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Short communication

Solubilization of cytochrome *c* in organic media with fluoroalkyl end-capped *N*-(1,1-dimethyl-3-oxobutyl)acrylamide oligomer: a new approach to fluorinated biocatalyst in organic media

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Abstract

Self-assembled molecular aggregates of fluoroalkyl end-capped *N*-(1,1-dimethyl-3-oxobutyl)acrylamide oligomer can solubilize cytochrome *c* in organic media such as methanol, although the corresponding non-fluorinated polymer cannot solubilize cytochrome *c* in organic media. Interestingly, the resulting fluorinated oligomer–cytochrome *c* aggregate was found to act effectively as a new fluorinated biocatalyst for the oxidation of pinacyanol chloride with hydrogen peroxide in the non-aqueous methanol.

Keywords: Fluorinated oligomer; Molecular aggregate; Cytochrome *c*; Solubilization; Organic media; Fluorinated biocatalyst

Fluoroalkyl end-capped oligomers are attractive functional materials due to exhibiting various unique properties such as biological activity and the formation of self-assembled molecular aggregates with the aggregations of end-capped fluoroalkyl segments which cannot be achieved by the corresponding randomly fluoroalkylated polymers, fluoroalkylated block-polymers, and non-fluorinated polymers [1]. For example, it was demonstrated that fluoroalkyl end-capped trimethylvinylsilane–acrylic acid cooligomers can form the molecular aggregates with the aggregation of terminal fluoroalkyl segments, and these fluorinated cooligomers possess a selective and potent anti-HIV-1 activity in vitro [2]. Very recently, we have also found that self-assembled molecular aggregates of fluoroalkyl end-capped *N*-(1,1-dimethyl-3-oxobutyl)acrylamide oligomer can recognize selectively hydrophilic amino and *N*,*N*-dimethylamino compounds such as methylene blue, acriflavine hydrochloride and luminol as guest molecules, although the corresponding non-fluorinated oligomer cannot recognize these compounds at all [3,4]. Therefore, it is very interesting to apply these molecular aggregates to a new fluorinated biocatalyst (a polymer–enzyme complex) system in organic media from the viewpoint of the development of novel synthetic organic chemistry. Especially, the use of organic solvents as reaction media has recently increased the diversity of biocatalyses due to the numerous advantages in employing enzymes in organic media [5]. Recently, there have been a variety of reports on organic media-soluble enzymes which were chemically or physically modified

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with polymers and surfactants [6]; however, studies on the organic media-soluble enzymes modified with fluorinated polymers have been hitherto very limited. From this point of view, the development of unique fluorinated biocatalyst system is deeply desirable. In this communication, we would like to report on the solubilization of cytochrome *c* in organic media with fluoroalkyl end-capped *N*-(1,1-dimethyl-3-oxobutyl) acrylamide oligomer, and the application to new fluorinated biocatalyst in organic media.

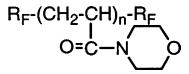
Fluoroalkyl end-capped *N*-(1,1-dimethyl-3-oxobutyl)-acrylamide oligomers (R_F—(DOBAA)$_n$—R_F) were prepared according to our previously reported method [3]. We have already reported that R_F—(DOBAA)$_n$—R_F can form the molecular assemblies with the aggregations of the end-capped fluoroalkyl segments, and this molecular aggregate consists of around 10 fluorinated oligomeric molecules by the static light scattering measurements [3]. Therefore, it is expected that R_F—(DOBAA)$_n$—R_F should act as a suitable host molecule for various biocatalyses. In particular, cytochrome *c* is well-known to be a water-soluble heme protein and can catalyze the oxidation of organosulfides and hydrocarbons in the aqueous organic solvents [7]. Therefore, the exploration of fluorinated molecular aggregates-cytochrome *c* complexes should open a new route for the development of the fluorinated biocatalyses. In fact, we have tested the solubility of cytochrome *c* in methanol with R_F—(DOBAA)$_n$—R_F ($M_n = 7900$; $R_F = CF(CF_3)$-OCF_2CF-$(CF_3)OC_3F_7$). A typical procedure for solubilizing cytochrome *c* is as follows. Cytochrome *c* powder (5 mg) was added in a methanol solution (5 ml) of R_F—(DOBAA)$_n$—R_F ($R_F = CF(CF_3)OCF_2CF(CF_3)$-$OC_3F_7$; 2 g dm^{-3}), and was stirred for 2 h at room temperature. After stirring, cytochrome *c* was found to be completely soluble in the methanol solution, and this solution changed from the colorless to the purple-red solution. UV–vis spectra of cytochrome *c* in the methanol solution shows an absorption band (λ_{max}) at 400 nm. In contrast, non-fluorinated DOBAA polymer did not solubilize cytochrome *c* in methanol under similar conditions, and most of cytochrome *c* powder was recovered. This finding suggests that cytochrome *c* is a potential guest molecule for self-assembled molecular aggregates of R_F—(DOBAA)$_n$—R_F with aggregations of the end-capped fluoroalkyl segments to exhibit a good solubility in methanol. To study the effect of fluoroalkyl end-capped oligomers on the solubility of cytochrome *c* in methanol, we tested several fluoroalkyl end-capped oligomers under similar conditions. The results were shown in Table 1.

