Cryobiology 69 (2014) 223–228

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Anti-ice nucleating activity of polyphenol compounds against silver iodide ☆

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ARTICLE INFO

Article history:
Received 7 April 2014
Accepted 21 July 2014
Available online 30 July 2014

Keywords:
Ice nucleation
Supercooling
Polyphenol
Antifreeze protein
Silver iodide
Emulsion

ABSTRACT

Freeze-avoiding organisms survive sub-zero temperatures without freezing in several ways, such as removal of ice nucleating agents (INAs), production of polyols, and dehydration. Another way is production of anti-ice nucleating agents (anti-INAs), such as has been reported for several antifreeze proteins (AFPs) and polyphenols, that inhibit ice nucleation by inactivating INAs. In this study, the anti-ice nucleating activity of five polyphenol compounds, including flavonoid and tannin compounds of both biological and synthetic origin, against silver iodide (AgI) was examined by measuring the ice nucleation temperature in emulsified polyphenol solutions containing AgI particles. The emulsified solutions eliminated the influence of contamination by unidentified INAs, thus enabling examination of the anti-ice nucleating activity of the polyphenols against AgI alone. Results showed that all five polyphenol compounds used here have anti-ice nucleating activities that are unique compared with other known anti-INAs, such as fish AFPs (type I and III) and synthetic polymers (poly(vinyl alcohol), poly(vinylpyrrolidone) and poly(ethylene glycol)). All five polyphenols completely inactivated the ice nucleating activity of AgI even at relatively low temperatures, and the first ice nucleation event was observed at temperatures between −14.1 and −19.4 °C, compared with between −8.6 and −11.8 °C for the fish AFPs and three synthetic polymers. These anti-ice nucleating activities of the polyphenols at such low temperatures are promising properties for practical applications where freezing should be prevented.

Introduction

Organisms living in cold environments have adapted themselves to subzero temperatures mainly by two different strategies, namely, freeze tolerance or freeze avoidance [2,3,5,11,38]. In freeze tolerance, to prevent intracellular ice formation, extracellular ice formation is not prevented but rather enhanced. In freeze avoidance, however, not only intracellular but also extracellular ice formation is prevented so that the entire tissue completely avoids freezing and thus stays supercooled. In either case, intracellular ice formation must be prevented because it is lethal to organisms.

In nature, intracellular ice formation can be inhibited in several ways, such as removal of ice nucleating agents (INAs), production of polyols, and dehydration [3,5,28,29,38]. Another way is production of anti-ice nucleating agents (anti-INAs) that inactivate INAs to stabilize the supercooled state of water [2,38]. Typical anti-INAs are several antifreeze proteins (AFPs), which are found in plants [2,12], invertebrates [3,24,25,28,32], and ectothermic vertebrates [8,26]. AFPs are also known to have other functions against ice growth, such as thermal hysteresis activity [6,27] and ice recrystallization inhibition [17,18,35], which play important roles mainly in freeze-tolerant organisms [2,38]. Many types of polyphenol compounds produced in plants, especially flavonoids and tannins isolated from xylem parenchyma cells, are also recognized as anti-INAs [16,19–21,30].

Apart from biological anti-INAs, certain synthetic polymers, such as polyglycerol (PGL) [36], poly(vinyl alcohol) (PVA) [13,15,36,37], poly(vinylpyrrolidone) (PVP) [15], and poly(ethylene glycol) (PEG) [15], various synthetic polyphenols [20,21], and several quaternary ammonium salts [31] also act as anti-INAs. Technical applications of these polymers and polyphenols as anti-INAs are promising due to their cost-effectiveness compared with biological anti-INAs.

There have been two major hypotheses to explain the function of anti-INAs; one is that anti-INA molecules inactivate the ice nucleating activity of INAs by masking specific ice nucleating sites

☆ *Statement of funding:* This study was supported by a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science (JSPS) (21360102 to T.I.).

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http://dx.doi.org/10.1016/j.cryobiol.2014.07.009
0011-2240/

on INA surfaces [13,15,21,26,32,33,36], and the other is that anti-INA molecules inhibit ice nucleation by directly affecting ice-like water clusters or ice embryos [13,32,33,36]. In this study, we considered the former hypothesis more probable based on our previous studies, which showed that AFP type III has no influence on ice nucleation in the absence of effective INAs [14], whereas it can effectively inhibit ice nucleation in the presence of effective INAs [15].

According to this hypothesis in which anti-INA molecules mask ice nucleating sites on INA surfaces, the effectiveness of a specific anti-INA would depend on the type of INA. Such dependencies have been proved by using a droplet freezing assay in which ice nucleation is examined by using numerous droplets with diameters on the order of millimeters, for various combinations of anti-INAs and INAs [13,19–21,30,36]. In these experiments, however, completely eliminating the influence of unidentified INAs contaminating the water was difficult, due to the large volume of droplets (1 μL [36], 2 μL [19–21,30], 100 μL [13]). To investigate the interaction of a specific combination of anti-INA and INA in detail, it is desirable to remove such unidentified INAs because several anti-INAs were reported to interact with unidentified air-borne INAs and thus either inactivate or enhance the ice nucleating activities of unidentified INAs [13,21,33].

