OA2.2 - Mimicking low temperatures in photosystem I using a room temperature trehalose glass

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Analyzing proteins in a glassy matrix can yield information on how protein dynamics affect their function. However, traditional experimental conditions can make measurements difficult, because they often require high concentrations of glycerol and cryogenic temperatures. To overcome these obstacles, a disaccharide known as trehalose can be used. Trehalose has long been known to stabilize biomolecules, and due to its unusually high glass transition temperature, can form a glass even when moderately hydrated. By embedding Photosystem I (PS I) complexes in a trehalose glass matrix at 11% relative humidity, it is possible to study an immobilized protein at room temperature and thereby separate the effects of temperature from those of protein motion. Measurements of forward electron transfer and charge recombination on glassy samples show a significant deviation from the behavior of PS I in solution. Forward electron transfer rates from A_1^- → F_X are slowed from 20 ns to 80 ns on the B-side, and from 200 ns to 13 μs on the A-side, values that closely resemble rates observed in a low temperature glycerol glass. Additionally, charge recombination rates changed from a largely monophasic decay in solution, to several broadly distributed phases in glass. By sequentially removing electron transfer cofactors, it was possible to determine the origin of the various phases and to model the effects on the PS I complex. While primary charge separation remains unaffected, electron transfer is blocked to varying degrees at every cofactor, and recombination from F_X occurs in an inhomogeneous fashion. Comparison with low temperature glycerol glasses reveals a similar effect on electron transfer. Transient EPR measurements at both X- and W-band suggests that the protein structure is largely unchanged in the glassy matrix. By measuring the spin correlated radical pair, $P_{700}^{\cdot+}A_1^{\cdot-}$, it is clear that the distance and relative orientation of the quinone are unchanged from the protein in solution. Further, the lack of an intersystem crossing triplet indicates that the antennae chlorophylls have not been sufficiently altered to be decoupled from energy transfer to the primary donor. This study demonstrates that a trehalose glass can mimic the effects of cryogenic temperatures while at room temperature without significantly distorting protein structure. (Supported by grant 1613022 from the National Science Foundation)