Dimethyl sulfoxide enhances both cellulose dissolution ability and biocompatibility of a carboxylate-type liquid zwitterion

Journal Name

LETTER

Dimethyl sulfoxide enhances both cellulose dissolution ability and biocompatibility of a carboxylate-type liquid zwitterion

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Heri Satria[a,b], Kosuke Kuroda[a*], Yota Tsuge[c], Kazuaki Ninomiya[c], and Kenji Takahashi[a]

Cellulose dissolution ability of a liquid zwitterion, the most biocompatible cellulose solvent, was improved by adding a co-solvent, dimethylsulfoxide. Moreover, biocompatibility of the liquid zwitterion was also improved by adding dimethylsulfoxide although it is toxic relative to the liquid zwitterion. The mixture is the efficient and extremely biocompatible cellulose solvent.

Despite being the most abundant biopolymer on earth, cellulose faces challenges in biorefinery applications because of its poor solubility. The recalcitrance of cellulose is due to its highly crystalline structure. Therefore, efficient solvents are necessary to convert cellulose into biofuels or the other highly valuable chemical compounds.[1] Some solvents or solvent systems, e.g. *N*-methylmorpholine oxide[2], *N,N*-dimethylacetamide/lithium chloride[3], 1,3-dimethyl-2-imidazolidinone/lithium chloride[4], and dimethyl sulfoxide (DMSO)/tetrabutylammonium fluoride[5], can dissolve cellulose directly. Recently, ionic liquids (ILs) which are liquid salts below 100 °C, have been highlighted for their ability to dissolve cellulose. Swatloski *et al.* have reported that 1-butyl-3-methylimidazolium chloride can dissolve 10 wt% of cellulose at 100 °C.[6] ILs containing carboxylate, dialkylphosphate, or alkylphosphonate anions have also been reported to have superior cellulose solubility.[7-12] Currently, ILs are recognized as one of the most effective solvents for dissolving cellulose.

However, ILs must overcome some critical challenges before they can be practically applied in biorefinery.[13, 14] One of their problematic characteristics is their toxicity to microorganisms when bioconversion is used in biorefinery. ILs show toxicity towards microorganisms by destructing their cell membranes via a two-step mechanism.[15] First, cations of ILs are electrostatically atrracted to anionic phospholipids of cell membranes. Then, the ILs insert the hydrophobic alkyl chain of their cations (called the cation tail) into the microorganism's cell membrane via hydrophobic interactions.

To overcome the problem of toxicity, our group has previously developed a biocompatible and cellulose-dissolving zwitterion[16], carboxylate-type liquid zwitterion (OE_2imC_3C, Fig. 1) as an analogue of cellulose dissolving ILs. The structure has no hydrophobic cation tail, which contributes to the toxicity, and has a similar polarity to other ILs capable of dissolving cellulose. Consequently, OE_2imC_3C has showed cellulose dissolution ability and the highest biocompatibility among all cellulose solvents. However, OE_2imC_3C has high viscosity, which limits its capability to dissolve cellulose. For example, the solubility of cellulose in OE_2imC_3C is 6 wt% at 100 °C due to its high viscosity (details later), which is lower than 1-butyl-3-methylimidazolium chloride (10 wt%).[6] It is the critical problem to overcome before practical use.

DMSO is a relatively polar aprotic solvent and has been often used as a co-solvent for the dissolution of cellulose with ILs. It is reported that DMSO can reduce the viscosity of ILs without hindering their ability to dissolve cellulose.[17-22] In this study, we investigated the solubility of cellulose in OE_2imC_3C/DMSO mixtures. In addition, the toxicity to *Escherichia coli* (*E. coli*) growth was also investigated and surprisingly improved by addition of DMSO, although DMSO is less biocompatible than pure OE_2imC_3C (but even DMSO is generally considered biocompatible).

Figure 2a shows cellulose solubility in OE_2imC_3C/DMSO mixtures at 100 °C. Pure OE_2imC_3C was capable dissolving up to 6 wt% of cellulose. However, at this concentration, the solution became too viscous to stir. Thus, we could not confirm whether 6 wt% was the true maximum solubility in pure OE_2imC_3C. We found that the addition of DMSO accelerated the dissolution of cellulose. The OE_2imC_3C/DMSO

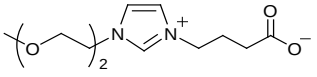

Fig. 1 A structure of OE_2imC_3C.

