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Analysis of supercooling activities of surfactants

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ABSTRACT

Supercooling-promoting activities (SCAs) of 25 kinds of surfactants including non-ionic, anionic, cationic and amphoteric types were examined in solutions (buffered Milli-Q water, BMQW) containing the ice nucleation bacterium (INB) *Erwinia ananas*, silver iodide (AgI) or BMQW alone, which unintentionally contained unidentified ice nucleators, by a droplet freezing assay. Most of the surfactants exhibited SCA in solutions containing AgI but not in solutions containing the INB *E. ananas* or BMQW alone. SCAs of many surfactants in solutions containing AgI were very high compared with those of previously reported supercooling-promoting substances. Cationic surfactants, hexadecyltrimethylammonium bromide (C16TAB) and hexadecyltrimethylammonium chloride (C16TAC), at concentrations of 0.01% (w/v) exhibited SCA of 11.8 °C, which is the highest SCA so far reported. These surfactants also showed high SCAs at very low concentrations in solutions containing AgI. C16TAB exhibited SCA of 5.7 °C at a concentration of 0.0005% (w/v).

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Introduction

Freezing of water is controlled by ice nucleation substances, which make freezing temperatures higher [21,22], anti-freeze substances including anti-freeze proteins and glycoproteins, which inhibit ice crystal growth after ice nucleation [2,4,5], and supercooling-promoting substances, which make freezing temperatures lower.

Although there have not been many studies on supercooling-promoting substances, recent studies have revealed the existence of various kinds of supercooling-promoting substances of biological origin, such as protein from bacteria [12], some anti-freeze proteins [3,25], some anti-freeze glycoproteins [19], polysaccharide from bacteria [29], several kinds of terpenoids [13], several kinds of phenylpropanoids [11], many kinds of flavonoid glycosides [8–10,14,15] and many kinds of tannin-related compounds [16,23]. Poly (glycerol) (PGL) [27], poly (vinyl alcohol) [28], poly (vinyl pyrrolidone) [6] and poly (ethylene glycol) [6] have also been identified as supercooling-promoting substances of non-biological origin.

The possibility of application of supercooling-promoting substances to inhibit recrystallization during warming for cryopreservation of biological organs by a vitrification procedure has long

been examined [27,28]. Recently, supercooling-promoting substances have been successfully applied to low temperature preservation of biological organs at subzero temperatures by supercooling [20] as well as to cryopreservation of biological materials by promoting vitrification with diluted vitrification solution [7] and by inhibiting recrystallization during warming of vitrification solution [1]. For further applications of supercooling-promoting substances in a variety of fields, especially in cryobiology, further discovery of novel supercooling-promoting substances may be useful. Since surfactants are amphipathic compounds as are many previously identified supercooling-promoting substances, the present study was carried out to investigate the supercooling-promoting activity (SCA) of surfactants that are commercially sold for use in foods, medical products, cosmetics and paints. The results showed that many of the surfactants analyzed had specific SCA.

Materials and methods

Twenty-five surfactants were examined in this study (Table 1). The surfactants included 12 non-ionic surfactants (Fig. 1), five anionic surfactants (Fig. 2), four cationic surfactants (Fig. 2) and four amphoteric surfactants (Fig. 3). The effects of these surfactants on freezing of water were analyzed by a droplet freezing assay as described previously [15,16]. Both control and experimental solutions were prepared as described previously [15,16]. Milli-Q water

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Table 1
Supercooling-promoting activity (SCA) and ice nucleation-enhancing activity (INA) of various surfactants in solutions with different ice nucleators.^{a,b}

