Specific heat of melanin at temperatures below 3 K

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The specific heat of synthetic melanin, cooled *in vitro*, has been measured over the temperature range 0.3–3 K. Below 1 K the specific heat has a magnitude and a $T^{1.3}$ temperature dependence similar to that of metmyoglobin and of polymer glasses. A phase transition previously reported near 2 K for dried melanin was not reproduced.

Experimental evidence[1] has indicated that some biological macromolecules may harbor a broad energy spectrum of localized, low-energy excitations similar to those found in amorphous solids such as the oxide glasses.[2] The presence of these excitations in glasses is revealed in many properties. In the specific heat, for example, the excitations produce a term roughly linear in temperature T which dominates the thermal-phonon contribution at $T \lesssim 1$ K. For biopolymers,[3] only one recent study of metmyoglobin[4] has extended specific-heat measurements well below 1 K. Therefore, we report here specific-heat measurements on a second system, synthetic melanin, for the temperature range 0.3–3 K. In brief, we find the specific heats of metmyoglobin and melanin to be essentially identical, and to reveal a term linear in temperature which is similar in magnitude to that found in nonbiological polymer glasses.

Natural melanins provide the pigmentation of the skin and occur in other areas of the human body.[5] The natural melanins are heterogeneous, high-molecular-weight polymers consisting of 20–50% protein. Synthetic melanins are readily and reproducibly prepared by oxidation of hydroxyaromatics, and have properties closely related to those of natural melanins.[5] Specific-heat measurements on both natural and synthetic melanins have been reported[6] for temperatures down to 1.5 K, and those results will be discussed below. These earlier measurements were obtained on dried samples. The present samples, by contrast, were cooled *in vitro* to be certain that the character of the molecules had not been altered by drying.[5,7]

Our samples of synthetic melanin were derived[8] from a precursor of 3,4-dihydroxyphenyl DL-alanine (DOPA). A solution of 10 g of DOPA in 2 l of H_2O was adjusted to a pH of 8 with the addition of concentrated NH_4OH. Air was bubbled through the stirred solution for three days, then the product was precipitated by lowering the pH to 2 with the addition of concentrated HCl. The product was washed several times with 0.01M HCl and, finally, with H_2O. The resulting black material contained ≈ 90 wt.% H_2O, the water content being determined by massing a sample before and after drying in air at 95 °C for several days.

We wished to reduce the free-water content so as to enhance the specific-heat signal contributed by the melanin alone. To this end we froze and thawed the aqueous suspension to segregate the melanin from free water, centrifuged the result, and decanted the water. This procedure lowered the H_2O content for two samples to 87% and 45% by weight. The resulting samples exhibited the electron-paramagnetic-resonance spectrum expected for melanin.

The calorimeter was a 0.28-g, 2-cm^3-capacity, thin-wall cup constructed of an elastic SC8 epoxy[9] to withstand a volume change during freezing of the sample. This cup was isolated from the refrigerator by dry sapphire plates.[10] An electrical heater with superconducting leads was embedded within the sample, and a carbon-chip resistance thermometer was attached to the outside of the cup. The carbon thermometer was calibrated *in situ* against a germanium resistance thermometer, which in turn had been calibrated on the EPT76 temperature scale derived from National Bureau of Standards superconducting fixed points using cerium magnesium nitrate magnetic thermometry for interpolation. The signal-to-noise ratio of the specific-heat measurements was enhanced with a signal-averaging technique.[11]

The heat capacity measured included contributions from the melanin, the H_2O, and the addenda (cup). We therefore needed to know the heat capacity of the calorimeter cup and the specific heat of ice. For this purpose we measured the heat capacity of the cup with 0.78 g of H_2O and again with 1.8 g of H_2O. The H_2O had been partially degassed by boiling. The data are presented in Fig. 1. The two measurements permitted a computation of the heat capacity of the cup and of H_2O frozen at a cooling rate of ≈ 1.5 K/min at atmospheric pressure. The results are indicated by the respective curves in Fig. 1. The heat capacity deduced for the calorimeter is in good agreement with the measured heat capacity of the epoxy,[9] which contributed more than 95% of the addenda heat capacity. Also, the specific heat of ice is in good agreement with published data[12] obtained for temperatures $\gtrsim 2$ K. The Debye temperature of ice reported in Ref. 12 was 226 K, which may be compared with the present value of 225 K obtained by assuming, as in Ref. 12, three vibrational degrees of freedom for each H_2O molecule. At the lowest temperatures, C/T^3 for a perfect crystal is expected to be constant, which would be represented by a horizontal line in Fig. 1. By contrast, C/T^3 of ice from the present measurements (below 1 K) increases slightly with decreasing temperature. This feature may arise from a very small concentration of orientational impurities or defects creating low-energy excitations. We use the fitted specific heat of ice