[a] Faculty of Biological Science and Technology, Institute of Science and Engineering, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan.
[b] Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung, Jl. Soemantri Brojonegoro No.1 Bandar Lampung 35145, Indonesia.
[c] Institute for Frontier Science Initiative, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan.

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

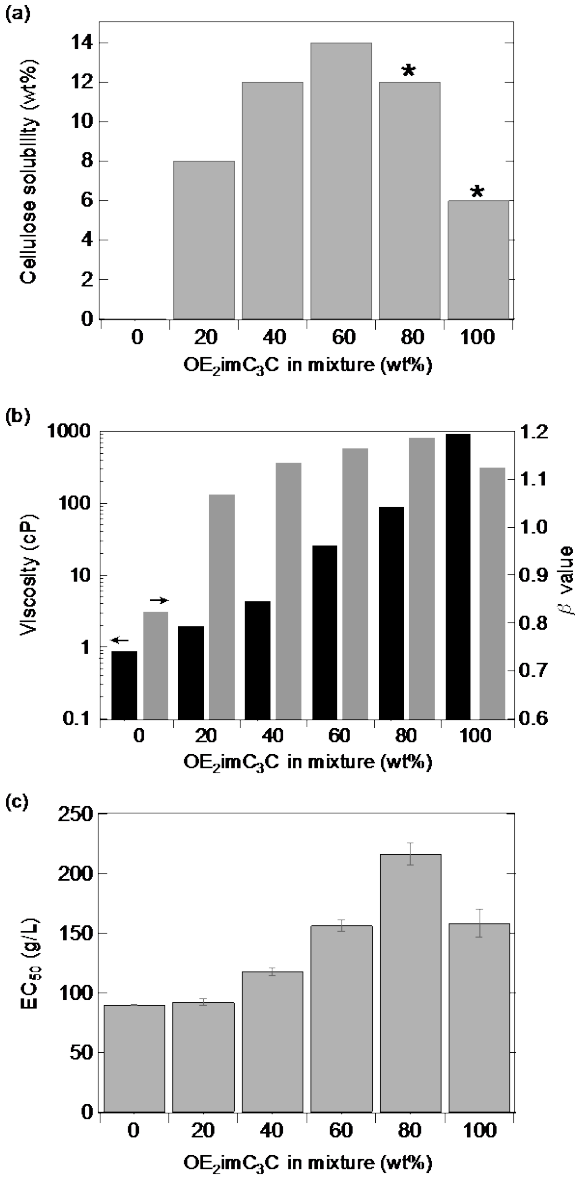

Fig. 2 (a)Cellulose solubility, (b)viscosity and β value, and (c)EC_{50} of OE_2imC_3C/DMSO. *Solubility of cellulose could not be evaluated anymore because the mixture could not be stirred due to the high viscosity. The viscosity was measured at 80 °C.

mixture (80/20) dissolved up to 12 wt% while the mixture faced a similar problem with pure OE_2imC_3C. The OE_2imC_3C/DMSO mixture (60/40) achieved the highest solubility of cellulose at 14 wt%. When 15 wt% of cellulose was added, the mixture remained stirrable but did not dissolve the cellulose. The solubility was much improved by addition of DMSO and intermediate between chloride-type ILs[6, 7] (cf. 1-butyl-3-methylimidazolium chloride: 10 wt%; 1-allyl-3-methylimidazolium chloride: 11 wt% at the same temperature) and carboxylate-type ILs[7] (1-ethyl-3-methylimidazolium formate: more than 20 wt%). This result indicates that OE_2imC_3C/DMSO mixture is ready to use. The dissolution ability of the OE_2imC_3C/DMSO mixture decreased when the OE_2imC_3C concentration is less than 60 wt%. The solubility was 12 and 8 wt% in the OE_2imC_3C/DMSO mixtures (40/60 and 20/80), respectively. This trend is similar to that of previously reported cellulose-dissolving ILs.[21]

To clarify the reason for the increase of cellulose solubility, we measured the viscosity of each mixture (Figure 2b). We measured the viscosity at 80 °C due to the high viscosity of OE_2imC_3C. Addition of DMSO caused the viscosity of the mixture to decrease almost exponentially. The OE_2imC_3C/DMSO mixture (60/40), which showed the highest solubility, had a much lower viscosity (26.2 cP at 80 °C) compared to that of pure OE_2imC_3C (935.2 cP at 80 °C), suggesting that low viscosity is related to cellulose solubility. We here would like to stress that OE_2imC_3C/DMSO solutions show similar viscosity to general carboxylate-type ILs[23] (cf. 1-ethyl-3-methylimidazolium acetate: 18 cP at 70 °C). It is noted that the viscosity of OE_2imC_3C/DMSO (60/40) at 30 °C was 175.8 cP while that of pure OE_2imC_3C is too high to be measured at the same temperature.