Substances	Abbreviation	Conc. (mg/mL)	Molarity ^c (mM)	SCA (°C) or INA (–°C) ^d Ice nucleators			Origin
				<i>E. ananas</i> (2 mg/mL)	Silver iodide (0.5 mM)	BMQW alone (unidentified ice nucleators)	
<i>Non-ionic surfactants</i>							
Triton X-100	TX-100	0.1	–	0.1 ± 0.3	6.9 ± 0.6*	–0.9 ± 1.4	Nacalai Tesque (Japan)
Triton X-114	TX-114	0.1	–	–0.4 ± 0.4	7.1 ± 0.6*	–0.2 ± 0.7	Wako Pure Chemical Industries, Ltd. (Japan)
Nonidet P-40	NP40	0.1	–	0.0 ± 0.2	10.9 ± 2.4*	–1.5 ± 2.5	Nacalai Tesque (Japan)
Tween 20	TW20	0.1	–	–0.4 ± 0.6	6.7 ± 1.0*	–0.4 ± 1.0	Wako Pure Chemical Industries, Ltd. (Japan)
Tween 80	TW80	0.1	–	0.1 ± 0.2	5.0 ± 1.3*	0.4 ± 0.6	Wako Pure Chemical Industries, Ltd. (Japan)
Polyethylene glycol monostearate n(=):40	PEGMS40	0.1	–	0.1 ± 0.2	9.3 ± 1.7*	–1.2 ± 1.1	Tokyo Chemical Industry Co., Ltd. (Japan)
Emulgen 2025G	E2025G	0.1	–	0.1 ± 0.3	11.5 ± 2.3*	–0.4 ± 1.3	Kao Corp. (Japan)
Rheodol TW-P 120	TW120	0.1	–	–0.1 ± 0.2	6.6 ± 2.5*	–0.6 ± 1.7	Kao Corp. (Japan)
Rheodol SP-L 10	SPL-10	0.1	–	–0.1 ± 0.1	4.5 ± 0.5*	0.4 ± 0.7	Kao Corp. (Japan)
Aminon PK 02S	PK02S	0.1	–	0.1 ± 0.1	7.8 ± 2.7*	–0.6 ± 0.2	Kao Corp. (Japan)
Megaface F-477	F477	0.1	–	0.1 ± 0.2	9.0 ± 1.8*	–2.2 ± 2.2	DIC Corp. (Japan)
l-O-n-Octyl-β-D-glucopyranoside	OG	0.1	0.3	0.0 ± 0.1	2.2 ± 1.4	–0.1 ± 1.1	Wako Pure Chemical Industries, Ltd. (Japan)
<i>Anionic surfactants</i>							
Sodium oleate	SO	0.1	0.3	0.1 ± 0.1	2.8 ± 1.1	1.9 ± 1.2	Tokyo Chemical Industry Co., Ltd. (Japan)
Sodium cholate	SC	0.1	0.2	0.1 ± 0.2	4.3 ± 1.7*	2.2 ± 2.2	Tokyo Chemical Industry Co., Ltd. (Japan)
Sodium dimethyl 5-sulfoisophthalate	SDSIP	0.1	0.3	0.1 ± 0.2	0.2 ± 0.2	–0.1 ± 1.4	Tokyo Chemical Industry Co., Ltd. (Japan)
Dodecene-1 LAS	D1LAS	0.1	0.3	0.6 ± 0.3*	3.9 ± 1.1*	–0.9 ± 1.2	Tokyo Chemical Industry Co., Ltd. (Japan)
Sodium monododecyl phosphate	SMP	0.1	–	0.2 ± 0.2	2.8 ± 1.0	–1.2 ± 2.7	Tokyo Chemical Industry Co., Ltd. (Japan)
<i>Cationic surfactants</i>							
Hexyltrimethylammonium bromide	C6TAB	0.1	0.4	0.0 ± 0.1	6.3 ± 0.6*	–0.1 ± 1.7	Tokyo Chemical Industry Co., Ltd. (Japan)
Hexadecyltrimethylammonium bromide	C16TAB	0.1	0.3	0.6 ± 0.2*	11.8 ± 1.9*	0.3 ± 1.6	Tokyo Chemical Industry Co., Ltd. (Japan)
Hexadecyltrimethylammonium chloride	C16TAC	0.1	0.3	0.5 ± 0.3*	11.8 ± 0.8*	0.3 ± 1.1	Tokyo Chemical Industry Co., Ltd. (Japan)
Acetamin 86	A86	0.1	–	0.0 ± 0.1	2.7 ± 0.5	–3.3 ± 2.0*	Kao Corp. (Japan)
<i>Amphoteric surfactants</i>							
Amphitol 20N	20N	0.1	–	0.0 ± 0.2	7.3 ± 0.7*	–0.3 ± 2.1	Kao Corp. (Japan)
Amphitol 20AB	20AB	0.1	–	–0.1 ± 0.1	4.1 ± 0.7*	0.6 ± 1.8	Kao Corp. (Japan)
Myristyl sulfobetaine	MS	0.1	0.3	–0.1 ± 0.2	10.3 ± 0.5*	–1.3 ± 1.5	Tokyo Chemical Industry Co., Ltd. (Japan)
CHAPS	CHAPS	0.1	0.2	0.0 ± 0.1	7.1 ± 2.3*	–0.2 ± 2.0	Nacalai Tesque (Japan)

^a SCA (indicated by °C) and INA (indicated by –°C) were obtained from the difference in FT₅₀ between control and experimental solutions as analyzed by a droplet freezing assay.

^b Presence of significant difference in SCA or INA was determined by Dunnett's test and is indicated by an asterisk (**p* < 0.05).

^c Molarity (mM) was shown only in surfactants that chemical structures were published.

^d Each value is the mean ± SD (*n* = 5). *n* = a separate droplet freezing assay with more than 20 droplets.

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