Influence of terminal structure of triethylene glycol-modified polyrotaxanes on their physicochemical properties and biomaterials functions

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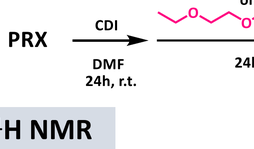
Introduction

β-Cyclodextrin (β-CD)

- Interact with cholesterol in the plasma membrane.
- Rapid renal excretion due to low molecular weight.
- The cellular internalization efficiency is typically low.

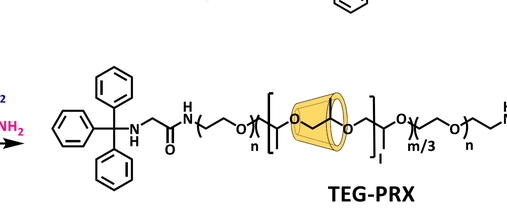
High dose required but causes side effects.



Polyrotaxane (PRX)

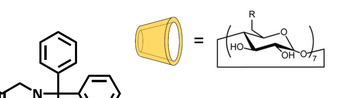
- Because the hydrophobic cavity of β-CDs is occupied with a polymer chain, the threaded β-CDs in the PRXs do not extract cholesterol from the plasma membrane, thus avoiding toxicity.
- High molecular weight avoids renal excretion.

We have proposed the use of PRX as a delivery carrier for β-CDs to reduce the toxicity and improve pharmacokinetics of β-CDs.



Chemical modification with hydrophilic groups

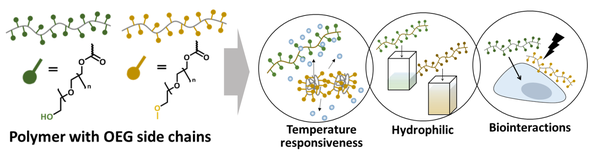
2-(2-hydroxyethoxy)ethyl carbamate group (HEE)



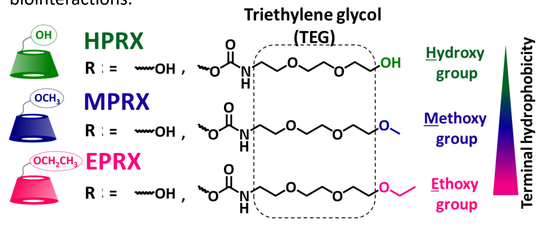
The chemical modification on PRXs is essential for these pharmacological applications because the PRXs are not soluble in aqueous media.

In this study...

The terminal structure of oligoethylene glycol (OEG) side chains is known to affect the temperature responsiveness, hydrophilic, and biointeractions of various OEG-grafted polymers.



We focused on triethylene glycol (TEG) chains for the chemical modification of PRXs and investigate their physicochemical properties and biointeractions.



Elucidating the structural effects of TEG-tethered chains in TEG–PRXs shall significantly aid the molecular design of OEG-modified PRXs as well as in improving the therapeutic effects of OEG-modified PRXs in cholesterol-relating metabolic diseases.

