Thermochimica Acta, 155 (1989) 103–107
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

ASSOCIATION BETWEEN ICE NUCLEI AND FRACTURE INTERFACES IN SUCROSE:WATER GLASSES

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(Received 23 March 1989)

ABSTRACT

When 65% sucrose: H_2O is cooled to $-130\,^{\circ}C$ in a DSC, a broad glass transition is seen at about $-70\,^{\circ}C$. During warming the transition is followed by a large devitrification and melting. Cryomicroscopy shows that nuclei form in fractures produced by cooling. During warming, ice is seen to grow from the cracks into the supersaturated melt surrounding them. Prevention of cracking with plasticizing additives inhibits massive ice formation.

INTRODUCTION

When samples of 65% (w/w) sucrose: H_2O are cooled to $-130\,^{\circ}C$ at moderate rates in a DSC, no freezing is observed even though there is freezable water in the sample. Instead, only a broad glass transition is observed below $-70\,^{\circ}C$. During warming, this glass melts reversibly, but melting is followed at $-60\,^{\circ}C$ and above by a devitrification which intensifies above $-35\,^{\circ}C$. These observations are entirely consistent with the supplemented phase diagram produced many years ago by Rasmussen and MacKenzie [1] on which a 65% solution has a T_g of $-79\,^{\circ}C$, beyond the measurable homogeneous nucleation temperature, T_h, but nonetheless intersects the T_g at $-79\,^{\circ}C$ and T_d at $-52\,^{\circ}C$. The origin of the nuclei producing ice in the sample during warming is not defined. It has been assumed that homogeneous ice nuclei are formed in the sample during cooling, but that viscosity interferes with their growth until the temperature is raised above T_d. The observations reported here cast doubt upon this interpretation.

EXPERIMENTAL

The 65% sucrose: water samples were compounded by weight in scintillation vials and dissolved at $85\,^{\circ}C$. Samples of 4 μl were placed between

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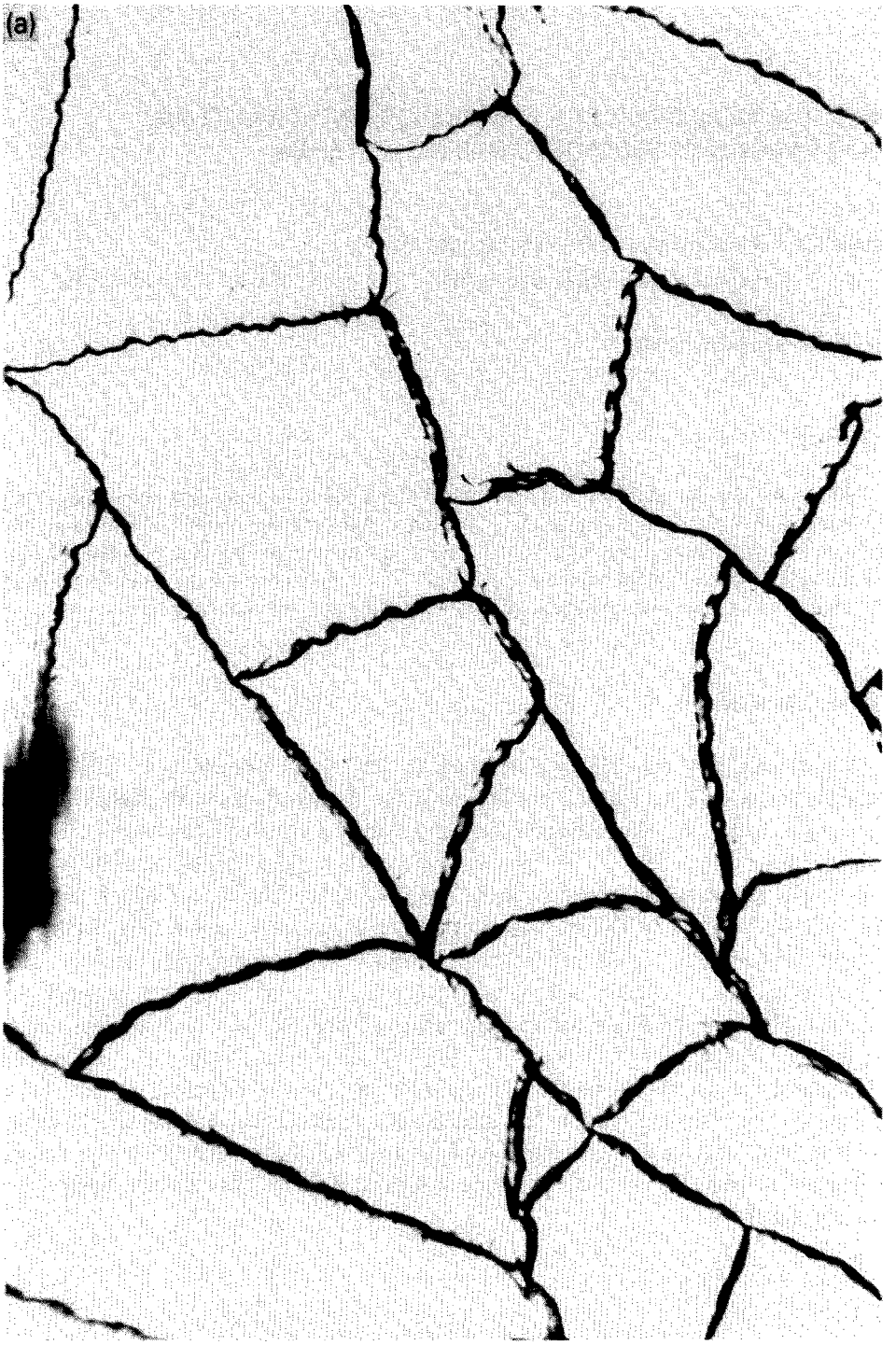

