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Analysis of supercooling activity of tannin-related polyphenols ☆

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ABSTRACT

Based on the discovery of novel supercooling-promoting hydrolyzable gallotannins from deep supercooling xylem parenchyma cells (XPCs) in Katsura tree (see Wang et al. (2012) [38]), supercooling capability of a wide variety of tannin-related polyphenols (TRPs) was examined in order to find more effective supercooling-promoting substances for their applications. The TRPs examined were single compounds including six kinds of hydrolyzable tannins, 11 kinds of catechin derivatives, two kinds of structural analogs of catechin and six kinds of phenolcarboxylic acid derivatives, 11 kinds of polyphenol mixtures and five kinds of crude plant tannin extracts. The effects of these TRPs on freezing were examined by droplet freezing assays using various solutions containing different kinds of identified ice nucleators such as the ice nucleation bacterium (INB) *Erwinia ananas*, the INB *Xanthomonas campestris*, silver iodide and phloroglucinol as well as a solution containing only unintentionally included unidentified airborne ice nucleators. Among the 41 kinds of TRPs examined, all of the hydrolyzable tannins, catechin derivatives, polyphenol mixtures and crude plant tannin extracts as well as a few structural analogs of catechin and phenolcarboxylic acid derivatives exhibited supercooling-promoting activity (SCA) with significant differences ($p > 0.05$) from at least one of the solutions containing different kinds of ice nucleators. It should be noted that there were no TRPs exhibiting INA in all solutions containing identified ice nucleators, whereas there were many TRPs exhibiting INA with significant differences in solutions containing unidentified ice nucleators alone. An emulsion freezing assay confirmed that these TRPs did not essentially affect homogeneous ice nucleation temperatures. It is thought that not only SCA but also INA in the TRPs are produced by interactions with heterogeneous ice nucleators, not by direct interaction with water molecules. In the present study, several TRPs that might be useful for applications due to their high SCA in many solutions were identified.

Introduction

Substances that affect freezing of water include heterogeneous ice nucleation substances, which raise freezing temperatures by enhancing ice nucleation [35,36], anti-freeze substances such as anti-freeze proteins and glycoproteins, which inhibit ice crystal growth after ice nucleation [3,10,11], and anti-ice nucleation substances, which reduce freezing temperatures by promoting supercooling due to inhibition of ice nucleation [15–17,23,24,38].

Among these various types of freeze-controlling substances, there have been few studies on anti-ice nucleation (supercooling-promoting) substances, and until recently only 16 kinds of single compounds such as proteins [5,12,19,39] including antifreeze proteins [5,12] and antifreeze glycoproteins [7,12,28,39], polysaccharides [43], terpenoids [20], phenylpropanoids [18], polyvinyl alcohol (PVA) [12,41,42] and polyglycerol [41] in addition to crude extracts from a few kinds of plant seeds [2] had been identified as supercooling-promoting substances (for a list, see Kasuga et al. [15,16]).

Recent studies on the mechanisms of winter adaptation of xylem parenchyma cells (XPCs) in trees by deep supercooling, however, have disclosed the presence of supercooling-promoting activity (SCA) in crude xylem extracts from several hardwood and softwood trees that contained deep supercooling XPCs [15,26]. Furthermore, in studies on the mechanisms of deep supercooling of XPCs, four kinds of flavonol glycosides were identified as novel supercooling-promoting substances in deep supercooling XPCs of Katsura tree [16,23]. Based on similarity in chemical structures with supercooling-promoting flavonol glycosides in XPCs, a further 26 flavonoid glycosides have been revealed to have SCA in many solutions containing different kinds of ice nucleators [17,24].

A more recent study on the mechanisms of deep supercooling in XPCs of trees has further revealed four kinds of novel supercooling-promoting hydrolyzable gallotannins, including 2,2′,5-tri-*O*-galloyl-α,β-D-hamamelose (tri-GHam), 1,2,6-tri-*O*-galloyl-β-D-glucopyranose (tri-GGlc), 1,2,3,6-tetra-*O*-galloyl-β-D-glucopyranose

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(tet-GGlc) and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (pent-GGlc), in deep supercooling XPCs of Katsura tree [38].

Further discovery of supercooling-promoting substances that have high SCA toward diverse kinds of solutions may be useful for a wide variety of applications that require supercooling of water to lower temperatures, including supercooling preservation [33] as well as successful cryopreservation [14] in biological materials. In the present study, based on the discovery of novel supercooling-promoting hydrolyzable gallotannins from deep supercooling XPCs [38], supercooling activity of a wide variety of tannin-related polyphenols (TRPs) was examined in solutions containing different kinds of ice nucleators.