To determine the reason for decreased cellulose solubility in the mixtures (40/60 and 20/80), β value of Kamlet-Taft parameters[24] of each mixture was measured as reported[25] (Figure 2b). The β value describes hydrogen bond basicity, and it is known as a key factor in disrupting the hydrogen bond networks between cellulose chains.[7, 26] β value somewhat decreased in the mixtures (40/60 and 20/80). The β value is reported to have a rough correlation with cellulose solubility[26], and it may be responsible for low solubility of cellulose in the OE_2imC_3C/DMSO mixtures (20/80 and 40/60). Another hypothesis regarding the low solubility in this region is the molar ratio of OE_2imC_3C to the OH groups of cellulose. In the mixture exhibiting maximum solubility, 14 wt% cellulose in OE_2imC_3C/DMSO (60/40), the molar ratio of OE_2imC_3C/OH is 1.00. In contrast, the molar ratios of OE_2imC_3C/OH in the cellulose-saturated OE_2imC_3C/DMSO mixtures (12 and 8 wt% cellulose in 40/60 and 20/80) are only 0.60 and 0.45, respectively. In the case of cellulose dissolved in excess of pure 1-ethyl-3-methylimidazolium acetate, a popular carboxylate-type IL, it is reported that one OH group makes a hydrogen bond with 0.92 ILs.[27] Therefore, while a ratio of 1.00 is sufficient to solubilize cellulose, ratios of 0.60 and 0.45 seem to be relatively low. Regarding the difference in the molar ratio of OE_2imC_3C/OH between the mixtures (60/40 and 20/80), the β values and cluster structure[28] of OE_2imC_3C/DMSO may also be involved, but further investigation is required.

We investigated the toxicity of the mixtures to *E. coli* growth (Figure 2c), by means of EC_{50}, which is the critical concentration of chemical compounds required for inhibiting the growth of microorganisms (details in Experimental section). The EC_{50} of pure OE_2imC_3C was 159 g/L was almost 1.7-fold higher than that of DMSO (90 g/L). Therefore, it was confirmed that the toxicity of OE_2imC_3C was even lower than that of DMSO, a known biocompatible organic compound often used as a solvent for adding hydrophobic compounds to cultures. Remarkably, the EC_{50} value increased to 217 g/L in the mixture (80/20): a lower toxicity than that of either pure OE_2imC_3C, although the addition of DMSO was expected to decrease the EC_{50}. It is noted that this value is extremely high because EC_{50} of 1-ethyl-3-methylimidazolium acetate is only 9 g/L[16]. In the

mixture (60/40), the EC_{50} decreased to 157 g/L, which is close to that of the pure OE_2imC_3C. Further, addition of high concentration of DMSO to OE_2imC_3C (40/60 and 20/80) caused the EC_{50} of the mixtures to decline to 118 and 93 g/L respectively. As expected, the EC_{50} of the solutions with high concentration DMSO became nearly equal to that of pure DMSO.

In order to explain the trend of the EC_{50} in Figure 2c, contribution of each solvent to the total EC_{50} was separately calculated. The contribution of OE_2imC_3C to the total EC_{50} in the OE_2imC_3C/DMSO (80/20) was 173 g/L (namely, that of DMSO and total EC_{50} were 44 and 217 g/L, respectively). Because this calculated contribution is higher than the EC_{50} of pure OE_2imC_3C (159 g/L), there may be positive synergistic effect. It may be caused by strong interaction of cations with DMSO[28] although further investigation is required to clarify. We think that there also seems to be another possibility—it is not synergistic effect—because 159 and 173 g/L is not so different and could be in error (see error bars in Figure 2c, and details are in discussed in ESI, the text for Fig. S1). In contrast, in the mixtures (60/40, 40/60, and 20/80), the EC_{50} values of each component were 94/62, 47/70, and 19/74 g/L (OE_2imC_3C/DMSO), respectively; it appears that the toxicity does not come from only one of the components, because the values are not similar to the EC_{50} of either of the pure OE_2imC_3C or pure DMSO (159 or 90 g/L). This observation may indicate a negative synergetic effect between OE_2imC_3C and DMSO when the DMSO concentration is over 20 wt%. The reason of positive/negative synergistic effect depending on the DMSO concentration is presumably due to forming ion clusters in DMSO at higher concentration.[28] In conclusion, the capability of OE_2imC_3C and DMSO mixtures to dissolve cellulose and their toxicity towards *E. coli* were evaluated. The addition of DMSO significantly increased the cellulose solubility. Notably, the mixtures with 20-60 wt% DMSO showed two fold higher cellulose solubility compared to that of pure OE_2imC_3C. Regarding the toxicity of the mixtures to *E. coli*, addition of 20 wt% DMSO unexpectedly improved the biocompatibility, despite DMSO having higher toxicity than that of OE_2imC_3C. From all results, OE_2imC_3C/DMSO (80/20) is the first solvent satisfying both efficient cellulose dissolution and utilization of microorganisms: the mixture is a promising solvent for biomass via bioconversion.