Fig. 1. Nucleation (a) and growth (b) of ice from the surfaces of cracks in sucrose: water glassy melts.


(b)

16-mm diameter, 170-μm thick coverslips and into a temperature controlled microscope stage (Linkam THM600). The temperature was lowered at either 5 or 10 °C min^{-1}, to about −130 °C and raised to ambient at 10 °C min^{-1}. Our interophometric microscope (ausJena 'Interphako', Zeiss Jena outside the U.S.A.) has a long working-distance condenser and objectives. The 'shearing method' was used for image contrast and for measuring path-length differences of the test material and small glass reference samples. Images were recorded on videotape or 35-mm film.

RESULTS

Samples of 65% sucrose solution cooled at 10 °C min^{-1} manifested no change in appearance between ambient temperature and −120 °C except in optical path length. During warming, small ice crystals sometimes appeared at temperatures below −40 °C, but did not grow rapidly until the temperature was raised to about −32 °C. They tended to be few in number, perhaps two or three per microscope field, and were usually associated with visible inclusions in the solution. When the temperature was lowered below −120 °C, to perhaps −148 °C, however, cracks suddenly appeared in the previously transparent glass. They were especially prominent if the sample was removed and plunged directly into liquid nitrogen. During warming, these cracks disappeared above the T_g, −79 °C, as the glass melted. At T_d, −52 °C, the cracks reappeared and as the temperature increased they broadened and darkened. It became apparent that the dark material was ice as spicules began to overgrow the transparent areas between the cracks (Fig. 1). Melting began at about −25 °C and the inhomogeneities in the solution, visible by interference contrast, slowly diffused to invisibility. When 5-μl samples were placed into DSC pans and cooled and warmed under similar conditions, there was no evidence for ice formation until the temperature had decreased below −90 °C. No ice formed in samples cooled to −140 °C in the DSC if the sample in the sample pan was covered with 2-methyl butane.

DISCUSSION

Ice formation during warming has long been recognized as the kiss of death for cryobiologically preserved cells and tissues and its prevention or amelioration has involved a major portion of our recent research effort. The traditional approach has been to warm rapidly. In cells which are tolerant of osmotic stress, such as human monocytes, moderate concentrations of cryoprotectant and warming at 80 °C min^{-1} suffice [2]. Unfortunately, in

more sensitive tissues, higher rates are required, and in organs as large as the human kidney, this requirement ranges from heroic to impracticable [3].

We have been assuming that homogeneous nuclei do form during cooling at, perhaps, $-70\,^{\circ}C$, but that they are unable to grow appreciably in solutions so close to their glass transitions. Their growth would resume when the specimens were warmed. The present experiments indicate that the ice nuclei form only at temperatures well below the glass transition of the 65% sucrose solution, and it is difficult to rationalize how even a hundred water molecules might reorganize to form a nucleus in what is for all practical purposes a dense and uniform solid.

The observations we have reported here support the alternative that fracture faces of cracks formed in the cooled glass not only provide free volume for heterogeneous nuclei to develop in, but a high energy surface for them to develop on. Even in the DSC, eliminating the air interface with 2-methylbutanol eliminated most nuclei. Specimen cracking has been recognized as an obstacle to cryopreservation since early experiments [4]. Our observations imply that the prevention of cracking is important not only for gross morphological preservation, but also for the suppression of ice growth during warming and the easing of conditions required for successful recovery of cryopreserved biological specimens.

ACKNOWLEDGEMENTS

We thank H.T. Meryman for his support and criticism, and Patrick Mehl and Allen Hirsh for valuable suggestions. Supported in part by NIH Grants BSRG 2 S07 RR05737 and GM 17959.

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