Short Communication

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RECRYSTALLIZATION MODELS FOR THE THERMOGRAM OF *SACCHAROMYCES CEREVISIAE*

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The differential scanning calorimeter reveals some information about the low-temperature characteristics of yeasts and bacteria with regard to the physical state of the cellular water. The features of sub-zero thawing thermograms thus obtained depend on the species of micro-organisms used (1) and their fine structure varies with the culture age of the specimen (2). The thermogram of *Saccharomyces cerevisiae* gave a characteristic response below its melting temperature, which might be attributed to the glass-transition and recrystallization of that part of the cellular water which remained in a vitrified state while the specimen was being rapidly frozen prior to the thawing period (1, 3, 4).

Neither bulk water nor homogeneous solutions are known to show such response in a relevant temperature range. Hence, the part of the cellular water that gives rise to the glass-transition and recrystallization mentioned above seems to exist either in a somewhat different physical state or in a different molecular environment from that of bulk water. No information is available at present about the site and status of the cellular water that gives the characteristic response. In this connection, the authors carried out similar thermometric experiments using some model systems that are composed of an organic component and water in the form of a mesomorphic or gel phase.

The specimens used were agar gel containing 75% water, potassium oleate-water mixture (7:3), and cetyl alcohol containing 5% water. The potassium oleate-water system makes a lyotropic liquid crystalline in the range of 30% to 60% water content, while a small amount of water in cetyl alcohol seems to be incorporated in a solid-like state at room temperature (5).

Twenty milligrams of each specimen was transferred to aluminium measuring pans, rapidly frozen by immersion into boiling liquid nitrogen, and rewarmed at a constant rate to take the thawing thermograms by the use of a low-temperature differential scanning calorimeter. The average freezing rate was estimated as 10°/sec and the constant thawing rate was 5°/min.

Fig. 1. Thawing thermogram of a rapidly frozen agar gel containing 75% water at a warming rate of 5°/min.

Fig. 2. Thawing thermogram of a rapidly frozen potassium oleate—water mixture (7:3) obtained under the same experimental conditions as in Fig. 1.

Three thermograms for these model systems are shown in Figs. 1, 2, and 3. The thermogram in Fig. 4 is that of *S. cerevisiae* given for the sake of comparison. The agar gel and oleate-water mixture show similar patterns of glass transition and recrystallization at different temperatures in the sub-zero range.

Fig. 3. Thawing thermogram of a rapidly frozen cetyl alcohol containing 5% water obtained under the same experimental conditions as in Fig. 1.

Fig. 4. Thawing thermogram of rapidly frozen cells of *Saccharomyces cerevisiae* IAM 4171 obtained under the same experimental conditions as in Fig. 1. T_G , glass transition temperature; T_R , recrystallization temperature; T_M , melting temperature.

These data do not suggest any definite conclusion as to the site of the relevant cellular water. However, taking into consideration the fact that the agar gel containing approximately the same amount of water shows a characteristic peak at a temperature similar to that of *S. cerevisiae*, this gel system seems to deserve the closest attention. The agar gel forms a polymer matrix structure, by which water is confined in separate small compartments within the gel structure. The water thus retained in the gel hydrates to hydrophilic sites of the polymer molecules. Because of this hydration and geometric compartmentalization, the water in agar gel appears to have lost some of its freedom of movement. This reduced freedom of movement might retard the ice-growing rate during the freezing process, thus leaving part of the gel water in a vitrified state to be recrystallized during the thawing period to follow. It is of interest that the yeast has a well-developed cell wall composed of polysaccharides around the cell membrane. One possibility is that the water contained in this part of the microbial cells causes the recrystallization peak observed with *S. cerevisiae* around at -20° . However, much more work remains to be done before any elaborate discussions can be made.

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