Transformable Supramolecular Materials for Reversible PEGylation of Protein Drugs

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Kumamoto University

Problem of Protein Drugs

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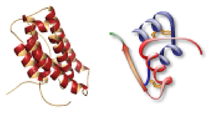
Problems of protein drugs

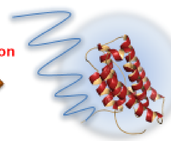


- Low-physicochemical stability ····· Quality
- Low-enzymatic stability ····· Activity
- Immunogenicity ····· Safety
- Low-blood retention ····· Activity
- High cost ····· Cost

PEG[1]ylation of protein 1) Polyethylene glycol

- Low-physicochemical stability
- Immunogenicity
- Low-blood retention

PEGylation

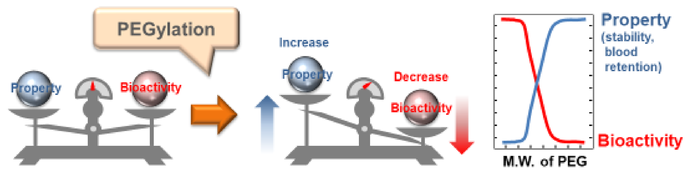


- Improvement of stability
- Decrease of immunogenicity
- Increase of blood retention

PEGylation is a promising method to improve these problems of proteins !!

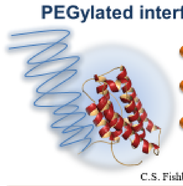
Problem of Bioactivity in PEGylated Proteins

2



Effect of PEGylation

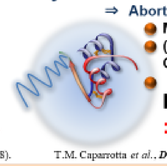
PEGylated interferon (Pegasys®)



- M.W. of PEG: 40 kDa (Branched)
- Circulating half-life : More than 73-fold
- Bioactivity : Reduction to 7%

C.S. Fishburn, *J. Pharm. Sci.*, 97, 4167-4183 (2008).

PEGylated insulin (LY2605541, Phase III) ⇒ Abort a project



- M.W. of PEG: 20 kDa (Linear)
- Circulating half-life : More than 10-fold
- Bioactivity : Reduction to 6%

T.M. Caparrotta *et al.*, *Diabetes Obes. Metab.*, 16, 388-395 (2014).

Decrease of bioactivity of protein caused by PEGylation is a serious problem.

Mixing-type PEGylation Materials

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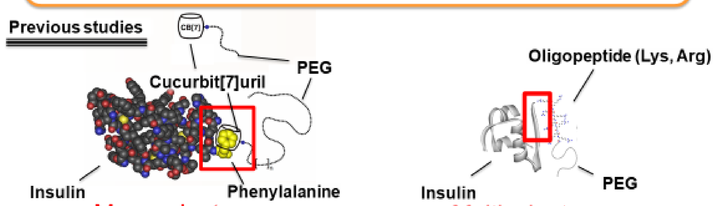
Recently, mixing-type PEGylation materials have attracted considerable attention due to their reversible properties.

Advantages of mixing-type PEGylation material

- Usability
- Retention of the bioactivity due to non-covalent interaction

Previous studies



M. Webber *et al.*, *Proc. Natl. Acad. Sci. USA*, 113, 14189-14194 (2016).
C.L. Maikawa *et al.*, *Nat. Biomed. Eng.*, 4, 507-517 (2020).
D. Tsiourvas *et al.*, *J. Colloid Interface Sci.*, 384, 61-72 (2012).

Mixing-type PEGylation materials which can form stable complex are desired.

Polyrotaxane (Transformable Polymer)

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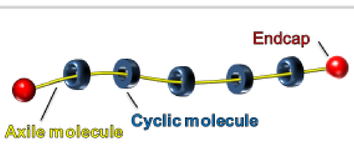


Polyrotaxane (PRX) 1) Cyclodextrin

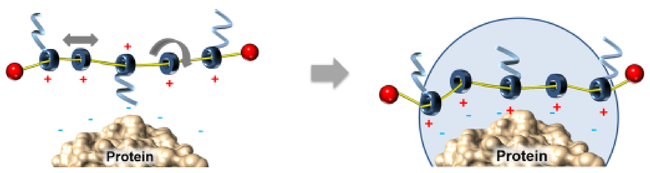
Interlocked molecule obtained by threading axle molecule through cyclic molecules (CyDs[1]) and capping their terminals with bulky compounds

A. Harada *et al.*, *Nature*, 356, 325-327 (1992).

- Flexible supramolecular material
- Movable properties of cyclic molecules

Modification of PEG & interactive group → *e.g. amino group*

PRX can transform corresponding to protein's shape and can interact strongly and multivalently !?



