Applications of natural deep eutectic solvents to extraction and preservation of biomolecules

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CHAPTER 7

General conclusion and perspective

1. Conclusions

Ionic liquids (ILs) have been considered as green solvents for over 20 years due to their characteristic features such as chemical and thermal stability, nonflammability, nonvolatility, and high solubilizing capacity. However, due to their toxicity and nondegradable property, have turned scientist to search for a more promising solvents, and then the term of deep eutectic solvents (DES) was introduced. DES are mixtures of a quaternary ammonium salt with hydrogen bond donors such as organic acids, urea, or glycerol, to form a complex with the halide anion of the quaternary ammonium salt. DES are believed to be generally nontoxic, easy to prepare, accessible, and cheap (Abbot et al. 2004; Jhong et al. 2009). Choi et al. (2011) proposed that Nature have applied the principles of ILs and DES, and the term of natural deep eutectic solvents (NADESs) was presented (**Chapter 2**).

Natural deep eutectic solvents (NADESs) are new generation of green solvents which are composed of natural ingredients such as sugars, amino acids, organic acids, and organic bases, and mix together under certain conditions to become liquid. NADESs have several advantages such as low melting point, nontoxic, zero vapor pressure, nonflammability, no environmental hazards, and sustainable production. The concept of NADESs is that everywhere in Nature, there is a third liquid phase, apart from water and lipid phase, which could solubilize medium polar compounds that are poorly soluble in water or lipids. This could explain the role of NADESs in extreme conditions, e.g. cold and drought resistance, desiccation and resurrection plants, and also as media for biosynthetic pathways (**Chapter 3**). NADESs is also believed to be involved in digesting process of insect-eating plants, e.g. *Drosera* species. *Drosera* obtained their nutrients from insects that are trapped by sticky exudates produced by glands on the tentacles. Digestive enzymes are released to digest the insects, and this process could take up for two days. From ^{1}H NMR and GC-MS measurement, the exudate of *Drosera* contains several NADESs components such as glucose, fructose, *myo*-inositol, arabinose, malic acid, lactic acid, mannose, and galactose. (**Chapter 4**).

To further explore the possibility of NADESs in protein preservation, two proteases enzymes were tested, namely protease (isolated from *Streptomyces griseus)*, and bromelain from pineapple fruit (**Chapter 5**). These enzymes were stored in several NADESs with different water concentration for 14 days under day light. It showed that NADESs had the ability to stabilize proteases in different water content, and NADESs also protect the enzymes from microorganism growth. Macromolecules like DNA and RNA are susceptible to degradation under certain environmental conditions, and NADESs may be involved in stabilizing these genetic materials in extreme conditions. DNA and RNA of *Drosophila melanogaster* were well preserved in NADESs even after 12 months, which could explain the role of NADESs in stabilizing and preserving macromolecules (**Chapter 6**). NADESs are also potential solvents for keeping isolated DNA for short time storage.

2. Perspectives

Natural deep eutectic solvents (NADESs) are promising green solvents which can be used in various applications, including secondary metabolite extraction, enzymatic reaction, solvents for medium polarity compounds, protein stabilizer, and media for drugs and cosmetics. The use of NADESs can be developed to a larger scale with intended to reduce the use of organic solvents in industrial fields. The combinations of NADESs can be expanded as their ingredients are abundant in Nature, and they can be tailored to a specific application.

The obstacles in using NADESs are the viscosity and nonvolatility, which makes NADESs difficult to remove from the materials. However, in food and pharmaceutical industry, NADESs can be used directly, in case of extraction process, some extra procedure is necessary to eliminate the solvents. Some methods can be applied, including supercritical CO_2 extraction, liquid-liquid and liquid-solid extraction may be employed (Dai et al. 2013).

Another important application of NADESs is in pharmaceutical fields, where NADESs can be used as media to dissolve poorly water-soluble compounds and directly administered to the patients. The natural components of NADESs make it safe for oral use, as for injection, specific NADESs combination may be applied to avoid any unwanted reactions of the body. The search for the right combinations is a challenge since injection forms need to be sterile, compatible with active compounds, hypoallergenic, and stable in body biofluids.

As for preservation of macromolecules, little information is available. The stability of DNA and RNA solution in NADESs was reported, yet the utilization of NADESs as cryoprotectants for DNA and RNA under extreme condition is need to be explored, as well as preserving bacteria in nonaqueous environment.

The invention of NADESs has contributed a great deal of diverse biotechnology or biological engineering applications. It may explain many cellular processes which may be applied in laboratory and even in industrial scale. The limitless NADESs combinations which are found in Nature will generate various applications, and specific combination will result in more suitable conditions for desired applications.

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