Ionic liquid-based transmission electron microscopy for herpes simplex virus type 1

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Received: 29 March 2018 / Accepted: 2 April 2018 / Published online: 13 April 2018

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Keywords Ionic liquid · Transmission electron microscopy · Biological specimens · Kamlet-Taft parameters · Herpes simplex virus type 1

Introduction

Ionic liquid (IL), which is often called room-temperature ionic liquid, ambient-temperature ionic liquid, and ambient-temperature molten salts, is a liquid salt composed entirely of cations and anions, and it melts below 298 K without any solvents. It is expected to be an innovative liquid material and a functional solvent due to its interesting physicochemical properties such as flame resistance, relatively high conductivity, negligible vapor pressure, and antistatic properties. Various next-generation applications in science and technology with IL have been proposed to date (Torimoto et al. 2010; Wasserscheid and Welton 2008; Eftekhari 2017). In 2006, it was reported that IL droplets could be observed by scanning electron microscopy (SEM) without charging behavior (Kuwabata et al. 2006). Subsequent efforts are being made to develop novel IL-based electron microscopy techniques to capitalize on the fact that a thin IL layer spread on insulating materials and biological specimens works as a conductive layer, similar to a thin gold or carbon layer (Torimoto et al. 2010; Tsuda et al. 2011; Eftekhari 2017). In this study, a facile IL-based pretreatment method for transmission electron microscopy (TEM) observation of herpes simplex virus type 1 (HSV) is suggested as an alternative to a conventional, complicated, and

laborious pretreatment protocol. The aim of this study is to explore a relationship between the IL species and the resulting TEM images by using Kamlet-Taft parameters.

Materials and methods

The preparation and purification methods of ILs, such as 1-ethyl-3-methylimidazolium tetrafluoroborate ([EtMeIm][BF₄]) and choline lactate ([Ch][CH₃CH(OH)CO₂]), used in this study have been described in a previous article in detail (Tsuda et al. 2011). The TEM observation was conducted by a Hitachi H-7650. The IL-based pretreatment protocol for TEM observation of HSV and liposome is depicted in Scheme 1. The polarity factors of the ILs to be used for the pretreatment process were determined from the UV-Vis data of ILs with dyes and the equations for obtaining Kamlet-Taft parameters (Reichardt 1994; Fukaya et al. 2007; Lee et al. 2008). The dyes used were Reichardt's dye (90%, Sigma-Aldrich), 4-nitroaniline (> 99%, Wako) and *N,N*-diethyl-4-nitroaniline (97%, Wako). The UV-Vis spectroscopy was conducted at room temperature (ca. 298 K). The HSV was supplied by Professor Yasuo Suda's laboratory (Kagoshima University, Japan). The liposome was prepared by vigorously agitating a 22.7 g L⁻¹ COATSOME EL-01-N (NOF) aqueous solution and allowing it to stand for more than 30 min.

Results and discussion

A precise pretreatment protocol, consisting of an aldehyde fixation, a dehydration process, a freeze-drying process, and a gold sputtering (or a carbon vapor deposition) process, is usually required before the observation of hydrous biological specimens by SEM and TEM. An IL-based pretreatment protocol for hydrous specimens to rapidly obtain high-resolution

This article is part of a Special Issue on 'Ionic Liquids and Biomolecules' edited by Antonio Benedetto and Hans-Joachim Galla

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Scheme 1 Ionic liquid-based pretreatment process for TEM observation of biological specimens

SEM images without artifact has been reported (Tsuda et al. 2011, 2014a; Eftekhari 2017). However, there is no report on the protocol for TEM observation of a virus, as it is believed that TEM observation of hydrous specimens always requires extremely dry or frozen conditions to completely avoid water vaporization. One of our recent achievements on SEM observation of water-absorbing polymers, pretreated with ILs, suggests that the vaporization is considerably suppressed by the interactions among the water molecules, the cations and anions and/or the anomalous ordered IL layer structure, like solid salt, on the specimens (Tsuda et al. 2014b; Wasserscheid and Welton 2008). Based on this result, an attempt has been made to establish a novel protocol with IL for TEM observation of HSV, a typical novel that is easy to handle. The protocol does not require any specialized equipment or special skills as depicted in Scheme 1 and can be completed in about 60 min.