Strategy ~Reversible PEGylation Material~

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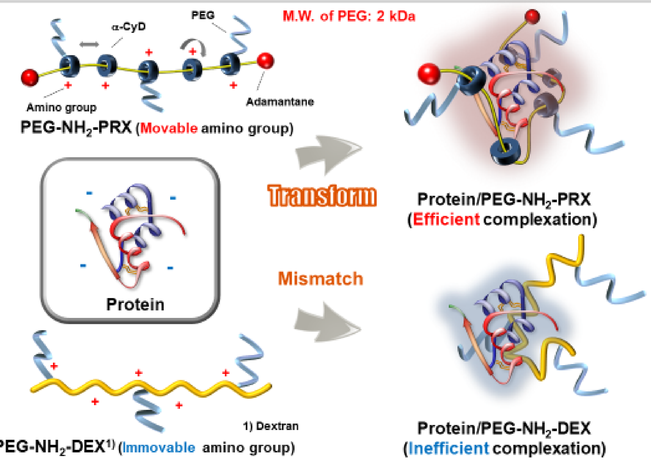

Purpose

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Development of Transformable Supramolecular Materials for Reversible PEGylation of Protein Drugs without Loss of Activity

Outline

- Complex formation of insulin/PEG-NH_2-PRX (Ultrafiltration)
- *In vivo* bioactivity of insulin/PEG-NH_2-PRX (Healthy and type 2 diabetic rats)
- Shaking stability of IgG/PEG-NH_2-PRX

Complex Formation of Insulin/PEG-NH_2-PRX

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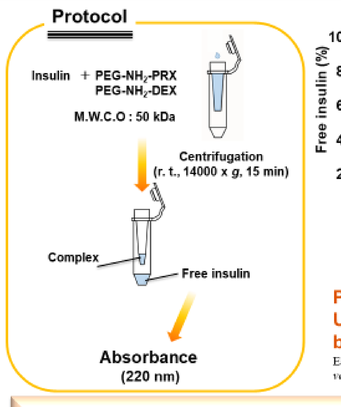

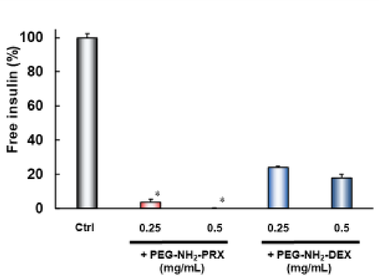

Permeation of Insulin through Ultrafiltration Membrane in Phosphate-buffered Saline

Each value represents the mean ± S.E. of 6 experiments. *p<0.05 *versus* PEG-NH_2-DEX.

PEG-NH_2-PRX could form complex with insulin more efficiently than PEG-NH_2-DEX.

In Vivo Bioactivity of Insulin/PEG-NH_2-PRX (Healthy Rats)

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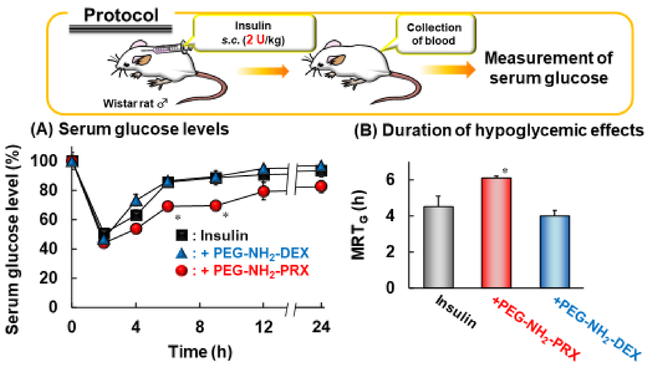

(A) Serum Glucose Levels and (B) Duration of Hypoglycemic Effects of Insulins after Subcutaneous Administration of Insulins to Healthy Rats

Each point represents the mean ± S.E. of 4 experiments. *p<0.05 *versus* Insulin.

