Supplementary Information

Redox Chemistry in the Pigment Eumelanin as a Function of temperature using Broad Band Dielectric Spectroscopy

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Supplementary Figures

Fig. S1. The data is reproduced from [1]. (Top) Real component of the dielectric response versus frequency plots for melanin thin films exposed to H₂O (a) and D₂O (b). The imaginary component of the dielectric response can also be seen for each water type (c and d). The relative pressure/humidity
levels are indicated in the legend. The important thing to note is the qualitative similar behavior between the hydration dependence of the dielectric data for melanin (above) and the temperature dependent data shown in Figure 2. This same behavior implies the same conductivity mechanism is operative both as a function of hydration and temperatures, i.e. the comproportionation reaction.

**Fig. S2.** Activated behavior of the real conductivity of the two melanin samples measured at a frequency 1 Hz: nearly constant loss regime (temperatures below 240 K) is characterized by an activation energy $E_a \approx 0.026$ eV±20% in dry sample (open symbols, 0% RH); hopping regime in wet sample gives $E_a$ value of 0.52 eV±10% (closed symbols, 13.5% wt. of water) and in dry sample 0.43 eV±10% (orange curve, 0% RH). We used the real part of complex conductivity obtained at AC 1 Hz since it is the closest to DC values of conductivity which could not be obtained directly by means of our facilities at the lowest temperatures.

**Estimation of the activation energy for the comproportionation reaction.**

The overall reaction for comproportionation reaction is:

$$\text{H}_2\text{Q} + \text{Q} + 2\text{H}_2\text{O} \rightarrow 2\text{SQ}^\text{-} + 2\text{H}_3\text{O}^+,$$
where H₂Q is hydroquinone, Q is quinone, SQ⁻ is semiquinone and H₃O⁺ is hydronium. The pKₐ’s for DHI has been determined to be 9.54 and 13.09 [2] and is similar to DHICA. Thus:

\[ \text{H}_2\text{Q} + \text{H}_2\text{O} \rightarrow \text{HQ}^- + \text{H}_3\text{O}^+, \text{pK}_{a1} = 9.54 \]

and

\[ \text{HQ}^- + \text{H}_2\text{O} \rightarrow \text{Q}^{2-} + \text{H}_3\text{O}^+, \text{pK}_{a2} = 13.09. \]

Assuming ideal behavior we can write:

\[ K_{a1} = \frac{[\text{HQ}^-][\text{H}_3\text{O}^+]}{[\text{H}_2\text{Q}][\text{H}_2\text{O}]} = e^\frac{\Delta G_{r,a1}}{RT}, \]

\[ K_{a2} = \frac{[\text{Q}^{2-}][\text{H}_3\text{O}^+]}{[\text{HQ}^-][\text{H}_2\text{O}]} = e^\frac{\Delta G_{r,a2}}{RT}. \]

The Gibbs free energy of reaction for A1 can then be determined for each as follows:

\[ K_{a1} = 10^{-9.54} = \frac{[\text{HQ}^-][\text{H}_3\text{O}^+]}{[\text{H}_2\text{Q}][\text{H}_2\text{O}]} = e^\frac{\Delta G_{r,a1}}{RT}, \]

\[ 10^{-9.54} = e^\frac{\Delta G_{r,a1}}{RT}, \]

\[ \ln 10^{-9.54} = -\frac{\Delta G_{r,a1}}{RT}, \]

\[ -9.54 \ln 10 = -\frac{\Delta G_{r,a1}}{RT}, \]

\[ 9.54 \ln 10 = \frac{\Delta G_{r,a1}}{RT}, \]

\[ 9.54RT \ln 10 = \Delta G_{r,a1}. \]

Assuming room temperature of 298 K:
9.54 \times 8.314 \, JK^{-1} \, mol^{-1} \times 298 \, K \times \ln 10 = \Delta G_{r, a1},

23636 \, J mol^{-1} \times \ln 10 = \Delta G_{r, a1},

23636 \, J mol^{-1} \times 2.303 = \Delta G_{r, a1},

\Delta G_{r, a1} = 54433 \, J mol^{-1},

\Delta G_{r, a1} = 9.039 \times 10^{-20} \, J,

\Delta G_{r, a1} = 0.562 \, eV.

