Upload image

Aug 10, 2020

Conjugation of peptide fragment 579-601 of HIV-gp41 or fragments 308-331 or 421-438 of the HIV-gp120 with keyhole limpet hemocyanin (KLH).

Angel A Justiz-Vaillant[1]

[1]University of the West Indies St. Augustine

1 Works for me dx.doi.org/10.17504/protocols.io.bjhukj6w

University of the West Indies angel.vaillant@sta.uwi.edu

Angel Justiz-Vaillant
University of the West Indies St. Augustine

ABSTRACT

Chemical synthesis facilitates the generation of peptides which are very difficult to express in bacteria, peptide/protein backbone modification, the incorporation of unnatural amino acids, and the production or synthesis of D-proteins.

The C-terminal cysteine can be added to the amino acidic sequences of HIV peptides (fragment 579-601 of the HIV-gp41 and fragments 308-331 or 421-438 of the HIV-gp120). These peptide fragments were dimerized by cysteine oxidation with dimethyl-sulfoxide [1] to faciliutate their conjugation to keyhole limpet hemocyanin that acts as a carrier protein.

Reference:
1. Tam JP, Wu CR, Liu w, Zhang JW (1991) Disulfide bond formation in peptides by dimethyl sulfoxide. Scope and applications. J Am Chem Soc 113: 66576662.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Angel Justiz-Vaillant. Conjugation of peptide fragment 579-601 of HIV-gp41 or fragments 308-331 or 421-438 of the HIV-gp120 with keyhole limpet hemocyanin (KLH)..**protocols.io**https://dx.doi.org/10.17504/protocols.io.bjhukj6w

DOI

dx.doi.org/10.17504/protocols.io.bjhukj6w

PROTOCOL CITATION

Angel A Justiz-Vaillant 2020. Conjugation of peptide fragment 579-601 of HIV-gp41 or fragments 308-331 or 421-438 of the HIV-gp120 with keyhole limpet hemocyanin (KLH).. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjhukj6w

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Angel Justiz-Vaillant. Conjugation of peptide fragment 579-601 of HIV-gp41 or fragments 308-331 or 421-438 of the HIV-gp120 with keyhole limpet hemocyanin (KLH)..**protocols.io**https://dx.doi.org/10.17504/protocols.io.bjhukj6w

CREATED

Aug 10, 2020

LAST MODIFIED

Aug 10, 2020

PROTOCOL INTEGER ID

40212

GUIDELINES

The Protocol has a high level of reproducibility and has worked for many other HIV peptides.

MATERIALS

NAME	CATALOG #	VENDOR
Glutaraldehyde EM Grade 25%	G5882-50ML	Sigma Aldrich
10mg KLH (Keyhole Limpet Hemocyanin) (Immunological Grade)	786-088	G-Biosciences
Peptide 579-601 of HIV-gp41		
Peptide 308-331 HIV-gp120		
Peptide 421-438 HIV-gp120		

SAFETY WARNINGS

Side effects include skin irritation, nausea, headache, and shortness of breath.

BEFORE STARTING

Ensure that the HIV peptide requires a terminal cysteine, so a disulfide bond formation in the peptides by dimethyl sulfoxide is needed before the conjugation to KLH. For example, fragment 254-274 of the second conserved domain of HIV gp120 has a terminal cysteine, and this peptide can be conjugated to KLH without previous treatment with dimethyl sulfoxide.

1 These peptide fragment (579-601 from HIV-gp41) is dimerized by cysteine oxidation with dimethyl-sulfoxide. The HIV peptide is dissolved in 5% acetic acid to a final concentration of 5.1 mg/ml.

2 The pH of the medium is adjusted to 6 with 1 M (NH4)2CO3.

3 Dimethyl-sulfoxide is added to 20% of the final volume, and after four hours at room temperature (RT), the solute is extracted.

4 Then, the peptide is dissolved in 3 ml 5% trifluoroacetic acid and precipitated with 35 ml cold ether.

5 The precipitate is dialyzed against 1.2 liters of deionized water, pH 7 at 4°C overnight.

6 Then, 1 mg of keyhole limpet hemocyanin (KLH) is diluted in 2.1 ml 0.1 M borate buffer (1.24 g boric acid, 1.90 g sodium tetraborate, pH 10, in 500 mL deionized water).

7 In a 20 ml glass tube, with a gentle stirring, 1.1 µmol of the HIV synthetic peptide (with C-terminal cysteine added) and 0.22 mL 0.3% glutaraldehyde solution (ACS reagent grade, pH 5.5, Sigma-Aldrich) are slowly mixed at RT and left to

Citation: Angel A Justiz-Vaillant (08/10/2020). Conjugation of peptide fragment 579-601 of HIV-gp41 or fragments 308-331 or 421-438 of the HIV-gp120 withÃÂ keyhole limpet hemocyanin (KLH).. https://dx.doi.org/10.17504/protocols.io.bjhukj6w

stand for 1.50 hrs.

8 When a yellow coloration is observed this indicates that the conjugation process is successful.

9 To blocking the excess of glutaraldehyde, 0.26 ml of 1 M glycine (Sigma-Aldrich) is added.

10 The mix is left for 32 min at RT.

11 The HIV-hemocynin conjugate is then dialyzed against 1.3 liters 0.1 M of borate buffer, pH 8.4 through the night at 4°C.

12 Then the previous buffer is used to dialyze the preparations for 8 hrs at 4°C.

13 The dialysates is stored at 4°C until further use.

Citation: Angel A Justiz-Vaillant (08/10/2020). Conjugation of peptide fragment 579-601 of HIV-gp41 or fragments 308-331 or 421-438 of the HIV-gp120 withÃÂ keyhole limpet hemocyanin (KLH).. https://dx.doi.org/10.17504/protocols.io.bjhukj6w