Difficult Peptides

PEGylated Peptides

A recently discovered potential 23-mer peptide therapeutic (SBP1) is a binder to the spike protein of the SARS-CoV-2 virus. Easy access to such peptide sequences with options for further modification is essential to support the global efforts in research labs worldwide to fight against emerging diseases like COVID-19. In this study, we demonstrate the chemical synthesis and purification of N-terminally modified SBP1 with Biotin-$(PEG)_5$ for array applications with our PEC Research Kit. No chromatographic instrumentation is required, also enabling less-equipped lab environments to work with such otherwise inaccessible materials. The modified peptide proved to be functional in the desired assay.

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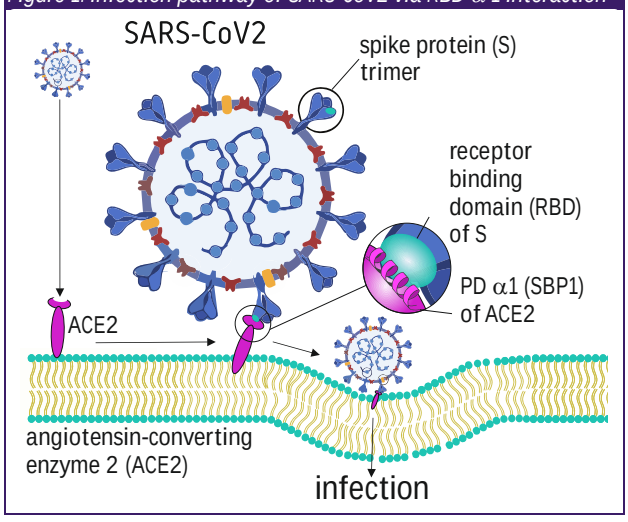
1. Introduction

The coronavirus disease 19 (COVID-19) caused by the new virus SARS-CoV-2 took over 303,000 lives worldwide (https://coronavirus.jhu.edu/map.html, as of 15th of May 2020). Recent studies revealed that both virus types, the severe acute respiratory syndrome coronavirus (SARS-CoV), and SARS-CoV-2 use the cellular receptor angiotensin-converting enzyme 2 (ACE2) to enter host cells. On the surface of the virus, spike protein trimers contain receptor-binding domains (RBD) on their tips. RBD binding to ACE2 causes internalization of the viral genes and infection of the host cell (Figure 1).[1]

Figure 1: Infection pathway of SARS-CoV2 via RBD α1 interaction



The workgroup of Brad Pentelute has found a sequence (spike protein binding peptide 1, SBP1) from the ACE2 PD α1 helix that is a strong binder to the RBD of the spike protein.[2]
Scienion AG, an industry-leading expert in array fabrication technologies, rapidly needed the SBP1 sequence baring biotin on its *N*-Terminus. A PEG-spacer should separate SPB1 from the biotin to ensure sufficient binding.
In this study, we show how to manufacture this modified peptide in a fast single PEC purification run. Also, the instrument-free access to such difficult peptides highlights the potential of our PEC technology, helping to make complex peptide-based study specimens available in less-equipped lab environments worldwide.

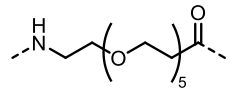
2. Method

Synthesis. The peptide (Table 1) was synthesized in a 100 µmol scale on Rink Amide AM Polystyrene resin. Single (≤13 aa) and double coupling (>13 aa) of amino acids (45 min. each coupling with 3 eq Fmoc-aa-OH, 3 eq Oxyma and 4 eq DIC) were applied. The Fmoc-$(PEG)_5$-OH and Fmoc-Lys(biotin)-OH building blocks were coupled manually each for 1 h. Capping after each coupling step (2 M Ac_2O, 2 M pyridine in DMF 1x5 min) ensured the selective coupling of the PEC-Linker in the last step only on the full-length peptide.
Linker RC+ (4 eq.) was coupled using DIPEA (6 eq.) and Oxyma (4 eq) in DMF for 2 h. TFA cleavage was performed for 2 h using 10 mL TFA/H_2O/DTT/TIS (84:8:6:2) .

Table 1: Pegylated peptide used in this study

ID	sequence	crude purity	final purity
#1	H_2N-K(biotin)-$(PEG)_5$-IEEQAKTFLDKFN-HEAEDLFYQS-$CONH_2$	18%	85%

-$(PEG)_5$- =



PEC purification (according to V2.1). The crude material was dissolved in 4.5 mL DMSO. Then, 500 µL citric acid/GdmCl buffer (0.1 M/7 M; pH 3.5) was . The dissolved peptide was added to 3 mL pre-conditioned suspension of activated filter material (150 µmol aldehyde content), and the immobilization was performed for 90 min. 0.5 ml 2 w% L-Cys in a citric acid buffer (pH = 4.5) was then added to block unreacted aldehydes and displace imines (15 min incubation time) before washing off impurities.

