

Biophysical Implications—The finding of long range electron-mediated redox activity in sugar glasses has biophysical implications and applications. On a biological/biophysical level, polysaccharide-derived hydrogen-bonding networks are likely to form in those biological systems that manifest anhydrobiosis. The present observations suggest that redox activity may be operative in such systems. Redox activity in these seemingly dormant systems may be important for low level intracellular signaling and for maintaining the average redox state of the composite system over an extended time period by minimizing autoxidation.

The reported phenomenon clearly has considerable potential as a biophysical tool. Not only do these biological systems present with novel electronic properties requiring further exploration of the nature of long range electron hopping mechanism, but they also provide a means of changing the redox state of proteins without conformational reorganization (due to the coupling of the protein to the rigid hydrogen bonding network of the trehalose glass). Thus, the rigid sugar matrix allows for the investigating of redox processes under conditions where there is no reorganization energy issue (26). The glassy matrix, by virtue of the near complete damping of conformational dynamics coupled to the mobility of surface waters, allows for the trapping and spectroscopic probing of functional intermediates under nonequilibrium conditions. For example the glass-facilitated reduction of the R state met derivative of HbA results in the production of a metastable deoxy-R state form of HbA. Similarly, this approach should yield ferrous cytochromes that retain the conformational distribution of the equilibrium population of the initial ferric species.

Implications for Technology—The finding that sugar-derived glasses support long range electron transfer is likely to be important in developing new electronic technologies (27). Perhaps most significant is that the above findings clearly raise the prospect of interfacing proteins with solid state devices. The glass confers conformational stability to embedded proteins under conditions where redox reactions still occur. In particular these results show that glass-stabilized redox proteins can be used to efficiently harvest electrons generated by either thermal or photo-initiated processes. Given the combination of these properties and the ease with which redox properties can be bio-engineered and optically modulated in redox center-containing proteins, it would appear that glass-embedded proteins represent an especially promising platform for harnessing the redox properties of proteins for use in robust solid state electronic and electro-optical devices. In addition, there are unique new opportunities for redox-based synthetic strategies. The glass traps the initial distribution of structures, not allowing the entering of new substrates or escaping of products from the
protein. Thus, the glass provides a platform for conducting redox chemistry on very well defined initially prepared and trapped species without the prospect of complex secondary reactions. The oxidized heme and the substrate-containing distal heme pocket of the hemeprotein now become a very well defined synthetic chamber with respect to reduction-initiated chemistry.

There have been schemes proposed and explored for using glucose as a source of usable electrons (28). These approaches are based on enzymatic reactions requiring aqueous environments whereas the present study indicates that glucose, fructose, or tagatose embedded in a solid glass matrix can be used to generate thermal electrons that are readily harvested by suitable redox active proteins. Thus, the combined addition of glucose (or fructose or tagatose) and a suitable electron harvesting protein into a glass matrix could provide the basis for robust thermal charging batteries. The current results suggest that such systems would be very efficient, with the proteins harvesting most of the thermally produced electrons (as well as photo-generated electrons for systems doped with a source of photoelectrons). Properties of the battery such as the charging temperature and the resulting voltage would be easily tuned by choice of sugar and redox protein.

Trehalose glass, because of its protein-stabilizing properties, is the basis for many powder formulations for protein and peptide-based pharmaceuticals (4–7, 29). These formulations have finite shelf life that is not readily explained given the stabilizing properties of the glass. The present study shows that these matrices are not inert. Trace amounts of reducing sugars in such materials can supply electrons that can ultimately find a suitable redox center within the glass. Similarly, one can anticipate thermally initiated long range electron transfer reactions between any two redox centers having appropriately different redox potentials. The design of sugar–glass–derived glassy matrices for use in long term storage of pharmaceuticals and food products must take into account this new redox capacity of the glass especially as a potential means of minimizing oxidative damage.

Acknowledgments—We thank Dr. Argyrides Argyrou (Albert Einstein College of Medicine) for technical advice and assistance, Dr. Bruce Palfey (University of Michigan) for samples of deazaflavin, Dr. Gil Levin of Biospherix (Annapolis, MD) for samples of tagatose, and Dr. John Olson, Rice University, for samples of mutant sperm whale myoglobins.

