NaCl–H_2O SYSTEMS AT TEMPERATURES BELOW 273 K, STUDIED BY DIFFERENTIAL SCANNI
CALORIMETRY

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SUMMARY

   Aqueous solutions of NaCl are studied by differential scanning calorimetry
(DSC) during cooling, at scan rate 2 K/min, to 218 K (-55°C) and subsequent hea-
ting to 288 K (+15°C). Hysteresis effects are reflected in the thermograms. Two
exothermic transitions are observed during the cooling, and two endothermic
transitions during the heating. The first exotherm is associated with the ini-
tial separation of ice from a subcooled NaCl solution. An exothermic transition
at 237 K (-36°C) appears as a pseudo eutectic solidification of a supersaturated
NaCl solution. The phase transitions observed are discussed with reference to
dehydration of biological cells during cooling.

INTRODUCTION

   Causes of freezing injuries to biological systems are not established in de-
tail, but the consensus is that separation of ice plays an important role in
the survival of frozen organisms (1,2). NaCl is of common occurrence in
cellular liquids, and as a contribution to current discussions about freezing
injuries we try, in the present paper, to elucidate the behaviour of NaCl solu-
tions during slow cooling and subsequent heating.

   General experimental and theoretical studies of aqueous solutions at subzero
temperatures are summarized in a recent review article (3). The analysis by
MacKenzie (4) of nucleation and crystallization by cooling is reported in ref.
(3). Our experimental data on NaCl–H_2O solutions is compared with MacKenzie's
description.

MATERIALS AND METHODS

   NaCl, analytical grade, from FERAK, Berlin, and double glass distilled water
are used.

   Perkin Elmer DSC-7, equipped with Intercooler II, is used for the calorime-
tric measurements. 15-18 mg samples, in sealed aluminium pans, are placed in
the calorimeter, with an empty pan as the reference. The scan rate is 2 K/min.

The heat effect due to the initial freezing of ice (Peak (I) in Fig. 1) is so large that the calorimeter looses control, unless special precautions are taken (5,6). In our experiments pieces of teflon sheet (mass: 39 mg; diameter: 6.3 mm; thickness: 0.7 mm) are placed between the sample holders and the pans; with this installation the thermal resistance of the calorimeter is raised so much that control is maintained. Calibration of the calorimeter, with gallium (T = 303 K. ΔH = 80.1 J/g) and water (T = 273 K), is made under heating conditions with the teflon sheets installed. Temperatures measured in cooling experiments are corrected for thermal lag (6).

The enthalpy of a transition is estimated from the integrated heat flow over the temperature range of the transition. The DSC-7 standard program is used for the calculation. Corrections due to differences in heat capacity of a sample before and after a transition (6) are ignored.

EXPERIMENTAL RESULTS

Fig. 1 shows thermograms (heat flow vs. temperature) measured during cooling and subsequent heating of an aqueous solution of 5.0% NaCl. The thermograms shown are examples out of a series measured on solutions with various concentrations of NaCl. For NaCl concentrations below 23% (the eutectic concentra-
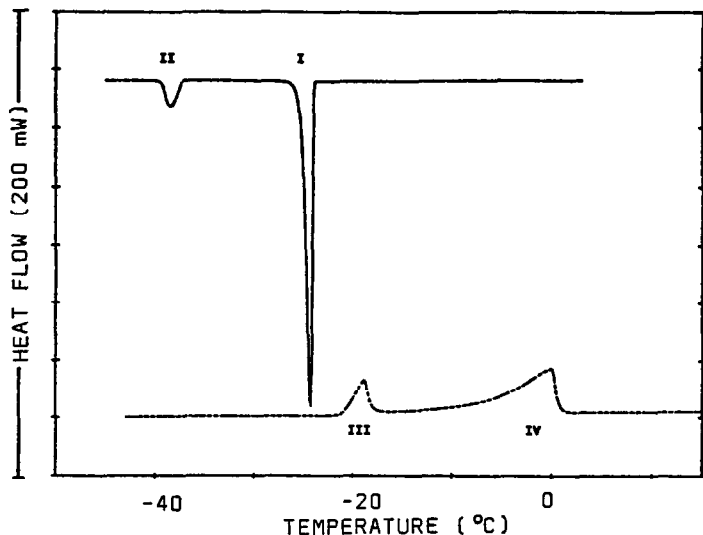

Fig. 1. DSC thermograms (heat flow vs. temperature) measured during cooling (———) and subsequent heating (—··—) of 5.0% NaCl in water. The scan rate was 2 K/min.

tion (7)) two peaks (exotherms) are observed during cooling to -55°C, and two peaks (endotherms) are observed during the subsequent heating to +15°C. In the following, the peaks and the corresponding temperatures and thermal transitions are called by a number, I-IV, according to their successive appearance in a cooling-heating cycle. The numbering is illustrated in Fig. 1.