It was gleaned from experience that biocompatibility of the ILs with the specimen was crucially important to obtain fine and artifact-free electron microscope images. The polarity factors of potential ILs suitable for the pretreatment process were determined by using the Kamlet-Taft parameters (Wasserscheid and Welton 2008). The obtained parameters are given in Table 1. α , β , π^* and E_T^N values are derived from the hydrogen bond donating ability, hydrogen bond accepting ability, dipolarity/polarizability and solvent polarity, respectively (Reichardt 1994; Lee et al. 2008). π^* and E_T^N values are nearly independent

of the IL species, while this is not the case with α and β . ILs with $[\text{BF}_4]^-$ anion show higher α and lower β values. Figure 1 shows TEM images of HSV pretreated with Scheme 1 using the ILs listed in Table 1. If the ILs with the value of β exceeding 0.95, i.e., $[\text{C}_2\text{mim}][\text{CH}_3\text{CO}_2]$, $[\text{C}_2\text{mim}][\text{CH}_3\text{CH}(\text{OH})\text{CO}_2]$ and $[\text{Ch}][\text{Lev}]$, were used, spherical HSV was observed (Fig. 1a, b, e). These TEM images are essentially identical to those taken by using a conventional approach (Hong Zhou et al. 1999; Grünwald et al. 2003). However, the other three ILs caused serious damage to the HSV. In fact, in the case of liposome, a similar tendency was observed (not shown). These results suggest that the value of β of the ILs should be considered, when the

Table 1 Kamlet-Taft parameters of the ionic liquids used in Fig. 1 and the result of TEM observation of liposome and HSV

Ionic liquids	α	β	π^*	E_T^N	Specimens	
					Liposome	HSV
$[\text{Ch}][\text{Lac}]$	0.59	0.80	1.12	0.68	○	×
$[\text{Ch}][\text{Lev}]$	0.55	0.98	0.96	0.61	○	○
$[\text{EtMeIm}][\text{OAc}]$	0.47	1.07	1.06	0.60	○	○
$[\text{EtMeIm}][\text{Lac}]$	0.50	0.95	1.08	0.62	○	○
$[\text{EtMeIm}][\text{BF}_4]$	0.65	0.34	1.05	0.69	×	×
$[\text{BuMeIm}][\text{BF}_4]$	0.63*	0.38*	1.05*	0.67*	×	×

*(Muldoon et al. 2001)

Fig. 1 TEM images of HSV (herpes simplex virus type 1) pretreated with Scheme 1. The used ILs were **a** [C₂mim][CH₃CO₂], **b** [C₂mim][CH₃CH(OH)CO₂], **c** [C₂mim][BF₄], **d** [C₄mim][BF₄], **e** [Ch][Lev], **f** [Ch][CH₃CH(OH)CO₂]

Scheme 1 is applied to the biological specimens in which the phospholipid bilayer is directly in contacts with the IL.

We found that the proposed innovative pretreatment process for TEM observation of HSV can produce fine and artifact-free electron microscope images in a short time, if appropriate ILs are used for the process. The value of β of the IL is the most important factor to obtain a preferred quality of the TEM image. This information will be useful in the application of IL-based pretreatment process to other hydrous biological specimens, e.g., enzymes, proteins and DNA.

Acknowledgements We thank Professor Yasuo Suda and Professor Masahiro Wakao (Kagoshima University, Japan) for their support in this research.

Compliance with ethical standards

Funding This research was supported by Core Research for Evolution Science and Technology (CREST) from Japan Science and Technology Agency (JST) and from Senri Life Science Foundation in Japan.

Conflict of interest Tetsuya Tsuda declares that he has no conflict of interest. Koshi Kawakami declares that he has no conflict of interest. Eiko Mochizuki declares that he has no conflict of interest. Susumu Kuwabata declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

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