Core-shell Type Resins for SPPS

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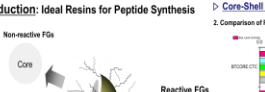
Abstract

Since Merrifield developed the solid-phase peptide synthesis (SPPS) method, gel type PS based resins, which still possess various short comings, have been used for peptide synthesis. For efficient peptide synthesis, various core-shell (CS) type resins [2-CTC resin, AM SURE resin, HiCore resin, etc] have been developed so that the reactive functional groups are mainly located on the shell layer of the resin for easy accessibility of reagents as well as efficient washing during SPPS. Due to this special structural feature, the CS type resins have shown high efficiency in various type of peptide synthesis, especially the difficult sequences, and photolytic cleavage reactions. When water compatible polymers such as polyethylene glycol (PEG) is grafted on PS resins they can be also used for on-bead screening of peptide ligand libraries. Recently, we have developed commercial scale production (~Kg scale) of CS PEG-g-PS resin (HiCore resin). Also, when properly designed, the HiCore resin could also be used for the purification of biomolecules.

Core-Shell Type Resins for Solid-phase Peptide Synthesis

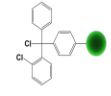
Introduction: Ideal Resins for Peptide Synthesis



Various Types of Core-Shell Type Resins

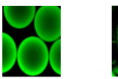
▷ Core-Shell Type BTCORE™ CTC

- Structure



- Production Scale : Multi-ton/ year
- Loading level : 0.8 ~1.0, 1.2~1.4 mmol/g resin

Cross-sectioned images of FITC-Trp-(Boc)-CTC resin



- 2-CTC (BTCORE™ CTC)
- 2-CTC (A Company)

- Performance

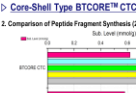
1. Synthesis of fully protected 3 fragments of T20 on BTCORE™ CTC

Sequence	Starting resin	Loading level (mmol/g)	Crude Yield	HPLC Purity
1-16	Fmoc-Gln-O-2-CTC-L	0.54	76%	84%
17-26	Fmoc-Leu-O-2-CTC-L	0.74	96%	95%
27-35	Fmoc-Trp(Boc)-O-2-CTC-L	0.69	92%	91%

- Fmoc-AA-OH (2eq.), HBTU/HOBt (2eq.), 30°C, 1h, without double coupling
- Cleavage : 1%TFA/DCM

▷ Core-Shell Type BTCORE™ CTC(continued)

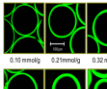
2. Comparison of Peptide Fragment Synthesis (27-35mer of T20)



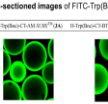
▷ AM SURE™ resin

- Structure

Thickness can be controllable in AM SURE™ resin



Cross-sectioned images of FITC-Trp(Boc)-CTC-AM Resins



Preparation of the Fmoc-Trp(Boc)-OH loaded CTC AM resins

- Performance

1. Synthesis of ACP (65-74) on Fmoc-Gly-CTC-AM resins

- Sequence : Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly
- Condition : purity after cleavage: 99% (CS) vs 79% (non CS)

▷ HiCore™ resin

- Structure & Property

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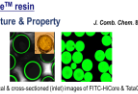
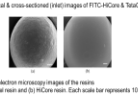
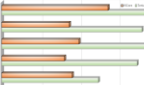


Confocal & cross-sectioned (inlet) images of FITC-HiCore & TentaGel.



Scanning electron microscopy images of the resins
(a) TentaGel resin and (b) HiCore resin. Each scale bar represents 10 μm.

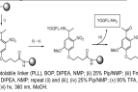
Nonspecific Protein Bindings on HiCore™ Resin



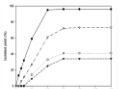
Bound proteins on HiCore (■) & TentaGel (■)

- Performance

Photocleavage Reactions on HiCore™ Resin



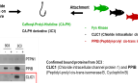
i) Fmoc-photolabile linker (PLL), BOP, DIPEA, NMP. ii) 20% Pip/NMP. iii) Fmoc-AA, BOP, HOBt, DIPEA, NMP, repeat (ii and iii). iv) 25% Pip/NMP. v) 95% TFA, 2.5% TES, 2.5% H2O. (vi) hv, 360 nm, MeOH.



Kinetic profiles of photolytic cleavage on each resin; from the top, (●) HiCore resin, (○) TG resin, (□) LL PS resin, (▶) HL PS resin

- **Core-shell structure : high efficiency in photo-cleavage reaction.**

Fishing proteins with peptide ligand on HiCore™ Resin



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Papers in prep (2024)

- Atopic Dermatitis related target proteins were identified from CA-PH & 3C3 attached **HiCore resin** by pull down assay method from cell extract

Summary

- Core-shell type resins are ideal solid supports for the peptide synthesis.
- Various kinds of core-shell type resins were developed for solid-phase peptide synthesis and bio-assay.
- Reaction kinetics of AA loading, couplings, and photolysis are superior compared to gel type resin because FGs are mostly located on the surface layer.
- Peptides with difficult amino acid sequences can be synthesized well on core-shell type resins with high purity.
- Peptide libraries can be assembled and screened successfully on HiCore resin.
- BeadTech Inc. is currently producing multi-ton scale BTCORE™ CTC and multi-kg scale HiCore™ Resin.

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