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Propolis-induced exclusion of colloids: Possible new mechanism of biological action

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ABSTRACT

Propolis is a natural product originating from life activity of honeybees. It exhibits wide range of biological properties applicable in medicine, the food industry, and cosmetics. Chemically, propolis is a complex and variable mixture with more than 300 identified biologically active components. Propolis’s many health-promoting effects are attributed to different biochemical mechanisms, mediated by often-concerted actions of some of its many constituents. Propolis is considered safe and biocompatible. Yet due to its intrinsic complexity, standardization of propolis preparations for medical use as well as prediction of e.g. pathogen-specific interactions becomes a non-trivial task. In this work we demonstrate a new physical mechanism of propolis action, largely independent of specific nuances of propolis chemistry, which may underlie some of its biological actions. We show that propolis-bearing surfaces generate an extensive exclusion zone (EZ) water layer. EZ is an interfacial region of water capable of excluding solutes ranging from ions to microorganisms. Propolis-generated EZ may constitute an effective barrier, physically disabling the approach of various pathogens to the propolis-functionalized surfaces. We suggest possible implications of this new mechanism for propolis-based prevention of respiratory infections.

1. Introduction

Propolis (bee glue) is produced by honeybees from resins collected from different parts of plants and their exudates, to which bees add their salivary enzymes and wax. Propolis is used by bees in construction of hives e.g. for sealing, lining, water impermeabilization, or protection from microbial and fungal growth. In its raw form, propolis is composed of resins (50–60%), wax (30–40%), essential oils (5–10%), pollen (~5%) and other substances such as vitamins and minerals [1,2]. Since ancient times it has been used in medicine, cosmetics, and food preservation because of its broad spectrum of biological activities [3,4] such as antibacterial [5–7], antifungal [7,8], antiviral [2,7], antioxidant [3,4,9,10], anti-inflammatory [11], antitumor [12], hepatoprotective [13], neuroprotective [14], cardioprotective [15], immunomodulatory [16] and others. All of those many actions of propolis are explained by variety of biochemical pathways due to plethora of propolis’ active compounds including flavonoids, phenolic acids, terpenes, aromatic acids and others [3,4]. Despite significant variations in propolis composition, depending on bee species and propolis origin, different propolis samples usually exhibit similar spectrum of biological actions [17]. Yet, the exact chemicals underlying any given propolis effect and defined biochemical mechanisms are not easy to identify [18]. In this report, we unravel a new physical mechanism that may contribute to manifestation of at least some of the biological activities of propolis.

Many experiments have demonstrated that next to various surfaces possessing hydrophilic functional groups, there is a layer of water that excludes solutes ranging from ions, organic molecules and proteins to polystyrene microspheres and bacterial cells [19–30]. Due to those properties, this layer of water has been given a generic name of “exclusion zone” (EZ). Relatively solute/colloid-free water can be collected next to EZ-promoting surfaces, embedded in solution containing various concentration of dissolved species (e.g. salts of seawater) or biotic and abiotic colloidal particles [30–33]. EZ can be generated by variety of surfaces of artificial, e.g. Nafion [19,22,26,27] and natural origin such as gels (agarose, agar, collagen, starch, gelatin) [21,23], health-promoting fats (ghee, coconut oil, lard) [24] or biological tissues (mussels or cornea) [22,24].

Recently, it has been demonstrated that EZ phenomena could be employed as a first line of defense against attachment of pathogenic microorganisms, as presence of EZ hampers the approach of any solutes or particles, including bacteria, to the EZ-generating surface [25]. Our experiments show that extensive EZ is formed also by propolis components. Although connection between EZ and antibacterial activity of propolis has not yet been made, propolis-functionalized surfaces, like...
textiles or wound dressings exhibit superior antibacterial properties [35,36].

2. Materials and methods

Natural propolis samples came from two geographically distant sources, Poland (Europe) and Washington State (US). Propolis from Poland was collected three years earlier and its physical appearance was dark brown, hard and brittle. Propolis from Washington State was freshly collected and appeared lighter in color and much softer than propolis from Europe.

0.45 g of each propolis sample was immersed in 3 ml of 70% ethanol at room temperature to prepare ethanolic extract of propolis (EEP). Experiments were performed with EEP after each of various times of extraction: 30 min, 24 h and 72 h. Additionally, EEP extract (72 h) was diluted with deionized water to contain 1.4% of ethanol in the final preparation, which constituted an emulsion of EEP in water.

Glass capillaries (internal diameter 1.12 mm, World Precision Instruments, Inc.), cut to the length of 1.5 cm were placed in a propolis preparation (EEP or its emulsion) for a minimum time of 30 min and maximum time of 24 h. Neither the time of extraction process nor time of capillary soaking had any statistically significant effect on experimental outcomes. After soaking in EEP, glass capillaries were air-dried at 30 °C for complete solvent removal, or otherwise used wet, immediately after removal from EEP, with excess solvent gently wiped with paper tissue (Kimwipes by Kimtech). Propolis-coated glass capillaries were then placed in aqueous solution containing a suspension of microspheres (Polybed Polystyrene 1 μm from Polyscience, cat no. 07310–15) and examined under optical microscopy for their ability to form zones void of microspheres. Additional information can be found in the Supplementary Material.