Experimental

Materials

OE_2imC_3C was synthesised as reported.[(1)] Avicel PH-101 was purchashed from Sigma-Aldrich.Co., Llc. DMSO was purchased from Nacalai Tesque Inc. The solvatochromic dyes, 4-nitroaniline was purchased from Tokyo Chemical Industries Co., Ltd. and *N*,*N*-diethyl-4-nitroaniline was purchased from Kanto Chemical Co., Inc. *E.coli* was purchased from ATCC. Tryptone, NaCl (Nacalai Tesque Inc.) and yeast extract (Becton, Dickinson and Company) were purchased and used for preparing lysogeny broth (LB) without purification. Viscometer (Brookfied DV-II+ Pro) was used for measurement of viscosity of OE_2imC_3C/DMSO.

Dissolution of cellulose

OE_2imC_3C/DMSO mixtures were prepared by mixing dry OE_2imC_3C and DMSO. Cellulose (1 wt%) was added into mixtures and the resulting solutions were stirred gently at 100 °C in an oil bath for 10 minutes. When cellulose was solubilised in the mixtures, the procedure was repeated until the maximum solubility of cellulose was achieved.

Measurement of β value of Kamlet-Taft parameters

Stock solutions of each solvatochromic dye, 4-nitroaniline (1 mg/mL) and *N*,*N*-diethyl-4-nitroaniline (1 mg/mL) were made with methanol. The solutions of 4-nitroaniline (30 μL) and *N*,*N*-diethyl-4-nitroaniline (30 μL) were taken into vials respectively, and were dried carefully under vacuum pressure. OE_2imC_3C/DMSO (200 μL) mixtures then were mixed into each dried dye. The homogenous mixtures were placed into quartz cells with 0.1 mm light-path length. The maximum absorption (λ_{max}) of the mixtures was determined to calculate the β value as following equations.

ν (dye) = $1/(\lambda_{max(dye)}10^{-4})$

$\beta = (1.035\,\nu_{(N,N\text{-diethyl-4-nitroaniline})} + 2.64 - \nu_{(4\text{-nitroaniline})})/2.80$

Assay of inhibition to growth of *E. coli* by OE_2imC_3C/DMSO mixtures

LB was made by mixing 10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, and 1 liter of ultra pure water. The OE_2imC_3C/DMSO mixture (5.0 g) was diluted by the LB (10 mL) to obtain a stock solution. OE_2imC_3C/DMSO/LB mixture solutions with various concentrations were prepared by dilution the stock solution with the LB. *E. coli* was pre-cultured aerobically at 37 °C in the test tube containing 2 mL of the LB. After pre-cultured, the *E. coli* cells were collected by centrifugation and inoculated into the OE_2imC_3C/DMSO/LB mixtures (2mL each tube) as to be an initial optical density at 600 nm (OD_{600}) of 0.1. The inoculated media were incubated at 37 °C for 24 h using a reciprocal shaker at 160 rpm, and the OD_{600} of solutions were measured. The median effective concentration (EC_{50}) concerning growth of *E. coli* was determined as concentration of the OE_2imC_3C/DMSO mixture at which the relative growth was reduced to a half of the value in pure medium.

Conflicts of interest

There are no conflicts to declare.

Acknowledgement

This research was supported in part by the COI program "Construction of next-generation infrastructure using innovative materials–Realization of a safe and secure society

that can coexist with the Earth for centuries", which is supported by MEXT and JST, the Advanced Low Carbon Technology Research and Development Program (ALCA) (No. 2100040 to K.T.) and the Cross-ministerial Strategic Innovation Promotion Program (SIP) of JST. This study was also partly supported by KAKENHI (18K14281) and Leading Initiative for Excellent Young Researchers from the Japan Society for the Promotion of Science.