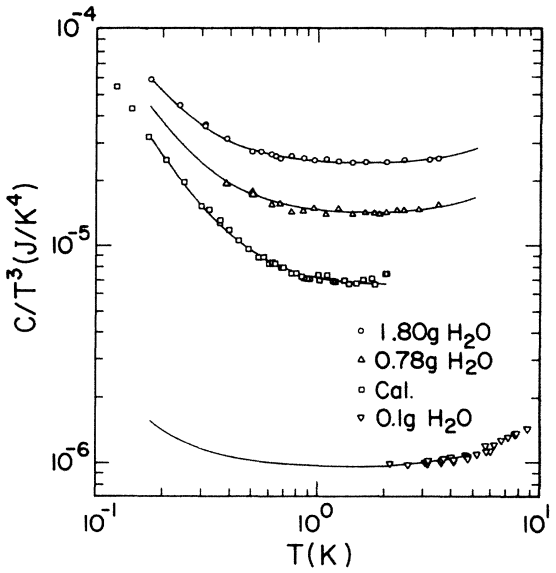

FIG. 1. Heat capacity C for the calorimeter filled with either 0.78 (△) or 1.80 (○) g of H_2O. Data have been divided by the cube of the temperature T to permit expansion of the vertical scale. Computer fits to these two data sets (shown by the respective solid lines assuming terms proportional to T, T^3, and T^5) permitted a computation of the heat capacity of ice, which may be compared with earlier data (▽, 0.1 g, Ref. 12), and a computation of the heat capacity of the calorimeter, which may be compared with earlier data for the epoxy (□, Ref. 9) from which the calorimeter was constructed.

only above 0.3 K, and so the presence of this effect in C/T^3 does not influence the following discussion.

The specific heats of the two samples of melanin in H_2O are shown in Fig. 2, the contribution from the calorimeter having been subtracted. The specific heat is larger for the sample containing the larger fraction of melanin. This suggests that the specific heat may be divided into two contributions, namely, that from the melanin and that from H_2O. Using the results of Fig. 1 for the specific heat

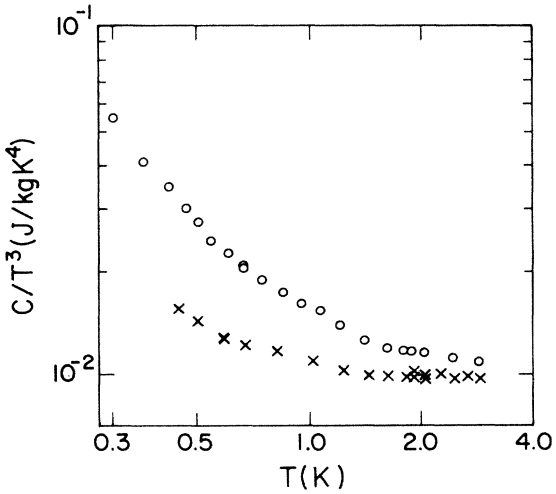

FIG. 2. The specific heat, divided by T^3, for melanin samples containing either 47 (○) or 89 (×) wt.% H_2O.

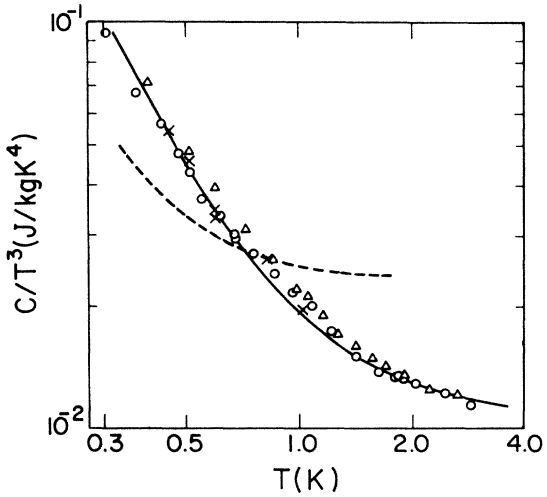

FIG. 3. The specific heat, divided by T^3, of melanin (○,×) obtained from Fig. 2 by subtracting the H_2O contribution. Also included for comparison are data for metmyoglobin (△) from Ref. 4 and, by the dashed line, the specific heat of a typical amorphous polymer (SC8, Ref. 9). The solid line indicates a conventional fit of the form $C = AT + BT^3$.

of ice, the subtraction of this contribution produces the specific heat of melanin shown in Fig. 3. Indeed, the two measurements give the same specific heat for synthetic melanin cooled *in vitro*. Note that the H_2O contribution which has been "removed" computationally is equal to that which can be removed physically by drying the sample at 95 °C for several days.