As shown in Table 1, fluorinated oligomers such as fluoroalkyl end-capped acryloylmorpholine oligomer (R_F—(ACMO)$_n$—R_F) and fluoroalkyl end-capped *N*-isopropylacrylamide oligomer (R_F—(NIPAM)$_n$—R_F) had

Table 1
Solubility of cytochrome *c*[a] in methanol with fluoroalkyl end-capped oligomers[b]

Oligomer	M_n	Solubility (%)[c]
R_F—(DOBAA)$_n$—R_F[d]	7900	100
—(DOBAA)$_n$—	29,000	1
R_F—(AMCO)$_n$—R_F[e]	4730	79
—(ACMO)$_n$—	3750	–[f]
R_F—(NIPAM)$_n$—R_F	1210	85
—(NIPAM)$_n$—	15,500	1

[a] Cytochrome *c*: 5 mg in oligomer methanol solution.
[b] Concentration of oligomer: 2 g dm^{-3} in MeOH.
[c] Solubility (%) of cytochrome *c* is based on the absorbance (λ_{max}: 400 nm) of cytochrome *c* in methanol solution of R_F—(DOBAA)$_n$—R_F ($R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$).
[d] $R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$.
[e] R_F—(ACMO)$_n$—R_F:



($R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$).
[f] —(ACMO)$_n$— was insoluble in methanol.

a solubility for cytochrome *c*. However, these fluorinated oligomers were found to be less effective than R_F—(DOBAA)$_n$—R_F. Additionally, non-fluorinated NIPAM oligomer (—(NIPAM)$_n$—) had no solubility for cytochrome *c*, and most of cytochrome *c* powder was recovered. Among these fluoroalkyl end-capped oilgomers, R_F—(DOBAA)$_n$—R_F was most effective. This result is not clarified at present; however, it is suggested that R_F—(DOBBA)$_n$—R_F oligomer could specifically bind cytochrome *c*. It is well-known that cytochrome *c* has 19 lysine residues and other ionic moieties on the surface [8]. These moieties should selectively function as effective binding sites of DOBAA segments in R_F—(DOBAA)$_n$—R_F to exhibit a good solubility in methanol. In fact, we have already reported that R_F—(DOBAA)$_n$—R_F oligomers can recognize selectively hydrophilic amino and *N*,*N*-dimethylamino compounds, and the DOBAA segments in oligomers could provide a suitable recognition site for these compounds [3].

To clarify the solubility of cytochrome *c* in methanol with R_F—(DOBAA)$_n$—R_F, experiments were conducted with varying concentrations of cytochrome *c*, and the result was shown in Fig. 1.