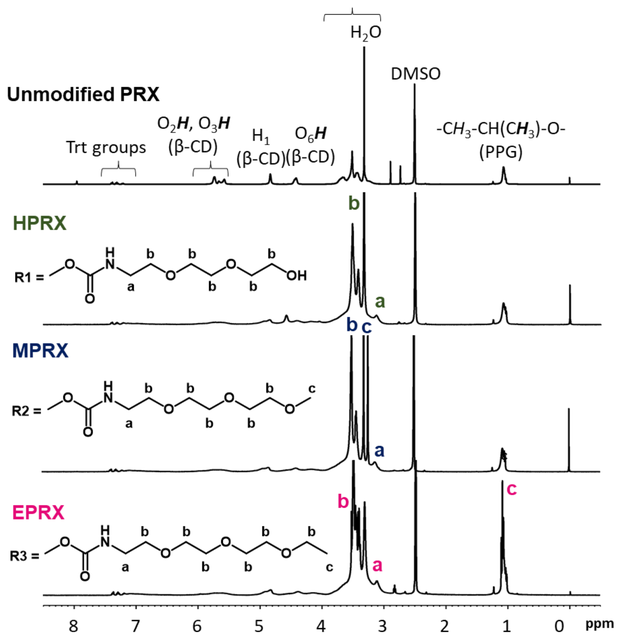
(1) Lutz, J.-F.; Akdemir, Ö.; Hoth, A., *J. Am. Chem. Soc.* **2006**, 128, 13046– 13047, (2) A. Tamura, N. Yui. *J. Control. Release* **2018**, 269, 148-158., (3) A. Tamura, M. Ohashi, N. Yui. *J. Biomater. Sci., Polym. Ed.*, **2017**, 28, 1124-1139., (4) A. Tamura, N. Yui. *Sci. Rep.* **2014**, 4, 4356

Synthesis and characterization of TEG-PRXs

Scheme

^{1}H NMR

- 400 MHz, $DMSO_{d6}$



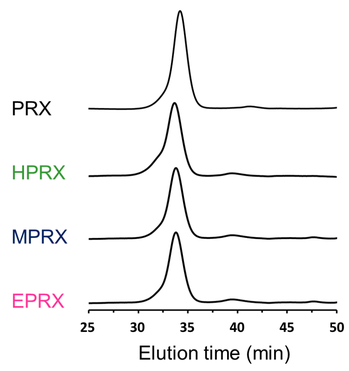
-CH_2-CH_2-O-(PEG)
-CH_2-CH(CH$_3$)-O-(PPG)
$H_{2\text{-}6}$(β-CD)

	terminal group of TEG-tethered chains	number of threading β-CDs	number of TEG-tethered chains on PRX (per β-CD)*	M_n	M_w/M_n
HPRX	-OH	12.3	49.2 (4.0)	28,700	1.23
MPRX	-OMe	12.3	55.4 (4.5)	30,600	1.23
EPRX	-OEt	12.3	71.3 (5.8)	34,800	1.21

*Determined by ^{1}H NMR in DMSO-d_6. The value in parentheses denotes the average number of TEG-tethered chains per threading β-CD in TEG–PRXs.

SEC charts

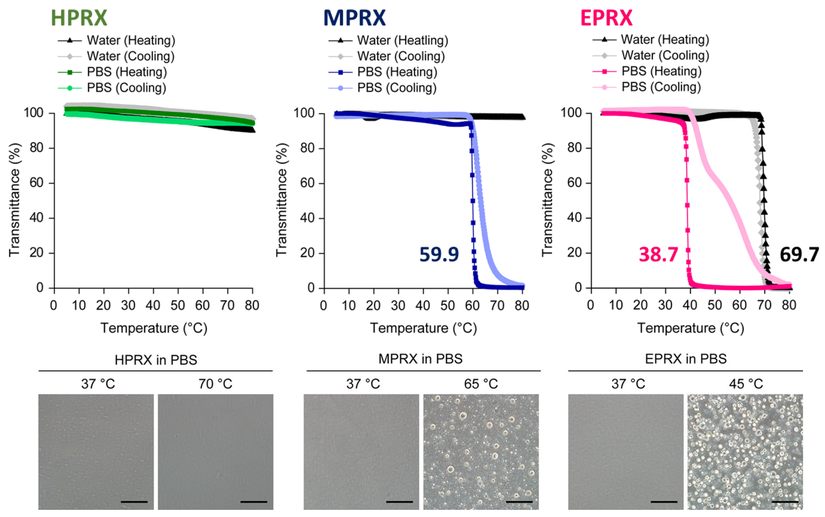
- TEG-PRXs (10 mg/mL in DMSO containing 10 mM LiBr)



