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How to Improve in Solubility of Water-Insoluble Cyclic Peptide with HA nanogel : Over 100,000-Fold Solubilization

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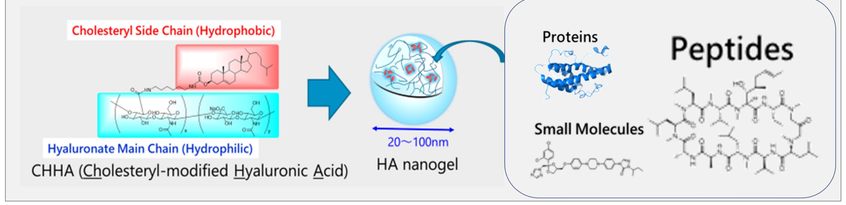
PURPOSE

Hyaluronic acid (HA) nanogel has hyaluronic acid in the main chain, a hydrophilic, biocompatible material with low toxicity, and a hydrophobic group in the side chain. A nano-ordered gel is spontaneously formed, due to its hydrophilic-hydrophobic interactions, in which it is possible to load small molecules, peptides, and proteins.(1,2) Unique characteristics are predicted, such as improved targeting ability, reduced toxicity and solubility of the active pharmaceutical ingredients (APIs). The general cyclic peptides have high potential as a novel modality with sufficient physicochemical properties, however sometimes encounter low kinetic solubility on some optimized peptides toward cell-permeable and oral peptide drugs.

Here in order to verify the compatibility of HA nanogel with cyclic peptide, we investigated the potential to enhance the solubility of these water-insoluble cyclic peptide, in addition we examined stability and injectability of HAnanogel formulation with Cholesteryl-modified Hyaluronic Acid (CHHA) as our HA nanogel.



METHOD(S)

For this study, we used CHHA whose degree of substitution by the cholesteryl group is 40 % in HA units, and whose molecular weight is 10 kDa.

Solubilization of cyclic peptide with HA nanogel and conventional solubilizer

The cyclic peptide powder was added to 1.8mL of CHHA solution in water and stirred with CHHA at 20 ℃ for 24 hours ([CHHA] = 16~24 mg/mL, [Peptide] = 2057~3994μM). Then, the solutions was filtered using a 0.22μm PES syringe filter. The solubility of peptide in HA nanogel formulation was analyzed using a high performance liquid chromatography. Loading capacity of peptide = [amount of encapsulated peptide] / [amount of CHHA].

The cyclic peptide A was combined with 10% Tween80 solution or 10% cyclodextrin derivative in the same manner as above hours ([solubilizer] = 100mg/mL, [Peptide A] = 909μM).

Measurement of viscosity

The viscosity of each HA nanogel formulation was determined at 23 ºC by using of an E-type Viscometer (type : RE85L, TOKI SANGYO, Japan)

RESULT(S)

Properties of PeptiDream's Cyclic Peptides

- Peptide A is the water-insoluble basic cyclic peptide (16 mer) and B is the water-insoluble neutral cyclic peptide (8 mer) including non-proteinogenic amino acids.
- **The formulation process is very simple and organic solvent free .**

Table 1. Basic physical properties of peptides

API Lot	Ring size (number of AA)	Net charge	Number of acidic AA	Number of basic AA	salt form	MF	MW	Kinetic sol (uM, pH7.4 in PB)	Remarks
PD_A	16	1	-1	2	2TFA	C108H147ClN24O22S	2201.02	0.03 (estimated by similar analogues)	Basic peptide with low solubility
PD_B	8	0	0	0	free	C53H97N9O9S	1036.47	0.03	Neutral peptides with low solubility

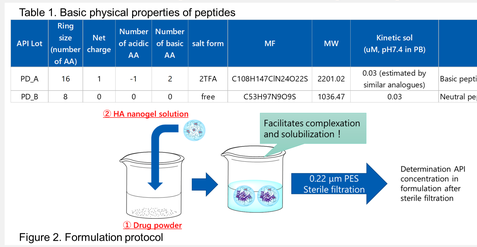


Figure 2. Formulation protocol

Over 100,000-Fold Solubilization with HA nanogel

- Water-insoluble cyclic peptides solution was stored at 20 ºC with/without HA nanogel.
- After 24 hr of stirring, Peptide was not dissolved at all in water, **while the HA nanogel containing Water-insoluble cyclic peptides was transparent.**
- This result indicates that HA nanogel can make a complex with cyclic peptides, which facilitates peptide solubilization.