TABLE 1

Onset temperature, T, and enthalpy change, ΔH, (measured per gram sample) of three transitions, I, II and III, observed during cooling and subsequent heating of aqueous NaCl solutions. w is the weight fraction of NaCl.
The transitions are indicated in Fig. 1, and further explained in the text.

W %	T(I) K	T(II) K	T(III) K	ΔH(I) J/g	ΔH(II) J/g	ΔH(III) J/g
0.24	259.7	236.0	252.0	-297.7	-0.9	1.6
0.50	259.0	239.0	252.0	-291.4	-2.7	4.0
0.90	258.8	237.1	252.1	-284.8	-4.9	8.6
2.00	253.8	237.1	252.0	-266.5	-11.6	20.1
3.03	257.2	237.1	252.2	-252.8	-17.8	31.1
3.97	252.7	237.5	252.1	-251.0	-24.6	41.4
5.03	250.6	237.2	252.2	-213.7	-27.5	
7.01	248.9	237.0	252.0	-210.1	-42.1	
8.90	244.7	238.2	252.1	-191.0	-54.0	
11.96	245.9	237.6	252.1	-161.9	-72.7	
15.16	238.1	236.2	252.0			
17.02	248.6	236.9	252.0			
19.08	241.7	236.6	252.2			
21.00	236.3	234.9	252.0			
21.89	237.0	235.5	252.0			
23.01	237.5	235.9	252.1			
w,eutec.						
23.95	229.1	228.4	252.2			
25.03	230.4	229.7	251.8			
25.49	234.3	233.6	251.9			
26.00	224.9	224.9	251.9			

The onset temperature and the enthalpy of the transitions I, II and III, of samples with various weight fractions of NaCl, w, are reported in Table 1.

T(I) is considered to be the temperature at which ice crystals appear in a NaCl solution, and ΔH(I) is the enthalpy of the initial separation of ice. Fig. 2 shows T(I) as a function of the weight fraction of NaCl.
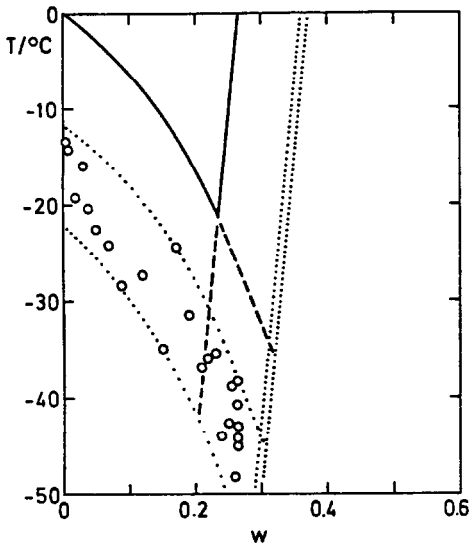
Fig. 2. Onset temperatures of peak I (initial separation of ice, see Fig. 1) observed during cooling of aqueous NaCl solutions at the rate 2 K/min. w is the weight fraction of NaCl.

The fully drawn curve shows the equilibrium phase diagram of NaCl-H$_2$O (7). The dotted curves enclose a temperature zone, within which onset of separation of ice is observed, and a zone associated with precipitation of NaCl from a supersaturated solution.
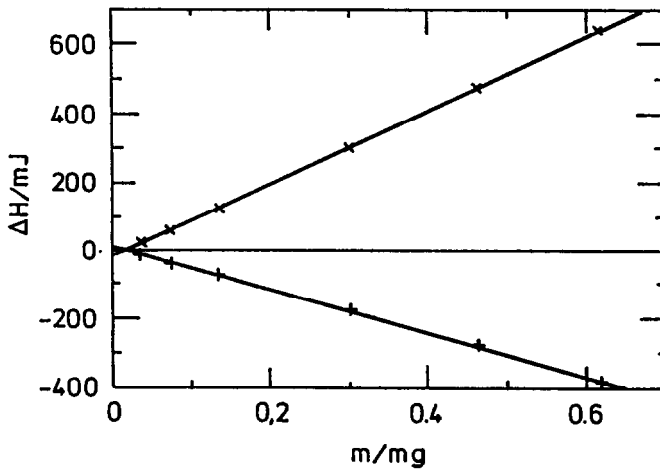