In Vivo Bioactivity of Insulin/PEG-NH_2-PRX (Type 2 Diabetic Rats)

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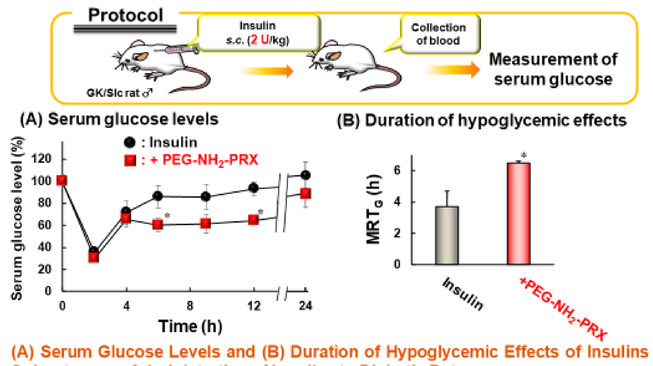

(A) Serum Glucose Levels and (B) Duration of Hypoglycemic Effects of Insulins after Subcutaneous Administration of Insulins to Diabetic Rats

Each point represents the mean ± S.E. of 4 experiments. *p<0.05 *versus* Insulin.

PEG-NH_2-PRX sustained hypoglycemic effect of insulin without loss of activity.

Shaking Stability of IgG/PEG-NH_2-PRX

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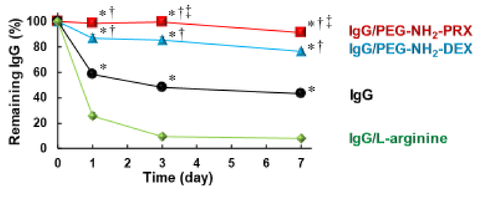
Antibody drug

- Weak against various stresses
  → Few additives which can improve especially shaking stress were reported.

IgG

S. Kiese *et al.*, *J. Pharm. Sci.*, 97, 4347-4366 (2008).
T. Kurinomaru *et al.*, *化学と生物* 53, 368-373 (2015).



Shaking Stability of IgG/PEG-NH_2-PRX in Phosphate-buffered Saline at 500 rpm

Each point represents the mean ± S.E. of 6 experiments. *p<0.05 *versus* IgG/L-arginine. †p<0.05 *versus* IgG. ‡p<0.05 *versus* IgG/PEG-NH_2-DEX.

PEG-NH_2-PRX improved the shaking stability of IgG compared to PEG-NH_2-DEX.

Conclusion

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- PEG-NH_2-PRX formed complex with insulin more efficiently than PEG-NH_2-DEX.
- PEG-NH_2-PRX sustained hypoglycemic effect of insulin in healthy and type 2 diabetic rats.
- PEG-NH_2-PRX improved the shaking stability of IgG compared to PEG-NH_2-DEX.

PEG-NH_2-PRX has the potential as a reversible PEGylation material for protein drugs without loss of activity.

Summary

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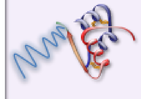
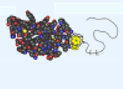
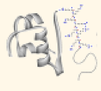
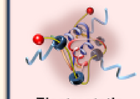
	LY2605541	PEG-cucurbit[7]uril	PEG-oligopeptide	PEG-NH_2-PRX
Schematic diagram				
Style of interaction	Covalent bond	Hydrophobic interaction	Electrostatic interaction	Electrostatic interaction
Interaction point	—	Monovalent	Multivalent	Multivalent (Transformable)
Bioactivity	Reduce	Retain	Not examined	Retain
Duration of Hypoglycemic effect	6 h	6 h	Not examined	12 h
Reference	R.A. Byrd *et al.*, *Toxicol. Pathol.*, 45, 402-415 (2017). ※ Abort a project at Phase Ⅲ	M. Webber *et al.*, *Proc. Natl. Acad. Sci. USA*, 113, 14189-14194 (2016). C.L. Maikawa *et al.*, *Nat. Biomed. Eng.*, 4, 507-517 (2020).	D. Tsiourvas *et al.*, *J. Colloid Interface Sci.*, 384, 61-72 (2012).	K. Utatsu *et al.*, *Mater. Today Bio*, 12, 100160 (2021).