CORRESPONDENCE/REBUTTAL

Comment on Color Loss in Ethanolic Solutions of Chlorophyll a

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In a recent paper published in this Journal, Shen et al.[1] investigated the effects of temperature and ethanol concentration on the stability of chlorophyll a. In the paper, the authors found that the degeneration of greenness in the ethanolic solutions of chlorophyll a follows first-order kinetics. The kinetic behavior of the color loss was found to show a limited variation throughout all of the ethanolic solutions from 1 to 60%. However, in the middle of the ethanol concentrations they examined (40%) the color loss was significantly accelerated by >1 order of magnitude. After analysis of their results, they proposed that the strongest interaction between water and ethanol molecules occurring at a 40% ethanol concentration is responsible for the highest rate of color loss. The authors then concluded that preservation of the chlorophyll pigment would least likely be achieved with a 40% ethanol concentration.

Whereas the authors clearly showed the kinetic characteristics of chlorophyll stability in the various ethanolic solutions on the basis of measurements of activation energy and rate constant for the various ethanol concentrations, we found the kinetic results could be further analyzed using the transition-state theory to elucidate a thermodynamic basis of chlorophyll stability. This approach has been widely employed in the study of molecular stability.[2,3] In this paper we show our analysis of the kinetics of the color loss of chlorophyll a in ethanolic solutions reported by Shen et al.[1] to elucidate a thermodynamic basis of the unique behavior of the pigment in a 40% ethanolic solution.

We first obtained the rate constant per second (k) by converting the rate constant per day from Table 2 of ref 1. Second, a rearranged version of the Eyring equation, eq 1, was applied to yield the standard molar Gibbs free energy of activation (ΔG_a°) at temperature T (K):

$$\Delta G_a^o = -RT \ln \frac{kh}{k_B T} \qquad (1)$$

In eq 1 R is the gas constant (8.3145 J/(K mol)), h is Planck's constant (6.6261×10^{-34} J s), and k_B is the Boltzmann constant (1.3807×10^{-23} J/K).[4] The resulting values of ΔG_a° are shown as a function of temperature in Figure 1A. As can be seen in Figure 1A, there is a significant correlation between ΔG_a° and temperature throughout the ethanol concentrations as R^2 values are >0.8, except for the 40% ethanolic solution. In all cases ΔG_a° is proportional to temperature. A noticeable feature is that the ΔG_a° value of the 40% ethanolic solution is significantly less than that of other ethanolic solutions. This feature was also shown in the Shen paper[1] in terms of activation energy, but a thermodynamic basis of the phenomenon was not included in their discussion.

To capture a thermodynamic basis of the result, standard molar enthalpy (ΔH_a°) and entropy (ΔS_a°) of activation of chlorophyll a color loss were obtained by linear regression in Figure 1A on the basis of eq 2. The slope and y-axis intercept of the linear regression in Figure 1A represent $-\Delta S_a^\circ$ and ΔH_a°, respectively.

$$\Delta G_a^\circ = \Delta H_a^\circ - T\Delta S_a^\circ \qquad (2)$$

The resulting values of ΔH_a° and ΔS_a° are shown in Figure 1B. In all cases ΔS_a° is a negative value, explaining the positive correlation between ΔG_a° for color loss of chlorophyll a and temperature shown in Figure 1A. In contrast, ΔH_a° values are positive throughout all of the ethanolic solutions. The magnitude of the thermodynamic variables indicates that both enthalpy and entropy contribute to the stability of chlorophyll a in ethanolic solutions. Interestingly, we observed a strong correlation between ΔH_a° and ΔS_a° throughout the ethanolic solutions as the R^2 value of the linear fitting in Figure 1B has a large value (0.9465). This feature is called enthalpy−entropy compensation, which is often observed in chemical and biological processes when enthalpy and entropy values vary significantly but the resulting free energy values remain relatively constant due to the compensatory behavior of enthalpy and entropy in the variations.[3,5−11] This explains the limited variability of kinetic characteristics of the color loss of the pigment in a wide range of ethanolic concentration observed in ref 1. The observation of enthalpy−entropy compensation in the color loss of chlorophyll a in ethanolic solutions becomes more evident if the linear fitting is conducted without the data of 40% ethanolic solution as the R^2 value is increased significantly to 0.9992 (Figure 1B). This suggests that the compensation between enthalpy and entropy is greatly diminished at 40% ethanolic solution, and this incomplete enthalpy−entropy compensation is the thermodynamic basis for the unique kinetic behavior of chlorophyll a in the 40% ethanolic solution observed in ref 1. The result of our thermodynamic analysis can be more clearly seen in Figure 1C, where three thermodynamic energy terms (ΔG_a°, ΔH_a°, and $T \times \Delta S_a^\circ$) are plotted as a function of ethanol concentration at 20 °C. Although our analysis explains the fastest color change of chlorophyll a occurring at a 40% ethanolic concentration on the basis of incomplete enthalpy−entropy compensation, a mechanistic understanding of the behavior needs more detailed studies with quantum and molecular mechanical calculations (for example, see ref 12).