3. Results and discussion

We used ethanolic extracts of propolis (EEP), which is the most common way of making propolis preparations. EEP is known for its highest content of biologically active propolis components, among which flavonoids comprise the main part [37]. Due to some limitations in medical use of strong ethanolic solution (70% alcohol in our case), we also prepared emulsions of EEP in water (1.4% alcohol). EEP deposited on a glass surfaces, either from original ethanolic solution or its emulsion, very consistently induced formation of extensive zone of particle-free water (Supplementary Material, Fig. S1 and S4). This exclusion zone (EZ) development started immediately after immersion of propolis-coated surface into microsphere-containing indicator solution (Fig. 1).

For surfaces coated with wet propolis extract, EZ fully developed within several minutes. For air-dried preparations, EZ progressed to its maximum size after about 40 min (Supplementary Material, Fig. S2 and S3). Therefore, EZ formation appears to unveil concomitantly with the hydration process. EZ was stable for approximately two hours and then progressively diminished. Shrinking with time is in agreement with some other observations and non-equilibrium nature of the EZ phenomena [35]. The exact mechanism of EZ-generation is still debated and is out of the scope of this communication [22,26,27]. Nevertheless, the recognized prerequisite for EZ formation is the presence of hydrophilic functional groups. Propolis is predominantly lipophilic in nature, but its constituents possess an abundance of hydrophilic -OH and -COOH functional groups [37], which may underlie the particle-exclusion capacity of the interfacial water next to propolis. The presence of those hydrophilic groups in all propolis samples, regardless of the source and variation of chemical composition, should assure the generic presence of EZ in the water next to propolis surfaces. Indeed, the EZ-promoting capacity of propolis observed in our experiments was largely independent of the propolis source and storage time. Three-year old propolis from Poland and freshly collected propolis from Washington State yielded comparable results (Fig. 1). Yet, their composition must have differed significantly as can be inferred not only from the sources’ spatial distance but also from physical appearance of raw materials and their ethanolic extracts (Fig. 2). Fresh propolis being much softer, lighter in color and leaving more residue after extraction implies larger amount of beeswax present in the raw sample.

Propolis layers deposited on the glass surface were invisible to the naked eye. Coated surfaces also appeared smooth under the microscope at the micrometer level (Fig. 1). Together with similar effect of propolis preparations of different concentration (EEP vs its emulsion) and comparable EZ-promoting capacity for different extraction times these observations indicate that only trace amounts of propolis components are sufficient to induce EZ phenomena.

4. Conclusions

The particle-repellent potential of propolis-functionalized surfaces results from the ability of propolis to induce a layer of EZ water. This ability in turn originates from the presence of hydrophilic functional groups abundant in all propolis samples, regardless of specific variations in chemical composition. Furthermore, due to the physical nature of the exclusion mechanism, this action of propolis should be very generic, rather than particle (or pathogen) specific. Indeed, EZ water has been recognized to exclude a variety of solutes starting from simple salt ions to microparticles and microorganisms [19–29]. Hence, colloidal particles in sizes ranging from viruses to bacteria should be effectively rejected. Therefore, some of the antimicrobial, antifungal or antiviral properties of propolis may, at least partially, result from physical blocking of the pathogens’ access to the potential site of infection.

In line with these considerations, the possible mechanism underlying antiviral activity of propolis was indeed suggested to involve inhibition of viral entry [3]. The latter notion is especially interesting from the perspective of ongoing pandemic of COVID-19, a disease caused by a new coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Propolis has been shown to be very effective in prevention of respiratory tract infections, many of which are caused by coronaviruses [38,39]. Based on our experimental results, it seems justified to suggest, that apart from oral administration, local application of propolis in the form of nasal or throat sprays, may help to prevent attachment of respiratory viruses to the host cells. Due to its lipophilic nature propolis can be expected to effectively stick to mucous lining of our air passages as the latter has also lipophilic character [40]. Consequently, viral entry may be blocked by a physical barrier in the form of propolis-generated EZ water layer, comprising additional mechanism of propolis antiviral action. In this way propolis could act as a first line of defense against respiratory pathogens.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.colcom.2020.100307.
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Fig. 1. Formation of exclusion zone (EZ) of water next to glass surface coated with (a) EEP from propolis from Poland; (b) EEP from propolis from Washington State; (c) emulsion of EEP in water; and (d) control sample showing non-coated glass capillary immersed in microsphere solution. Note that the coated surface appears smooth at the micrometer level. Any surface roughness of this scale would be discriminable at the image, as are discriminable individual 1-μm sized particles. Scale bars: 200 μm.

Fig. 2. Appearance of propolis preparations: (a) ethanolic extract (70% ethanol); (b) water extract and (c) emulsion (1.4% ethanol) of propolis from Poland; and (d) ethanolic extract (70% ethanol) of propolis from Washington State.
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