Fig. 3. The enthalpy, ΔH, measured of the "pseudo eutectic" solidification (II) of NaCl-water at 273 K (x), and of the eutectic melting (III) at 252 K (+). m is the amount of NaCl present in the samples studied.

During the cooling no transition is observed at the eutectic temperature of NaCl-H_2O (252 K) (7), but for NaCl concentrations below 23% (the eutectic concentration) an exotherm is observed at a temperature, T(II), close to 237 K (-36°C), independently of the concentration (see Fig. 1 and Table 1). The enthalpy of this transition, ΔH(II), and of the eutectic melting at 252 K, ΔH(III), is shown in Fig. 3 as function of the amount, m, of NaCl present in the samples studied.

In thermograms measured during heating peak(III) is followed by a broad endotherm (peak(IV) which reflects the gradual melting of ice in equilibrium with a more and more dilute NaCl solution.

DISCUSSION

The temperature, T(I), at which ice appears in NaCl solutions under the conditions of our cooling experiments is considerably lower than the equilibrium freezing temperature (7). Ice separates from a metastable (subcooled) solution, and the observed values of T(I) are scattered within a "freezing zone", such as it is illustrated in Fig. 2.

Subcooling, and scattering of freezing temperatures are phenomena in accordance with theories of separation of ice by heterogeneous nucleation, and with general experimental experiences from aqueous systems (3,4). The data in Table 1 illustrates the striking difference between the scattered (and concentration dependent) values of T(I) on one hand, and the nearly constant values of T(II) and T(III) on the other.

The amount of ice separated (per gram sample), at T(I), may tentatively be estimated as ΔH(I)/ΔH*, where ΔH* is the specific freezing enthalpy of pure water at the temperature T(I) (ΔH*(T) = [$-334-2.05$(T-273)]J/g (8)), and the heat of dilution of NaCl solutions (9) is ignored. The weight fraction, w', of NaCl in the remaining liquid phase, calculated as w' = w/(1-ΔH(I)/ΔH*), supports the view that equilibrium is established between ice and water (3,4). Further cooling presumably proceeds in accordance with the left hand branch of the equilibrium phase diagram, shown in Fig. 2.

Noteworthy is the observation that, during cooling, no exothermic transition is observed at the eutectic temperature (252 K, or -21°C). Below this temperature the liquid phase of a sample is supersaturated with NaCl. The transition (II) exhibits characteristics of eutectic phase transitions: T(II) is (nearly) independent of the NaCl concentration (Table 1), and ΔH(II) increases linearly with the amount of NaCl present (Fig. 3). Transition (II) thus appears as a pseudo-eutectic phase transition (solidification), which - just like the true eutectic melting at 252 K - includes the total amount of NaCl present in a sample, at some given concentration. As illustrated in Fig. 2 by the dashed ex-

58

tension of the left-hand branch of the equilibrium phase diagram, we suggest that solidification occurs when a narrow "zone of precipitation" of NaCl is reached. The total amount of NaCl remains in the liquid phase until the temperature T(II) = 237 K is reached.

The aim of the present investigation has been to contribute to the elucidation of conditions in partly frozen biological materials. During slow cooling of a biological system formation of ice in the extracellular liquids causes dehydration of the cells. The dehydration is driven by differences between the chemical potential of intra- and extracellular water. Our studies of aqueous NaCl solutions support the view that ice-water equilibrium is established in the extracellular liquids. In that case, the chemical potential of extracellular water is equal to the chemical potential of ice, and thus a function solely of the temperature. The amount of ice separated, and the concentration in the remaining cellular liquid depend on the nature and the amount of solute molecules, but the chemical potential of water, and thus the primary driving force of dehydration, depends only of the temperature.

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