The linker-modified peptide was incubated for 15 min with 500 mg DTT in 10 mL MeCN / 5 w% aq. $NaHCO_3$ (1:1) to reduce the azide of the linker. The activated filter material was washed to remove excess of DTT and formed DTT(ox).

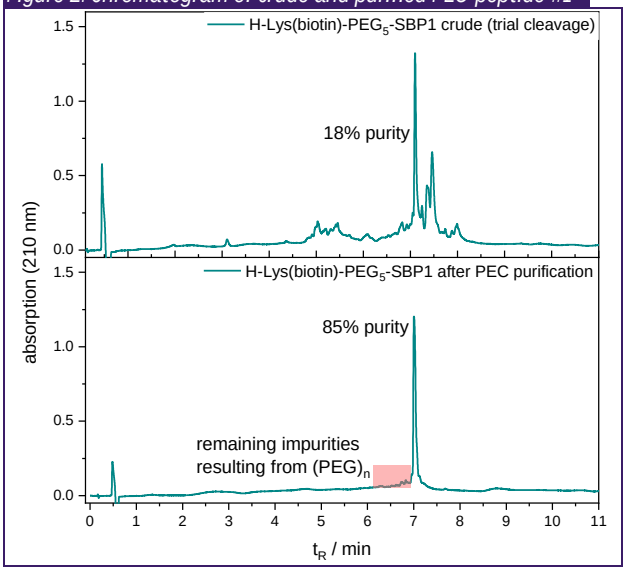
The PEC-Linker cleavage was initiated by adding TFA/H_2O (2:3). After 60 min, 0.2 mL TFA was added, and the filtrate was collected in 15 mL tubes. Each sample was washed/eluted twice with 0.2 mL 95% aq. TFA. Chilled diethyl ether was used to precipitate the peptides before lyophilization.

Analysis. UPLC-UV chromatograms and mass spectra were recorded with an analytical Acquity H-Class UPLC-ESI-MS system from Waters on a C-18 column (1.7 µm, 2.1 x 500 mm). As the mobile phase, mixtures of water (A) and MeCN (B) with 0.1% TFA were used.

3. Discussion

Synthesis of Biotin-PEG_5-SBP1. Trial cleavage after coupling of Fmoc-$(PEG)_5$-OH and Fmoc-Lys(biotin)-OH showed successful assembly of the desired product. However, we determined an overall low crude purity of 18% (Figure 2, top) with closely eluting impurities. The purity decrease most likely occurred during Fmoc-$(PEG)_5$-OH coupling since the purity before the coupling of this residue was higher (>70%), as determined by trial cleavage.

Figure 2: Chromatogram of crude and purified PEG-peptide #1



Despite the presence of the PEG-spacer and the hydrophobic biotin moiety, no particular issues during the PEC process occurred, and the routine procedures came to action. For example, the dissolution of the crude linker-modified peptide precipitate in DMSO worked well. Neither we observed specific problems during the loading of the activated filter material.

We obtained 31 mg of the purified peptide after ether precipitation with a product purity of 85%. The remaining impurities were conjugates with PEG-spacer of different lengths (n = 2, 3, 4, and 6) arising from polydisperse polymer building blocks.

Difficult PEGylated peptides. PEGylation has aggravating effects for purification by reversed-phase high-pressure liquid chromatography (RP-HPLC).[3] The reasons are (a) nonuniform polymers and (b) complex modulation of the retention time through the PEG-chain. Using PEC, the latter becomes irrelevant because of the chemo-selective separation principle. The dispersity of the PEG raw material can, however, be critical to the procedure, supported by the remaining impurity profile of the purified product. Yet, the peptide showed excellent binding behavior towards RBD in the nanomolar range, which further confirmed the previous findings.[2]

Machine-free purification of difficult peptides. This study reveals a significant benefit of PEC technology because it doesn't rely on chromatographic machinery. The PEC method works with chemicals, handled in fritted cartridges. Therefore, given that the supply of raw materials is possible, the manufacturing of purified complex peptides becomes feasible in organic synthesis or biochemical labs without access to chromatographic equipment.

4. Results at a glance

- obtain purified SARS-CoV2 inhibitor peptides with ready-to-use Research Kit
- apply the PEC routine procedure for PEGylated difficult peptides
- use monodisperse PEG building blocks for further purity optimization

References

[1] Wrapp D.; *et al. Science* 2020, 367, 1260-1263.
[2] Zhang G.; Pomplun S.; Loftis A. R.; Loas A.; Pentelute B. L. *bioRxiv* 2020.
[3] Park E. J.; Na D. H. *Analytical Chemistry* 2016, 88, 10848-10853.

About Belyntic

Belyntic GmbH is a chemistry-for-healthcare enterprise focusing on the purification of biopolymers, especially peptides. Belyntic offers the world's first broadly applicable peptide purification kits as well as development and implementation services of its proprietary Peptide Easy Clean (PEC) technology.

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