Temperature responsivity of TEG–PRXs

Transmittance measurement

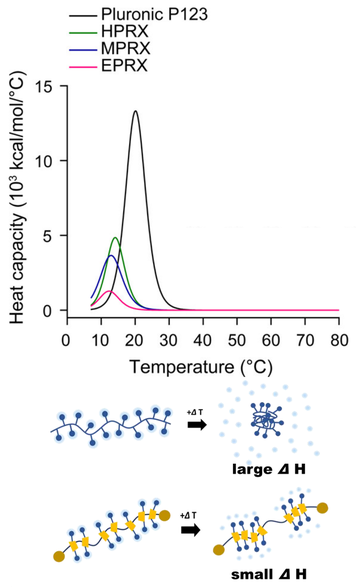
- TEG-PRXs (10 mg/mL in distilled water and PBS (10 mM NaH_2PO_4–Na_2HPO_4, 150 mM NaCl, pH 7.4))
- 600 nm using a V-550 UV–vis spectrophotometer
- Heating or cooling rate : 1.0 ° C/min



The T_{CP} values of TEG–PRXs varied depending on the terminal structure of TEG-tethered chains. This suggests that the main chain structure also affects the temperature-induced phase transition behaviors. Along with the temperature-dependent changes in transmittance, MPRX and EPRX formed coacervate droplets, spherical aqueous droplets comprising dense macromolecular solutions.

Differential scanning calorimetry (DSC)

- TEG-PRXs (10 mg/mL in PBS)

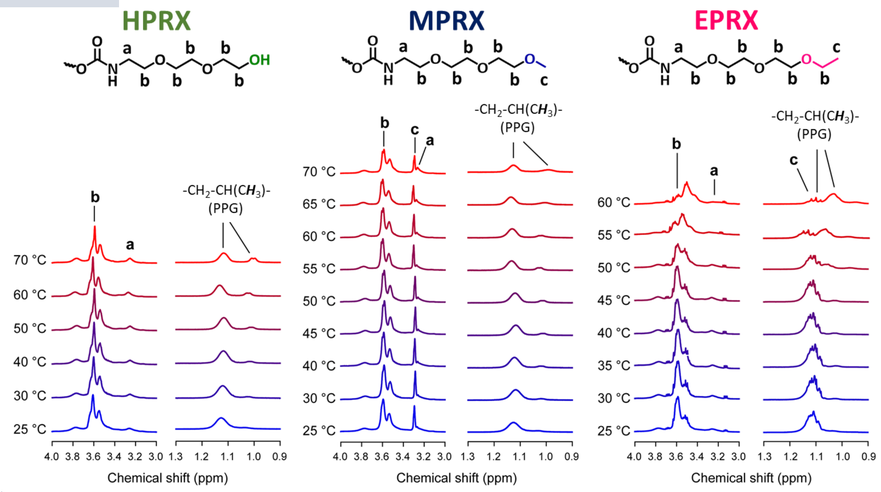


The TEG–PRXs did not undergo significant dehydration and conformational change in the temperature-induced phase transition.

Temperature-dependent structural change of TEG–PRXs

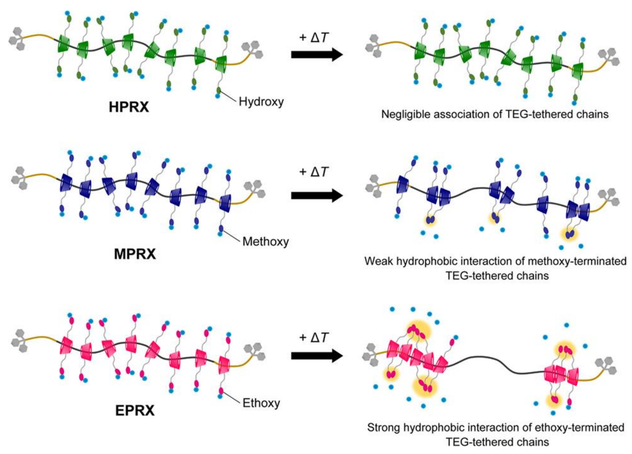
^{1}H NMR

- 400 MHz, deuterated PBS



For MPRX and EPRX, the intensity of TEG backbone (peak b) and the terminal group of the TEG-tethered chains (peak c) decreased slightly as the temperature increased.