Notes and references

1 D. Klemm, B. Heublein, H. P. Fink and A. Bohn, *Angew. Chem. Int. Ed. Engl.*, 2005, **44**, 3358-3393.
2 H. P. Fink, P. Weigel, H. J. Purz and J. Ganster, *Prog. Polym. Sci.*, 2001, **26**, 1473-1524.
3 C. L. Mccormick and T. R. Dawsey, *Macromolecules*, 1990, **23**, 3606-3610.
4 N. Tamai, D. Tatsumi and T. Matsumoto, *Biomacromolecules*, 2004, **5**, 422-432.
5 G. T. Ciacco, T. F. Liebert, E. Frollini and T. J. Heinze, *Cellulose*, 2003, **10**, 125-132.
6 R. P. Swatloski, S. K. Spear, J. D. Holbrey and R. D. Rogers, *JACS*, 2002, **124**, 4974-4975.
7 Y. Fukaya, A. Sugimoto and H. Ohno, *Biomacromolecules*, 2006, **7**, 3295-3297.
8 I. Kilpelainen, H. Xie, A. King, M. Granstrom, S. Heikkinen and D. S. Argyropoulos, *J. Agric. Food Chem.*, 2007, **55**, 9142-9148.
9 N. Sun, H. Rodriguez, M. Rahman and R. D. Rogers, *Chem. Commun.*, 2011, **47**, 1405-1421.
10 Y. Fukaya, K. Hayashi, M. Wada and H. Ohno, *Green Chem.*, 2008, **10**, 44-46.
11 H. Ohno and Y. Fukaya, *Chem. Lett.*, 2009, **38**, 2-7.
12 B. Zhao, L. Greiner and W. Leitner, *Rsc Advances*, 2012, **2**, 2476-2479.
13 F. Ganske and U. T. Bornscheuer, *Biotechnol. Lett.*, 2006, **28**, 465-469.
14 M. Ouellet, S. Datta, D. C. Dibble, P. R. Tamrakar, P. I. Benke, C. L. Li, S. Singh, K. L. Sale, P. D. Adams, J. D. Keasling, B. A. Simmons, B. M. Holmes and A. Mukhopadhyay, *Green Chem.*, 2011, **13**, 2743-2749.
15 G. S. Lim, J. Zidar, D. W. Cheong, S. Jaenicke and M. Klahn, *J. Phys. Chem. B*, 2014, **118**, 10444-10459.
16 K. Kuroda, H. Satria, K. Miyamura, Y. Tsuge, K. Ninomiya and K. Takahashi, *J. Am. Chem. Soc.*, 2017, **139**, 16052-16055.
17 L. Wu, S. H. Lee and T. Endo, *Bioresour. Technol.*, 2013, **140**, 90-96.
18 X. Zhang, W. W. Zhao, Y. J. Li, C. Li, Q. P. Yuan and G. Cheng, *Rsc Advances*, 2016, **6**, 62278-62285.
19 X. D. Li, Y. Zhang, J. W. Tang, A. Lan, Y. P. Yang, M. Gibril and M. H. Yu, *J Polym Res*, 2016, **23**.
20 D. A. Fort, R. C. Remsing, R. P. Swatloski, P. Moyna, G. Moyna and R. D. Rogers, *Green Chem.*, 2007, **9**, 63.
21 K. Ohira, K. Yoshida, S. Hayase and T. Itoh, *Chem. Lett.*, 2012, **41**, 987-989.
22 R. Rinaldi, *Chem Commun (Camb)*, 2011, **47**, 511-513.
23 E. Quijada-Maldonado, S. van der Boogaart, J. H. Lijbers, G. W. Meindersma and A. B. de Haan, *The Journal of Chemical Thermodynamics*, 2012, **51**, 51-58.
24 M. J. Kamlet and R. W. Taft, *JACS*, 1976, **98**, 377-383.
25 K. Kuroda, H. Kunimura, Y. Fukaya and H. Ohno, *Cellulose*, 2014, **21**, 2199-2206.
26 A. Brandt, J. Gräsvik, J. P. Hallett and T. Welton, *Green Chem.*, 2013, **15**, 550.
27 B. D. Rabideau, A. Agarwal and A. E. Ismail, *J. Phys. Chem. B*, 2013, **117**, 3469-3479.
28 C. Zhang, H. Kang, P. Li, Z. Liu, Y. Zhang, R. Liu, J.-f. Xiang and Y. Huang, *Cellulose*, 2016, **23**, 1165-1175.