Repeating the calculation for \Lambda 2 one gets:

\begin{align*}
K_{a2} &= 10^{-13.09} = \left[ \frac{Q^2}{[H_3O^+]} \right] \left[ \frac{[H^+] [H_2O]}{[HQ^-]} \right] = e^{-\frac{\Delta G_{r, a2}}{RT}}, \\
10^{-13.09} &= e^{-\frac{\Delta G_{r, a1}}{RT}}, \\
\ln 10^{-13.09} &= -\frac{\Delta G_{r, a1}}{RT}, \\
-13.09 \ln 10 &= -\frac{\Delta G_{r, a1}}{RT}, \\
13.09 \ln 10 &= \frac{\Delta G_{r, a1}}{RT}, \\
13.09 RT \ln 10 &= \Delta G_{r, a1}.
\end{align*}

Assuming room temperature of 298 K:

13.09 \times 8.314 \, JK^{-1} \, mol^{-1} \times 298 \, K \times \ln 10 = \Delta G_{r, a1},

32431 \, J mol^{-1} \times \ln 10 = \Delta G_{r, a1},
Now, turning to the redox chemistry reactions, we focus on the one electron half reactions. Currently, there is no information available for indole quinones, but there is information available for 1,2, benzoquinone units [3], [4]. This entity is a reasonable proxy since it is known that in melanin during synthesis break up of the indole quinone occur frequently [5], i.e. you will have 1,2-benzoquinone units. Thus

\[
Q + e^- \rightarrow SQ^-, \quad E_0 = -0.21 \text{ V}
\]

and

\[
SQ^- + e^- \rightarrow Q^{2-}, \quad E_0 = +0.043 \text{ V}.
\]

Working with the first reaction one gets:

\[
E_0 = -\frac{RT}{zF} \ln K_{red1},
\]

\[
E_0 = -\frac{RT}{zF} \ln K_{red1},
\]

\[
zFE_0 = -RT \ln K_{red1},
\]

\[
\Delta G_{r,red1} = -RT \ln K_{red1}.
\]

Hence, since we know \(E_0\), we have the Gibbs free energy

\[
\Delta G_{r,red1} = zFE_0 = 1 \times 9.6485 \times 10^4 \text{ J mol}^{-1} \times -0.21 \text{ V},
\]
\[ \Delta G_{r,\text{red}1} = -20261 \, J/mol, \]
\[ \Delta G_{r,\text{red}1} = -3.365 \times 10^{-20} \, J, \]
\[ \Delta G_{r,\text{red}1} = -0.21 \, eV. \]

Repeating the above calculation for the second half reaction:

\[ E_0 = \frac{RT}{zF} \ln K_{\text{red}2}, \]
\[ E_0 = \frac{RT}{zF} \ln K_{\text{red}2}, \]
\[ zFE_0 = -RT \ln K_{\text{red}2}, \]
\[ \Delta G_{r,\text{red}1} = -RT \ln K_{\text{red}2}. \]

Hence, since we know \( E_0 \), we have the Gibbs free energy

\[ \Delta G_{r,\text{red}1} = zFE_0 = 1 \times 9.6485 \times 10^4 \, Cmol^{-1} \times 0.043 \, V, \]
\[ \Delta G_{r,\text{red}1} = 4149 \, J/mol, \]
\[ \Delta G_{r,\text{red}1} = 6.89 \times 10^{-21} \, J, \]
\[ \Delta G_{r,\text{red}1} = 0.043 \, eV. \]

With all the above, we are able to estimate the Gibbs free energy of the overall comproportionation reaction. Recalling, the equilibrium constant for the overall reaction will have to have the form of

\[ K_{\text{comp}} = \frac{[SQ^-]^2[H_3O^+]^2}{[Q][H_2O][H_2O]^2}, \]

which can be constructed as follows:
\[
\frac{[HQ^-][H_2O^+][Q^2^-][H_2O^+][SQ^-][SQ^-]}{[H_2Q][H_2O][HQ^-][H_2O][Q^2^-][\mathbb{Q}^-][\mathbb{Q}^-]} = K_{a1}K_{a2}\frac{K_{\text{red}1}}{K_{\text{red}2}} = K_{\text{comp}}.
\]

This has the temperature dependence of:

\[
K_{\text{comp}} = e^{-\frac{\Delta G_{r,\text{comp}}}{RT}} = e^{-\frac{\Delta G_{r,a1}}{RT}}e^{-\frac{\Delta G_{r,a2}}{RT}}e^{-\frac{\Delta G_{r,\text{red}1}}{RT}}e^{-\frac{\Delta G_{r,\text{red}2}}{RT}}
\]

\[
K_{\text{comp}} = e^{\frac{(\Delta G_{r,a1} + \Delta G_{r,a2} + \Delta G_{r,\text{red}1} - \Delta G_{r,\text{red}2})}{RT}}.
\]