Materials and methods

Tannin-related polyphenols (TRPs)

The TRPs used in this study are shown in Tables 1 and 2. The TRPs were grouped into tannin-related single compounds including hydrolyzable tannins, catechin derivatives, structural analogs

Table 1
Supercooling-promoting activity (SCA) and ice nucleation-enhancing activity (INA) of tannin-related single compounds in solutions containing different ice nucleators.[a,b]m

Substances	Abbreviation	Conc. (mg/mL)	SCA (°C) or INA (−°C)[c] Ice nucleators					Origin
			E. ananas (2 mg/mL)	*X. campestris* (2 mg/mL)	Silver iodide (0.5 mM)	Phloroglucinol (120 mM)	None (air-borne ice nucleators)	
Hydrolyzable tannins								
1,2,6-tri-*O*-galloyl-ß-D-glucopyranose	tri-GGlc	1.0	1.5 ± 0.4*	0.6 ± 0.5	3.3 ± 0.9*	0.2 ± 0.3	5.1 ± 3.0*	Purified compound[d]
1,2,3,6-tetra-*O*-galloyl-ß-D-glucopyranose	tet-GGlc	1.0	2.9 ± 0.5*	3.0 ± 0.6*	3.7 ± 0.8*	1.0 ± 0.7	4.3 ± 2.5	Purified compound[d]
1,2,3,4,6-penta-*O*-galloyl-α and ß-D-glucopyranose	pent-GGlc	1.0	3.0 ± 0.2*	–[i]	2.2 ± 1.5	6.8 ± 3.7*	1.3 ± 2.8	Synthetic compound[e]
1-methyl-2,3,4,6-tetra-*O*-galloyl-α-D-glucopyranose	M-tet-GGlc	1.0	3.4 ± 0.2*	–[i]	2.6 ± 1.2*	5.1 ± 2.5*	1.1 ± 1.3	Synthetic compound[e]
2,2′,5-tri-*O*-galloyl-α,ß-D-hamamelose	tri-GHam	1.0	2.3 ± 0.7*	1.2 ± 0.3*	4.5 ± 0.6*	0.2 ± 0.4	4.3 ± 2.8	Purified compound[d]
Myo-inositol pergallate	MIPG	1.0	3.0 ± 0.2*	–[i]	3.0 ± 0.9*	0.6 ± 1.1	−0.4 ± 4.0	Synthetic compound[f]
Catechin derivatives								
(+)-Catechin	C	1.0	0.9 ± 0.4*	0.3 ± 0.4	4.2 ± 0.2*	0.4 ± 0.7	−9.0 ± 0.8*	Funakoshi Corp. (Japan)
(−)-Epicatechin	EC	1.0	0.7 ± 0.3	0.2 ± 0.4	4.7 ± 0.7*	0.2 ± 0.6	−9.8 ± 1.0*	Funakoshi Corp. (Japan)
(−)-Epigallocatechin	EgaC	1.0	0.8 ± 0.4*	0.1 ± 0.5	4.5 ± 0.3*	1.0 ± 0.4	−7.6 ± 1.1*	Funakoshi Corp. (Japan)
(−)-Epicatechin gallate	ECG	1.0	3.3 ± 0.3*	0.9 ± 0.3*	4.0 ± 1.7*	1.7 ± 0.5	2.3 ± 2.0	Wako Pure Chemical Industries, Ltd. (Japan)
(−)-Epigallocatechin gallate	EgaCG	1.0	3.3 ± 0.3*	0.9 ± 0.4*	3.6 ± 1.6*	0.5 ± 0.8	2.3 ± 2.6	Wako Pure Chemical Industries, Ltd. (Japan)
(−)-Gallocatechin gallate	gaCG	1.0	3.2 ± 0.2*	2.3 ± 0.5*	3.3 ± 1.3*	−0.2 ± 0.4	2.3 ± 2.3	Funakoshi Corp. (Japan)
(+)-catechin gallate	CG	1.0	0.4 ± 0.1	–[i]	5.8 ± 0.9*	–[i]	0.7 ± 5.8	Synthetic compound[g]
Procyanidin A2	PCA2	1.0	0.8 ± 0.1*	–[i]	4.1 ± 2.1*	–[i]	1.5 ± 1.3	Extrasynthese (France)
Procyanidin B1	PCB1	1.0	1.2 ± 0.2*	–[i]	2.5 ± 0.8*	–[i]	−4.6 ± 3.8	Extrasynthese (France)
Theaflavin	TF	1.0	1.9 ± 0.3*	–[i]	4.5 ± 0.7*	–[i]	−11.0 ± 2.1*	Wako Pure Chemical Industries, Ltd. (Japan)
Theaflavin-3,3′-digallate	TF-diG	1.0	2.6 ± 0.2*	–[i]	2.9 ± 1.1*	–[i]	−5.7 ± 4.1	Wako Pure Chemical Industries, Ltd. (Japan)
Structural analogs of catechin								
Quercetin 3-gallate	Q3G	1.0	−0.2 ± 0.8	–[i]	2.1 ± 1.7	–[ii]	−0.5 ± 5.3	Synthetic compound[h]
Ampelopsin 3-gallate	Amp3G	1.0	0.1 ± 0.3	–[i]	4.6 ± 0.6*	–[i]	−1.4 ± 5.9	Synthetic compound[g]
Phenolcarboxylic acid derivatives								
Gallic acid	GA	1.0	0.2 ± 0.3	–[ii]	1.6 ± 0.9	–[ii]	−3.0 ± 2.0	Tokyo Chemical Industry Co., Ltd. (Japan)
Methyl gallate	MG	1.0	−0.5 ± 0.4	–[i]	3.3 ± 1.2*	–[i]	−2.7 ± 1.7	Tokyo Chemical Industry Co., Ltd. (Japan)
Ellagic acid	EA	1.0	−0.3 ± 0.5	–[i]	0.5 ± 0.4	–[i]	−5.0 ± 1.8	Tokyo Chemical Industry Co., Ltd. (Japan)
Caffeic acid	CA	1.0	0.1 ± 0.1	–[i]	2.1 ± 0.7	–[i]	−6.1 ± 3.6*	Tokyo Chemical Industry Co., Ltd. (Japan)
Chlorogenic acid	ChlA	1.0	0.0 ± 0.2	–[i]	0.8 ± 0.6	–[i]	−5.4 ± 2.8	Tokyo Chemical Industry Co., Ltd. (Japan)
Rosmarinic acid	RA	1.0	0.7 ± 0.2	–[i]	3.0 ± 0.3*	–[i]	−10.7 ± 2.0*	Wako Pure Chemical Industries, Ltd. (Japan)