REFERENCES
1. Arguelles, J. C. (2000) Arch. Microbiol. 174, 217–224
2. Crowe, J. H., Hoekstra, F. A., and Crowe, L. M. (1992) Anhydrosibiosis 54, 579–599
3. Crowe, J. H., Carpenter, J. F., and Crowe, L. M. (1998) Annu. Rev. Physiol. 60, 73–103
4. Crowe L. M., David, S. R., and Crowe, J. H. (1966) Biophys. J. 7, 2087–2093
5. D’Alfonso, L., Collini, M., and Baldini, G. (2003) Eur. J. Biochem. 270, 2497–2504
6. Garzon-Rodriguez, W., Koval, R. L., Chongprasert, S., Krishnan, S., Randolph, T. W., Warne, N. W., and Carpenter, J. F. (2004) J. Pharm. Sci. 93, 684–696
7. Heller, M. C., Carpenter, J. F., and Randolph, T. W. (1999) Biotechnol. Bioeng. 63, 166–174
8. Hagen, S. J., Hofrichter, J., and Eaton, W. A. (1995) Science 269, 959–962
9. Gottfried, D., Peterson, E., Sheikh, A., Yang, M., Wang, J., and Friedman, J. (1996) J. Phys. Chem. 100, 12034–12042
10. Cordone, L., Galajda, P., Vitrano, E., Gassmann, A., Ostermann, A., and Parak, F. (1998) Eur. Biophys. J. 27, 173–176
11. Sastry, G. M., and Agmon, N. (1997) Biochemistry 36, 7097–7108
12. Dantsker, D., Samuni, U., Friedman, J. M., and Agmon, N. (2005) Biochim. Biophys. Acta 1749, 234–251
13. Ray, A., Friedman, B. A., and Friedman, J. M. (2002) J. Am. Chem. Soc. 124, 7270–7271
14. Dashnau, J. L., Zelent, B., and Vanderkooi, J. M. (2005) Biochim. Biophys. Acta 114, 71–83
15. Wright, W. W., Carlos Baez, J., and Vanderkooi, J. M. (2002) Anal. Biochem. 307, 167–172
16. Massey, V., and Hemmerich, P. (1978) Biochemistry 17, 9–16
17. Brunori, M., Giuffre, A., Nienhaus, K., Nienhaus, G. U., Scandurra, F. M., and Vahle, B. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 11983–11988
18. Rachmilewitz, E. A., Peisach, J., and Blumberg, W. E. (1971) J. Biol. Chem. 246, 3356–3366
19. Liu, W., Guo, X., and Guo, R. (2005) Int. J. Biol. Macromol. 37, 232–238
20. Christian, J. F., Unno, M., Sage, J. T., Champion, P. M., Chien, E., and Sligar, S. G. (1997) Biochemistry 36, 11198–11204
21. Sakai, H., Onuma, H., Umeyama, M., Takeoka, S., and Tsuchida, E. (2000) Biochemistry 39, 14595–14602
22. Wright, W. W., Guffanti, G. T., and Vanderkooi, J. M. (2003) Biophys. J. 85, 1980–1995
23. Abbruzzetti, S., Giuffrè, S., Sottini, S., Viappiani, C., and Cordone, L. (2005) Cell Biochem. Biophys. 43, 431–437
24. Cordone, L., Cottone, G., Giuffrè, S., Palazzo, G., Venturoli, G., and Viappiani, C. (2005) Biochim. Biophys. Acta 1749, 252–281
25. Liberzzi, F., Vitrano, E., and Cordone, L. (1999) Biophys. J. 76, 2727–2734
26. Hoffman, B. M., Celis, L. M., Cull, D. A., Patel, A. D., Seifert, J. L., Wheeler, K. E., Wang, I., Yao, J., Kurnikov, I. V., and Nocek, J. M. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 3554–3559
27. Gray, H. B., and Winkler, J. R. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 3534–3539
28. Chaudhuri, S. K., and Lovley, D. R. (2003) Nat. Biotechnol. 21, 1229–1232
29. Newman, Y. M., Ring, S. G., and Colaco, C. (1993) Biotechnol. Genet. Eng. Rev. 11, 263–294
30. Agmon, N. (2005) J. Phys. Chem. A 109, 13–35