Therefore:

\[
\Delta G_{r,\text{comp}} = \Delta G_{r,a1} + \Delta G_{r,a2} + \Delta G_{r,\text{red}1} - \Delta G_{r,\text{red}2}.
\]

\[
\Delta G_{r,\text{comp}} = 0.562 \text{ eV} + 0.771 \text{ eV} - 0.21 \text{ eV} - 0.043 \text{ eV}.
\]

\[
\Delta G_{r,\text{comp}} = 1.08 \text{ eV}.
\]

Overall reaction energy of 1.08 eV. We do note that this is a calculation done only based on monomers, however, the purpose of this calculation is to yield and approximate value for comparison to the data obtained in the study.

**Quinhydrone explanation**

To understand the background to our proposal, we give a short discussion on the structural knowledge of melanin. It is accepted that melanin is a structurally disordered material with constituent indolequinone building blocks with various redox states (see scheme in Fig.1). These blocks are cross-linked and organized into oligomer sheets [6]–[11], which then stack to form the aforementioned grains. Reduced (hydroquinones – H\(_2\)Q) and half-reduced (semiquinones - SQ) states are Brønsted acids where the pK\(_a\) of the generic melanin semiquinone is presumably closer to 6.8, which is the pK\(_a\) for the DHI monomer unit [12]. Furthermore, in the 1960-80s, presence of quinhydrone in melanin was intensively articulated mainly in EPR/ESR studies, since melanin is a quinone/hydroquinone system [13]–[15]. But this articulation has fallen by the way side in recent
years. The presence of quinhydrone in melanin is not an unreasonable supposition from knowledge of model quinone compounds. For example, $p$-benzoquinone, unlike melanin, can be relatively easily crystallized and studied with the X-ray diffraction technique. It was demonstrated that pressures of 1.5-3 GPa induce cooperative transfer of proton and/or electron in crystals of $p$-benzoquinhydrone derivatives leading to formation of stable $p$-benzosemiquinone [16], [17]. In 2006 Molčanov et al. [18] demonstrated by means of variable-temperature X-ray diffraction combined with EPR and DFT analysis, that $p$-benzosemiquinone can be considered as an excited state of $p$-benzoquinhydrone, and formation of $p$-benzosemiquinone from $p$-benzoquinhydrone can be induced by high pressure or X-ray irradiation under atmospheric pressure. This conversion happens by a single-crystal-to-single reaction and radical molecules are stabilized by low-barrier hydrogen bonds; in other words, great structural transformations are not needed for the chemical transition. At the same time, it is well established for the $p$-benzoquinones that quinhydrone complex is much more stable in solid dry state [19] and is rather labile in solution owing to water intercalative H-bonding interactions.

Unfortunately, the investigations of ability of more close analogs of melanin, i.e. $o$-quinones, to form $o$-quinhydrone were not performed intensively till now. We could find only one structural study devoted to formation of $ortho$-quinhydrone from phenanthrene-9,10-dione and phenathrene-9,10-diol [20]. The authors particularly underline stability of the novel compound in dry state and instability in solution.

Therefore, taking the above discussion into account, we propose an additional chemical pathway for melanin’s redox chemistry that is depicted in Figure 1, where we show the quinohydrone complex and the reaction pathways II and III. Introduction of water will move the system towards formation of anions, predominantly semiquinone anion $SQ^-$. According to the current most developed concept, the conductivity mechanism (at least in the dark) of melanin is due to the reactions IV and V, which is perturbed by the presence of water and yields protons and anionic semiquinones. These charge carriers provide the main contribution to the observed conductivity by their diffusion through the water matrix and $SQ^-$-anions [21]–[24]. At the same time, it should be noted that according to the
X-ray, infrared and thermally depolarization current [25]–[27] studies of dry melanin, certain portion of water molecules always remain incorporated in the material. Thus, the dimer complex of the quinhydrone with charge transfer has additional degrees of freedom in comparison with monomers. Appearance of water leads to disintegration of the complex and disappearance of the corresponding degrees of freedom, their contribution to the set of states and, as a consequence, to decrease of melanin (not water) heat capacity. At higher temperatures (above 220 K), certain increase of specific heat is observed in wet melanin that transforms into a decrease above ≈240 K. We associate its origin with the water transformation into liquid phase and its subsequent evaporation from the sample.

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