The influence of such unidentified INAs on ice nucleation temperature can be eliminated by using emulsified droplets a few to a few tens of microns in diameter [8,14,34], which is at least four orders of magnitude smaller in volume than those used in the droplet freezing assay. Inactivation of ice nucleating activity of silver iodide (AgI) when using fish AFPs or several synthetic polymers as anti-INAs was previously examined by using this emulsion freezing assay, while successfully eliminating the influence of unidentified INAs other than AgI [15].

In this study, the anti-ice nucleating activities of five polyphenol compounds (Table 1), including flavonoid and tannin compounds, against AgI were examined by using the emulsion freezing assay, and were then compared with previously reported results for five other known anti-INAs, namely, two fish AFPs and three synthetic polymers [15]. The results showed that each polyphenol compound has a unique anti-ice nucleating activity, and that compared with the fish AFPs and synthetic polymers, each polyphenol completely inactivated the ice nucleating activity of AgI even at relatively low temperatures.

Materials and methods

Materials

Table 1 summarizes the five polyphenol compounds used as anti-INAs in this study. As previously revealed by the droplet freezing assay, these five polyphenol compounds inactivate ice nucleating activity of AgI [20,21]. Q3(Glc)n is a single-component polyphenol, α-oligoglucosyl quercetin 3-*O*-β-D-glucopyranoside (Fig. 1A), which is classified as a flavonol glycoside [1,20]. According to the information provided by the supplier (San-Ei Gen F.F.I., Japan), the average molecular weight of Q3(Glc)n used in this study was about 800. The other four are polyphenol mixtures. SEgaCG is composed of several tea catechins, containing 95% (−)-epigallocatechin gallate (EgaCG) (Fig. 1B) [21]. TC is also composed of multiple types of tea catechins (Fig. 1B), although its accurate composition is unknown [21]. TA is composed of hydrolyzable gallotannins, containing mainly 1,2,3,4,6-pentagalloyl-β-D-glucopyranose (pent-GGlc) (Fig. 1C) [21]. OLG is mainly composed of proanthocyanidin oligomers and catechin type monomers. Fig. 1D shows a typical structure of proanthocyanidin oligomers [9,10,21]. According to the information provided by the supplier (Amino Up Chemical, Japan), the molecular weight of OLG used in this study was widely distributed between approximately 290 and 4000, with two peaks around 1000 and

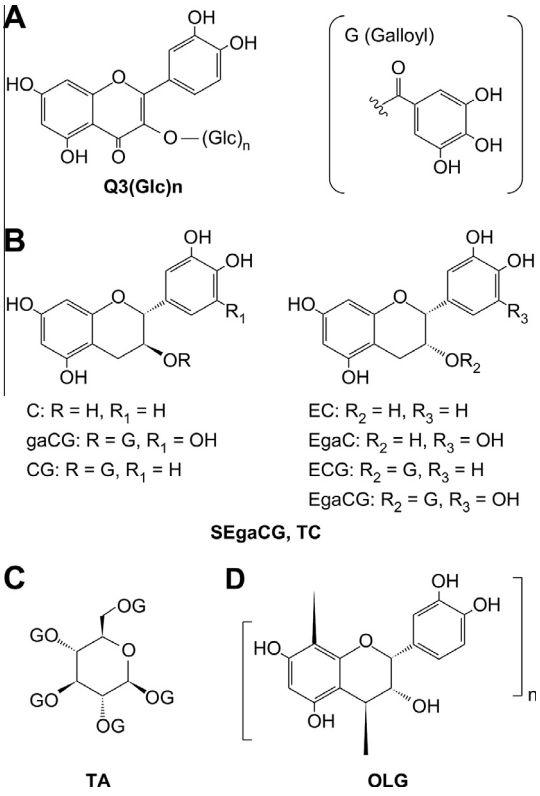

Fig. 1. Structural formulas of polyphenols used in this study.

Table 1
Polyphenol compounds used in this study.

Substance	Abbreviation	Classification	Main constituent	Origin
Sanmelin	Q3(Glc)n	Flavonoid (flavonol glycoside)	α-Oligoglucosyl quercetin 3-*O*-β-D-glucopyranoside	San-Ei Gen F.F.I. (Japan)
Sunphenon EGCg	SEgaCG	Flavonoid (flavanol)	(−)-Epigallocatechin gallate (EgaCG) (~95%), (+)-catechin (C), (−)-gallocatechin gallate (gaCG), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG)	Taiyo Kagaku (Japan)
Tea catechin	TC	Flavonoid (flavanol)	Tea catechin (>40%): C, gaCG, (+)-catechin gallate (CG), EC, (−)-epigallocatechin (EgaC), ECG, EgaCG, etc.	Genryoya.Com. (Japan)
Tannic acid	TA	Tannin (hydrolyzable tannin)	1,2,3,4,6-Pentagalloyl-β-D-glucopyranose (pent-GGlc)	Wako Pure Chemical Industries (Japan)
Oligonol	OLG	Tannin (condensed tannin)	Proanthocyanidin oligomers such as EC, procyanidin A2 (PCA2) and Ec-EgaCG, and catechin type monomers (~40%)	Amino Up Chemical (Japan)