Click Chemistry Protocols

Introduction

Classical click chemistry uses copper. Copper (I) catalyzes the 1,3-dipolar cycloaddition of alkynes with azides to form 1,2,3-triazoles.[1,2] The sources of copper (I) include: copper (I) iodide, copper (I) bromide, copper filings (copper) or copper sulfate (CuSO4), and reducing agents. However, due to the thermodynamic instability of Cu (I), it can be easily oxidized to inactive Cu (II), so it is usually necessary to use suitable chelating ligands to prepare copper catalysts.

TBTA covers some practical applications of click chemistry other than water binding reactions. The water-soluble tris (3-hydroxypropyltriazolylmethyl) amine (THPTA) click ligand further simplifies click chemistry by allowing the entire reaction to proceed in water, providing biocompatibility for click reactions.[3,4] The binding of THPTA's ligand to Cu (I) blocks the bioavailability of Cu (I) and improves potential toxic effects while maintaining the catalytic effect of click coupling. THPTA ligands can effectively label living cells while maintaining cell viability. [5]

In our hands, we found that THPTA is a highly efficient, one-click chemical ligand in a partially complete aqueous reaction. Marking can be done in just 15-30 minutes at room temperature. After at least one month of freezing, the ligand CuSO4 complex showed no loss of activity.

Example Protocols

This section contains some general reactions for click reactions. These can be used as a starting point for optimizing your specific click chemistry program.

Oligonucleotide and DNA labeling

1. Prepare the following stock solutions

- 200 mm THPTA ligand in water.
- 100 mm CuSO4 in water.
- Alkyne-labeled oligomers in water.
- 100mm sodium ascorbate in water
- DMSO / TBUOH or 10 mm azide in water

2. A few minutes before the reaction, CuSO4 and THPTA ligands were incubated at a ratio of 1: 2. This solution is stable after several weeks of freezing.
3. Add excess azide (4-50 eq) to the oligonucleotide / DNA solution.
4. Add 25 equivalents of THPTA / CuSO4.
5. Add 40 equivalents of sodium ascorbate.
6. Let the reaction stand at room temperature for 30-60 minutes.
7. Ethanol precipitation of oligomers or purifications.

Labeling of cell lysates

1. Prepare the following to prepare the following solution:

- 100 mM THPTA ligand in aqueous buffer or water.

- 20 mM CuSO4 in water.
- 300 mM sodium ascorbate in water.
- 2.5 mM alkyne or azide labeling reagent in water or DMSO

2. For each azide or alkyne-modified protein lysate sample, add the following sample to a 1.5 mL microcentrifuge tube and centrifuge briefly to mix.

- 50 µL of protein lysate (1-5 mg / mL) in protein extraction buffer.
- 90 µL PBS buffer.
- 20 µL of 2.5 mM corresponding azide (or alkyne) detection reagent in DMSO or water.

3. Add 10 µL of 100 mM THPTA solution, vortex briefly to mix.
4. Add 10 µL of 20 mM CuSO4 solution, vortex briefly to mix.
5. Add 10 µL of 300 mM sodium ascorbate solution to initiate click reaction, vortex briefly to mix.
6. Protect reaction from light and allow click reaction to incubate for 30 minutes at room temperature.
7. Proteins in lysate are now click labeled and ready for downstream processing and/or analysis.

Reference

[1] Rostovtsev, V. V., et al (2002). A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective “Ligation” of Azides and Terminal Alkynes. Angew. Chem. Int. Ed., 41(14), 2596–2599.

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[3] Chan, T.R., et al (2004). Polytriazoles as Copper(I)-Stabilizing Ligands in Catalysis. Org. Lett., 6(17), 2853-2855.

[4] (a) Presolski, S. I., et al (2011). Copper-Catalyzed Azide–Alkyne Click Chemistry for Bioconjugation. Current Protocols in Chemical Biology. 3(4), 153–162. (b) Hong, V., et al (2009). Analysis and Optimization of Copper-Catalyzed Azide– Alkyne Cycloaddition for Bioconjugation. Angew. Chem. Int. Ed., 48(52), 9879–9883.

[5] Hong, V., et al (2010). Labeling Live Cells by Copper-Catalyzed Alkyne–Azide Click Chemistry. Bioconjugate Chem., 21(10), 1912-1916.