In conclusion, our thermodynamic analysis indicates that the stability of chlorophyll a in ethanolic solutions is determined by the combination of ΔH_a° and ΔS_a°, and the contributions of each thermodynamic parameter depend on the ethanol concentration.
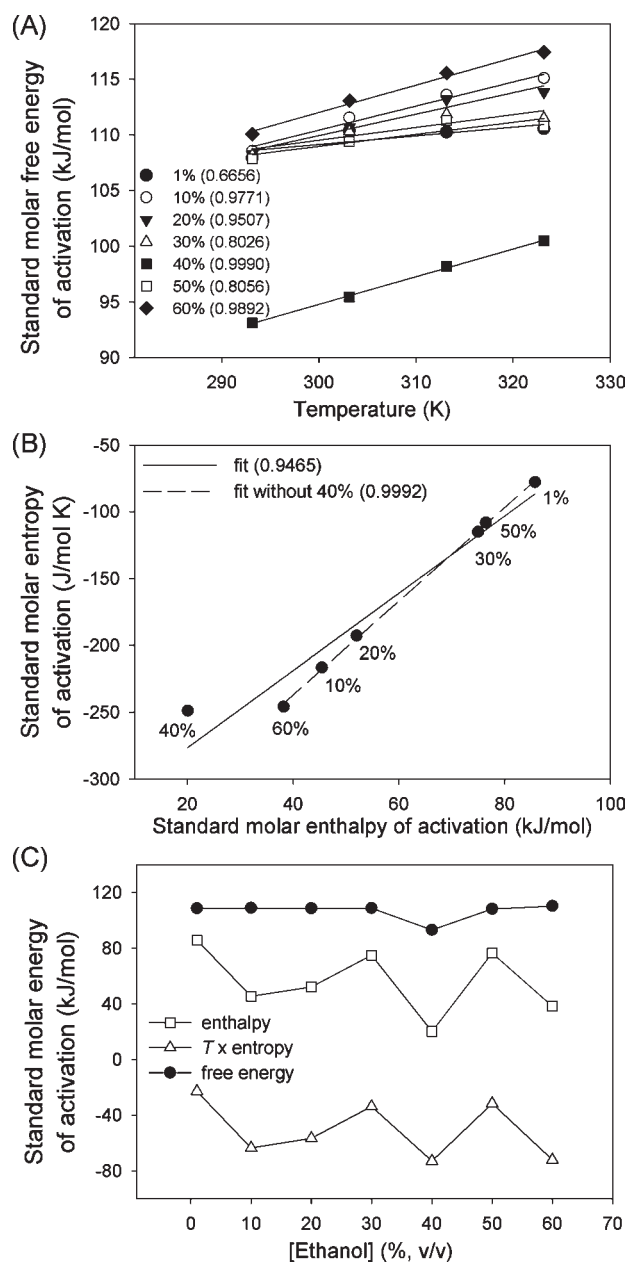
Figure 1. (A) Temperature-dependent standard molar free energy of activation for color loss of chlorophyll *a* in ethanolic solutions. Standard molar enthalpy and entropy of activation were obtained by linear fitting. R^2 of the fitting is shown in the legend. (B) Relationship between enthalpy and entropy of activation. R^2 values of two cases of fitting are shown in the legend. (C) Thermodynamic energy values of chlorophyll color loss at 20 °C. SigmaPlot (version 11, Systat Software Inc., San Jose, CA) was used for graph preparation and fitting.

Although there can be a wide variation in the values of ΔH_a° and ΔS_a° in the color loss of chlorophyll *a*, enthalpy—entropy compensation limits the variation of ΔG_a° value except for the 40% ethanolic solution, and this exception explains the unique kinetic characteristics of the ethanolic solution. Recently, several pigments have been investigated.[13−15] It will be of great interest to examine those additional pigments to determine if their stabilities exhibit enthalpy—entropy compensation as observed in this paper.

Finally, we would like to inform the readers that there were two errors in the original paper.[1] The authors of the original paper accepted our report of the errors in the previous version of this paper, and their correction of the errors is now available.[16]

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