As shown in Fig. 1, solubility of cytochrome *c* in methanol was found to increase with increasing the concentrations of R_F—(DOBAA)$_n$—R_F, and the solubility became almost constant above 2 g dm^{-3} of oligomer concentration. This finding would indicate that R_F—(DOBAA)$_n$—R_F could form the molecular aggregate above the concentration of 2 g dm^{-3} in organic media [3]. In addition, it was clarified that as the con-

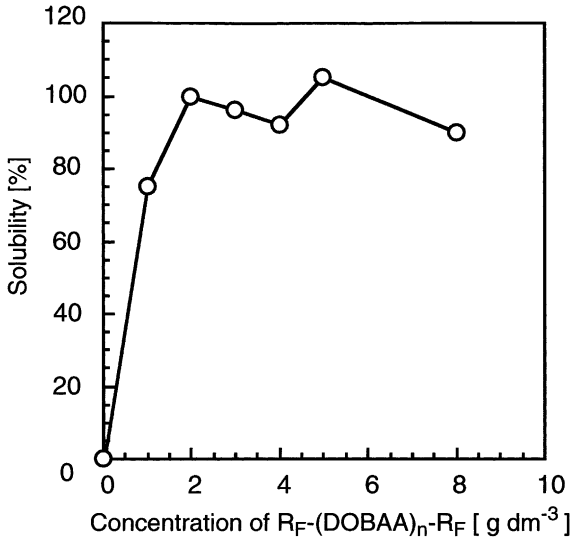


Fig. 1. Relationship between the solubility of cytochrome *c* and the concentration of R_F—(DOBAA)$_n$—R_F (R_F = $CF(CF_3)OCF_2$-$CF(CF_3)OC_3F_7$) oligomer. Concentration of cytochrome *c*: 5 mg in R_F—(DOBAA)$_n$—R_F methanol solution (5 ml).

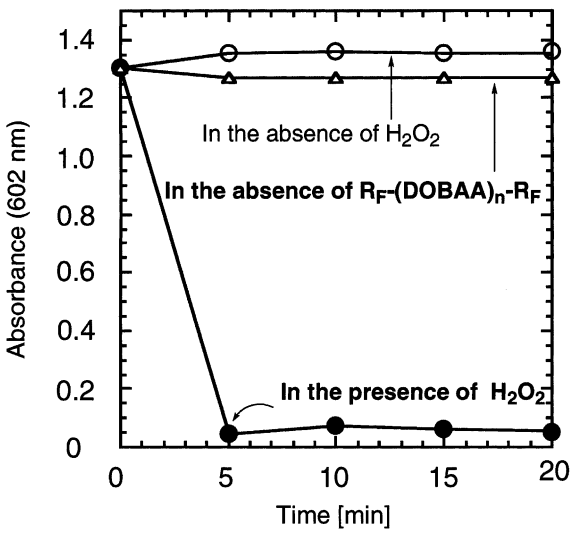


Fig. 2. Time course of catalytic oxidation of pinacyanol chloride by cytochrome-*c*-R_F—(DOBAA)$_n$—R_F in methanol. R_F— (DOBAA)$_n$—R_F (R_F = $CF(CF_3)OCF_2CF(CF_3)OC_3F_7$) 2 g dm^{-3} in methanol: 5 ml; cytochrome *c*: 5 mg in methanol; pinacyanol chloride: 185 mmol dm^{-3} in methanol (1 ml); H_2O_2: 71 mmol dm^{-3} in phosphate buffer (pH 7.0): 1 ml.

centration of cytochrome *c* increases (from 1 to 6 mg), the solubility of cytochrome *c* also increases at the 2 g dm^{-3} of oligomer concentration. In particular, when the concentration of cytochrome *c* is 6 mg dm^{-3}, the solubility increased slightly compared with the case of 5 mg dm^{-3}. However, in this case, cytochrome *c* powder was in part recovered. Therefore, the preferable conditions for the solubilization of cytochrome *c* in methanol are as follows; concentrations of R_F—(DOBAA)$_n$—R_F (R_F = $CF(CF_3)OCF_2CF(CF_3)OC_3F_7$) and cytochrome *c* are 2 g dm^{-3} and 5 mg dm^{-3}, respectively.

Hitherto, pinacyanol chloride (λ_{max}: 602 nm) is well known to be a convenient substrate to spectroscopically determine the activities of model catalysts in organic media [9]. Thus, we tried to investigate the potential for R_F—(DOBAA)$_n$—R_F–cytochrome *c* aggregate as a new fluorinated biocatalyst for the oxidation of pinacyanol chloride with hydrogen peroxide in the non-aqueous methanol. The result was shown in Fig. 2.

As shown in Fig. 2, UV-vis spectroscopy showed that R_F—(DOBAA)$_n$—R_F–cytochrome *c* aggregate afforded an excellent catalytic activity for the oxidation of pinacyanol chloride with H_2O_2, although no catalytic activity was observed in the case of the absence of hydrogen peroxide or the absence of R_F—(DOBAA)$_n$—R_F.

