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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).

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Angel A Justiz-Vaillant[1]

[1]University of the West Indies St. Augustine

University of the West In... angel.vaillant@sta.uwi.e...

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

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Protocol status: Working
We use this protocol and it's working

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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.

Guidelines

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

Materials

MATERIALS

- Glutaraldehyde EM Grade 25% **Sigma Aldrich Catalog #**G5882-50ML
- 10mg KLH (Keyhole Limpet Hemocyanin) (Immunological Grade) **G-Biosciences Catalog #**786-088
- 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 start

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 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.