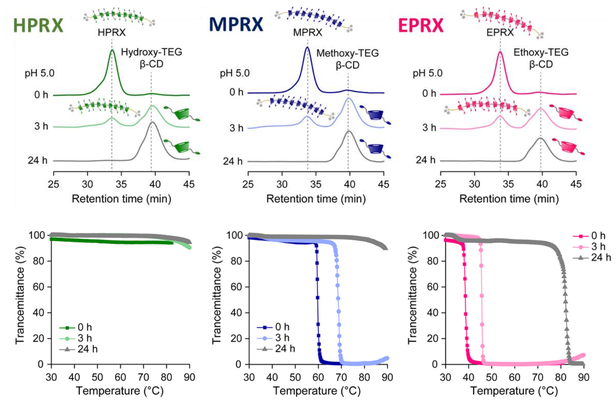
Molecular mechanism of phase transition



The TEG backbone occurred due to the association of the β-CDs along the polymer axle.

Degradation of TEG–PRXs

- SEC charts : TEG-PRXs (10 mg/mL in DMSO containing 10 mM LiBr)
- Phase-contrast micrographs Acetic acid buffer (pH5) (10 mg/mL)

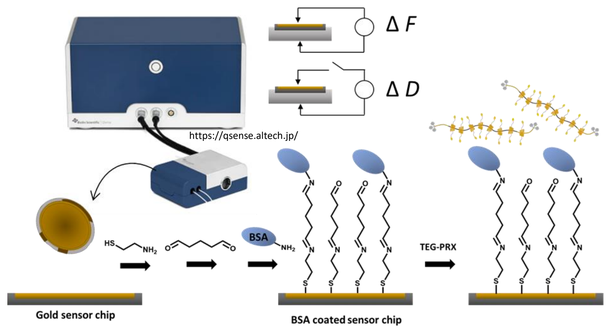


The supramolecular structure of the PRXs, wherein multiple β-CDs were interlocked along the axle polymer, contributed toward their temperature-induced phase transition at a lower temperature than the constituent free TEG-β-CDs.

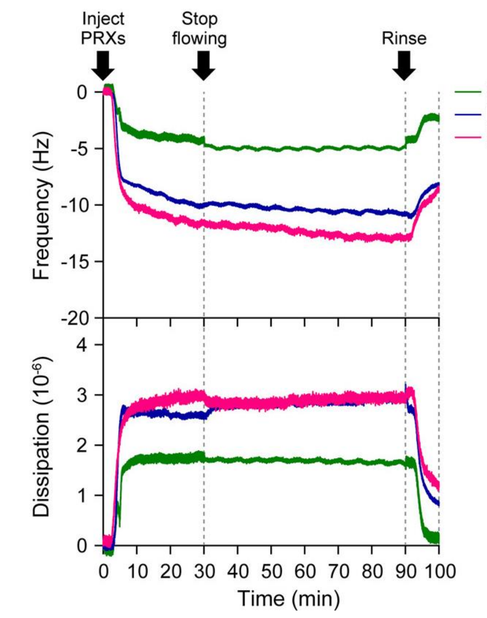
Biointeractions of TEG-PRXs

Microbalance with dissipation monitoring (QCM-D)

- BSA-immobilized gold surfaces treated with TEG-PRXs at 25 ° C.
- TEG–PRXs (1 mM threaded β-CD) in tris(hydroxymethyl)aminomethane (Tris) buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4)