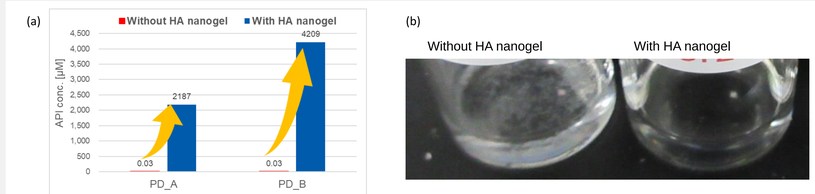


Figure 3. (a) Solubility of Water-insoluble cyclic peptides with (blue bar) and without (red bar) HA nanogel after incubating at 20 ºC for 24 hours. (b) The view of peptide B Formulation.

Formulation Viscosity and Injectability at 30G

- HA nanogel can be sterile filtered through a 0.22 μm filter.
- HA nanogel (27.5mg/mL) formulation is the low viscosity that can be passed through 30G.After solubilization, HA nanogel/peptide complex formulation viscosity was slightly higher than that of HA nanogel alone.

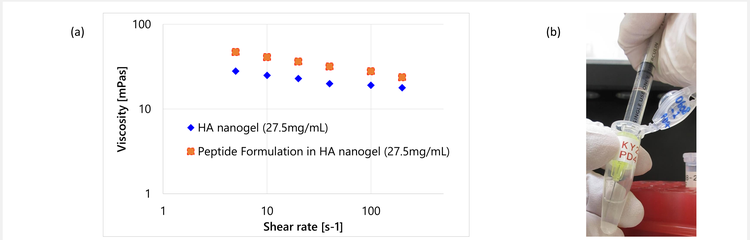


Figure 4. (a) Effect of shear rate on the viscosity with or without peptide A.
(b) HA nanogel formulation A and B can be aspirated with a 30G needle.

The Stability of HA nanogel Formulation

The stability of Tween80 formulation was dramatically poor at 40 ºC , 75%RH. The API concentration was less than half in one month. Two peaks derived from API were observed by HPLC.

- Cyclodextrin derivatives, one type of conventional solubilizer, showed no solubilizing ability at all.
- However, **the HA nanogel formulation showed a much higher stability**.
- HA nanogel (16mg/mL) can load 27% peptide A by weight.

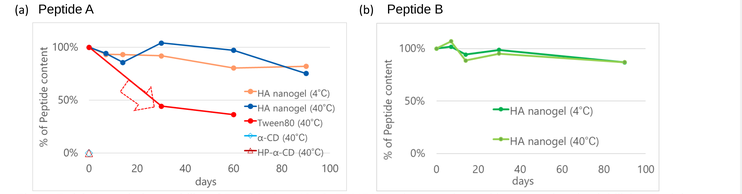


Figure 5. (a) Formulation stability of Peptide A at 40 ºC, 75%RH and comparison with conventional solubilizing excipients.
* HA nanogel (16mg/mL) formulation stability at 4 ºC, 75%RH in PP tube.
(b) HA nanogel (24mg/mL) Formulation stability of Peptide B at 4, 20, and 40℃, 75%RH in PP tube.

CONCLUSION(S)

From the above results, we have concluded that the water-insoluble basic or neutral cyclic peptide-containing HA nanogel formulation have enormous potential to enhance the solubility in water without organic solvent (DMSO, EtOH etc.). We believe HA nanogel can dramatically improve the solubility of your peptides and show a great impact on the patient's life and their family's life.

REFERENCES

1) Macromol. Biosci. 2012, 12, 475 – 483. 2) WO2010-053140

ACKNOWLEDGEMENTS

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