At temperatures above 1.5 K our data may be compared with the earlier results on synthetic melanin[6] shown in Fig. 4. In the earlier work, the melanin was intentionally dried and squeezed into a hard pellet retaining 1% H_2O. The two sets of data agree within $\approx 3\%$ up to $T \approx 1.8$ K, where the dried product undergoes a suggested magnetic phase transition[6] to a larger magnitude of C/T^3. The same feature appears to occur near 1.9 K in data for dried

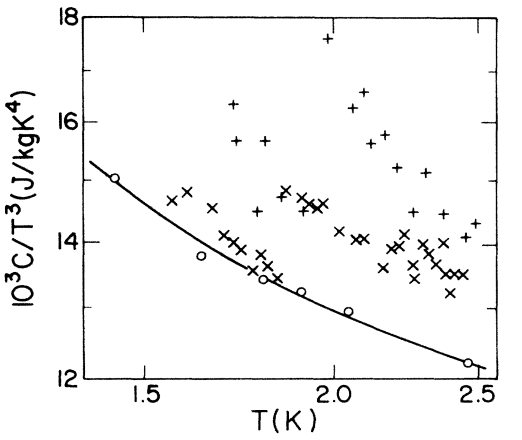

FIG. 4. The specific heat, divided by T^3, of melanins. ○, synthetic melanin cooled in vitro, present data (the solid line is the fit shown in Fig. 3); ×, dried synthetic melanin, Ref. 6; +, dried melanosome, Ref. 6.

melanosome extracted from a human tumor, also shown in Fig. 4. These features are *not* present in our melanin sample cooled *in vitro*. We note that unusual calorimetric behavior has been reported[13] for human serum albumin in H_2O within the temperature range 2.6–4.2 K, and an unidentified phase transition has been observed[14] near 1.7 K for deoxyhemoglobin solution in H_2O but *not* for oxyhemoglobin solution. Clearly, sample preparation and history are relevant to the calorimetric anomalies observed in biopolymers in the temperature range near 2 K.

We are aware of only one other biomolecule measured at temperatures below 1 K. The specific heat of crystalline metmyoglobin[4] containing residual $(NH_4)_2SO_4$ solution, included in Fig. 3, is essentially identical to that of the present melanin sample throughout the temperature range of measurement. The specific heats of the melanin and metmyoglobin are also qualitatively the same as for amorphous polymeric solids at $T < 1$ K, as indicated by the dashed line in Fig. 3. The temperature dependence of the specific heat is close to $T^{1.3}$ at the lowest temperatures. More conventionally the specific heats of glasses are fit by the expression $C = AT + BT^3$. This temperature dependence produces the solid curve in Fig. 3 with $A = 8.4 \times 10^{-3}$ J/kg K^2 and $B = 1.09 \times 10^{-2}$ J/kg K^4. It has been stated[3] that the value of A is much greater in magnitude for biopolymers than for nonbiological polymer glasses. This is clearly not true for the two biopolymers shown in Fig. 3, which thus far are the only two to be measured to temperatures well below 1 K.

In amorphous solids, the specific-heat contribution linear in temperature arises from localized two-level systems (TLS's) which also influence other low-temperature properties.[2] In metmyoglobin it has been shown[4] that, in addition to $C \propto T$ as seen in Fig. 3, the dielectric response is characteristic of the presence of TLS's. Also, relaxation measurements on myoglobin[15] suggest the presence of glasslike behavior and TLS's. Therefore, it is reasonable to assume that the low-temperature specific heats of the biopolymers in Fig. 3 are indeed contributed by TLS's as in inorganic polymer and oxide glasses.

In summary, the specific heat of synthetic melanin cooled *in vitro* is, at $T \lesssim 1$ K, similar to the specific heats of metmyoglobin and of nonbiological polymer glasses both in temperature dependence ($\propto T^{1.3}$) and magnitude. The phase transitions reported for dried melanin samples near 2 K were not reproduced.

Two questions remain unanswered. Will the specific heats of different biomolecules, when measured below 1 K, vary in magnitude by a factor of 1000 as suggested[3] by measurements obtained at higher temperatures? In inorganic glasses the range is only ≈ 10. Secondly, is the presence of the broad spectrum of low-energy excitations, revealed by the specific heat, related to the physiological function of biomolecules,[16] or merely a reflection of the highly disordered structure of those molecules as for inorganic glasses?

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