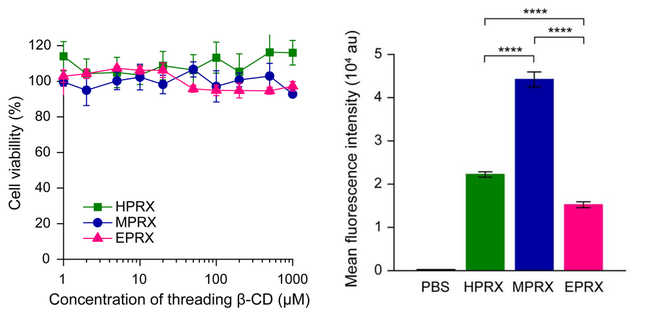


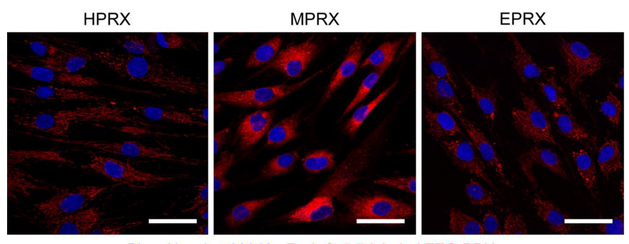
The ΔF and ΔD values of MPRX and EPRX were remarkably larger than those of HPRX, without any obvious difference in the interaction with BSA. These results suggest that the hydrophobic methoxy and ethoxy terminal groups of the TEG-tethered chains augmented the interaction with BSA.



Cell viability and Intracellular distribution

- Cell viability : Normal human skin fibroblasts (NHF) treated with HPRX, MPRX, and EPRX for 24 h.
- NHF cells treated with Cy5.5-HPRX, Cy5.5-MPRX, and Cy5.5-EPRX for 24 h at a concentration of 200 µM of threading β-CDs.



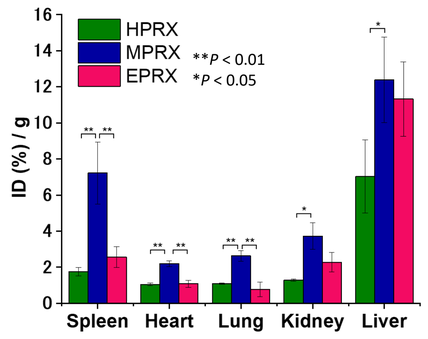


Blue: Hoechst 33342, Red: Cy5.5-labeled TEG-PRXs

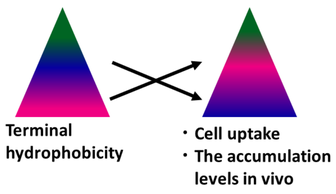
All Cy5.5-TEG-PRXs showed cellular uptake and the level of Cy5.5-MPRX was the highest.

Body distribution

- BALB/c, male, 10 weeks, 21~26 g/body, n=5
- Peritoneal administration, 200 µL/body,
- 200 mg/kg of Cy5.5-TEG-PRXs



The accumulation levels of MPRX in the liver was the highest among TEG-PRXs, consistent with the in vitro cellular uptake study.



The order of intracellular uptake levels and the accumulation levels of TEG–PRXs did not follow the hydrophobicity of terminal group structures.

Conclusion

- **The hydrophobicity of the terminal group structures of TEG-tethered chains on PRXSs affects the chemical and biological properties of the PRXs.**
- **Methoxy-terminated TEG is confirmed to be an effective chemical modification to improve the pharmaceutical applications of PRX.**
  - ✓ The methoxy-terminated TEG-PRX (MPRX) showed sufficient solubility in aqueous media and avoided the temperature-induced precipitation near the body temperature or culture temperature.
  - ✓ MPRX exhibited high interaction with model protein, intracellular uptake, and liver accumulation among three-series of TEG-PRXs.

Ohashi, M., Tamura, A., Yui. N., *Langmuir* **2021**, 37, 11102–11114