[a] SCA and INA were obtained from the difference in FT_{50} 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] Each value is the mean ± SD ($n = 5$). n = a separate droplet freezing assay with more than 20 droplets.

[d] Purified from Katsura tree by the authors [38].

[e] Synthetic compounds produced by the authors using the method of Ren et al. [29]. Chemical shifts of synthetic compounds correspond to data published by Ren et al. [29].

[f] Synthetic compounds produced by the authors using the method of Kawamoto et al. [21]. Chemical shifts: ESI-MS (negative) m/z $[M-H]^-$ calcd. for $C_{48}H_{35}O_{30}$, 1091.12186; found, 1091.12271. ^1H NMR (270 MHz, CD_3OD): δ 5.79 (2H, dd, $J = 2.8, 10.2$ Hz), 5.94 (1H, t, $J = 10.2$ Hz), 6.16 (1H, t, $J = 2.8$ Hz), 6.18 (2H, t, $J = 10.2$ Hz), 6.89 (2Hx2, s), 6.91 (2H, s), 6.92 (2Hx2, s), 7.19 (2H, s).

[g] Synthetic compounds produced by the authors. Chemical shifts were identical to those reported by Nonaka et al. [27] and Saito et al. [32].

[h] Synthetic compounds produced by the authors using the method of Farkas et al. [6]. Chemical shifts: ESI-MS (positive) m/z $[M + Na]^+$ calcd. for $C_{22}H_{14}O_{11}Na$, 477.043283; found, 477.04302. ^1H NMR (270 MHz, CD_3OD): δ 6.24 (1H, d, $J = 2.3$ Hz), 6.48 (1H, d, $J = 2.3$ Hz), 6.82 (1H, d, $J = 8.5$ Hz), 7.21 (3H, s), 7.33 (1H, dd, $J = 2.2, 8.5$ Hz), 7.37 (1H, d, $J = 2.2$ Hz).

[i] Not examined.

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