In this way, it is strongly expected that our present R_F—(DOBAA)$_n$—R_F–cytochrome *c* aggregate is applicable to novel fluorinated biocatalyst for variety of organic synthetic reactions. Further studies are actively in progress.

References

[1] Wang J, Mao G, Ober CK, Kramer E. J Polym Prepr (Am Chem Soc, Div Polym Chem) 1997;38:953;
Su Z, Wu D, Hsu SL, McCarthy T. J Polym Prepr (Am Chem Soc, Div Polym Chem) 1997;38:951;
Elman JF, Johs BD, Long TE, Koberstein JT. Macromolecules 1994;27:5341;
Affrossman S, Bertrand P, Hartshorne M, Kiff T, Leonard D, Pethrick RA, et al. Macromolecules 1996;29:5432;
Affrossman S, Hartshorne M, Kiff T, Pethrick RA, Richards RW. Macromolecules 1994;27:1588;
Xu B, Li L, Yekta A, Masoumi Z, Kanagalingam S, Winnik MA, et al. Langmuir 1997;13:2447;
Zhang H, Hogen-Esch T, Boschet F, Margaillian A. Polym Prepr (Am Chem Soc, Div Polym Chem) 1996;37:731;
Sawada H. Chem Rev 1996;96:1779;
Sawada H, Kawase T. Yuki Gosei Kagaku Kyokai Shi 1999;57:291;
Sawada H. J Fluorine Chem 2000;101:315 [2000;105:219];
Sawada H, Kawase T. Kobunshi Ronbunshu 2001;58:147 and 255.
[2] Sawada H, Itoh N, Kawase T, Mitani M, Nakajima H, Nishida M, et al. Langmuir 1994;10:994;
Sawada H, Tanba K, Itoh N, Hosoi C, Oue M, Baba M, et al. J Fluorine Chem 1996;77:51.
[3] Sawada H, Yoshino Y, Kurachi M, Kawase T, Takishita K, Tanedani T. Polymer 2000;41:397.
[4] Sawada H, Yoshino Y, Kawase T, Fujimori K. Eur Polym J 2000;36:1533.

[5] Cowan DA, Plant AR. ACS Symp Ser 1992;498:86;
Dordick JS. Enzyme Microb Technol 1989;11:194.
[6] Inada Y, Matsushima A, Hiroto M, Mishimura H, Kodera Y. Meth Enzymol 1994;242:65;
Mabrouk PA. ACS Symp Ser 1997;680:118;
Okahata Y, Fujimoto Y, Ijiro K. J Org Chem 1995;60:2244;
Okazaki S, Kamiya N, Goto M. Biotechnol Prog 1997;13:551;
Otamiri M, Adlercreutz P, Mattiasson B. Biotechnol Bioeng 1994;43:987;
Sugimura Y, Fukunaga K, Matsuno T, Nakao K, Goto M, Nakashio F. Biochem Eng J 1998;2:137;
Vakurov AV, Gladilin AK, Levashov AV, Khmelnitsky YL. Biotechnol Lett 1994;16:175;
Murakami Y, Hirata A. Biotechnol Tech 1999;13:545;
Murakami Y, Hoshi R, Hirata A. Biotechnol Lett 2001;23:125;
Murakami Y, Hirata A. J Biosci Bioeng 1999;88:441.
[7] Tinaco R, Vazquez-Duhalt R. Enzyme Microb Technol 1998;22:8;
Souppe J, Urrutigoity M. New J Chem 1989;13:503;
Hamachi I, Fujita A, Kunitake T. J Am Chem Soc 1997;119:9096;
Vazquez-Duhalt R, Semple KM, Westlake DWS, Fedorak PM. Enzyme Microb Technol 1993;15:936.
[8] Yamada T, Kikawa K, Shinoda S, Tsukube H. Tetrahedron Lett 1999;40:6967;
Yamada T, Shinoda S, Kikawa K, Ichimura A, Teraoka J, Takui T, et al. Inorg Chem 2000;39:3049.
[9] Glenn JK, Gold MH. Arch Biochem Biophys 1985;242:329.