Virus Research 256 (2018) 21–28

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Review

Recent trends in click chemistry as a promising technology for virus-related research

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ARTICLE INFO

Keywords:
Click chemistry
Virus
Labeling
Biosensor
Delivery
Diagnosis

ABSTRACT

Click chemistry involves reactions that were originally introduced and used in organic chemistry to generate substances by joining small units together with heteroatom linkages (C-X-C). Over the last few decades, click chemistry has been widely used in virus-related research. Using click chemistry, the virus particle as well as viral protein and nucleic acids can be labeled. Subsequently, the labeled virions or molecules can be tracked in real time. Here, we reviewed the recent applications of click reactions in virus-related research, including viral tracking, the design of antiviral agents, the diagnosis of viral infection, and virus-based delivery systems. This review provides an overview of the general principles and applications of click chemistry in virus-related research.

1. Introduction

Click chemistry describes reactions originally introduced and used in organic chemistry to generate substances by joining small units together with heteroatom linkages (C-X-C) (Kolb et al., 2001). Over the last few decades, click chemistry has been widely used in bioscience fields, such as chemical biology, drug development and bionanoparticles, as a promising tool to modify biomolecules, such as DNA, protein and virions (Ahmad Fuaad et al., 2013; Avti et al., 2013; Jewett and Bertozzi, 2010; Kolb et al., 2001; Li et al., 2013; Liu et al., 2017; Moses and Moorhouse, 2007; Thirumurugan et al., 2013; Yi et al., 2018).

There are two main steps in the reaction to efficiently synthesize biomolecules or substances by joining substrates of small molecules with specific biomolecules: 1) biomolecules A and B are labeled with azide and alkyne functional groups via click chemistry, respectively, and 2) azide-labeled molecule A and alkyne-labeled molecule B are conjugated to form a stable conjugate (Himo et al., 2005; Kolb et al., 2001; Liu et al., 2017; Moses and Moorhouse, 2007; Salic and Mitchison, 2008). Copper-catalyzed alkyne-azide cycloaddition (CuAAC) is one of the most widely used click reactions, which reacts efficiently at room temperature and is stable to most functional groups to generate stable products (Kolb et al., 2001; Moses and Moorhouse, 2007). To label cells *in situ* using CuAAC, an azide or alkyne is conjugated to a biomolecule in the cell, such as a nucleic acid, nucleoside, amino acid, monosaccharide or fatty acid, which is termed the biosynthetic incorporating reaction (Amblard et al., 2009; Salic and Mitchison, 2008). Subsequently, the complementary alkyne or azide labeled with the reporter group is linked with the biomolecules via click chemistry in the presence of catalytic copper(I) (Amblard et al., 2009; Salic and Mitchison, 2008). However, although the CuAAC reaction is effective for bioconjugation, the reaction is limited in live cells due to that the copper, as a catalyst, is cytotoxic. Thus, some copper-free click chemistry methods have been developed, such as Cu-free alkyne-azide cycloaddition (Jewett et al., 2010), strain-promoted alkyne-azide cycloaddition (SPAAC) (Sachin et al., 2012), strain-promoted inverse-electron-demand Diels-Alder cycloaddition (SPIEDAC) (Nikic et al., 2014), the thiol–ene reaction (Hoyle and Bowman, 2010), *etc*.

Using click chemistry, fluorophores or other reporter molecules attach to the specific biomolecules, allowing the biomolecules to be identified, located and characterized. Recently, click chemistry has been widely used in virus-related research (Best, 2009; Bruckman et al., 2008; Cowan et al., 2012; Gao et al., 2012; Generous et al., 2014; Kalveram et al., 2013; Lu et al., 2015; Nwe and Brechbiel, 2009; Rubino et al., 2012; Wang et al., 2018). In this review, we will introduce the application of click chemistry in virus-related research, including viral tracking, the design of antiviral agents, the diagnosis of viral infection, and virus-based delivery systems. Furthermore, the advantages and disadvantages of click chemistry for virus-related research were also discussed. This review provides an overview of the general principles and applications of click chemistry in virus-related research.

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https://doi.org/10.1016/j.virusres.2018.08.003
Received 8 June 2018; Received in revised form 27 July 2018; Accepted 2 August 2018
Available online 03 August 2018

2. Click chemistry in virus-related research

2.1. Viral tracking

To date, due to the lack of knowledge of viral infection, the pathogenesis of most viruses as well as the interaction between the virus and host cells is still unclear (Liu et al., 2017). Using click chemistry, viral protein and the virion can be clicked rapidly and quantitatively with small and highly stable tags, allowing the visualization of dynamic changes in labeled viral proteins or virions in cells and providing more knowledge of the interactions between the host cells and virus.

A classical click reaction for viral labeling is a protocol described by Bruckman (Bruckman et al., 2008). In this system, an alkyne group was quantitatively attached to the tyrosine residues on the surface of tobacco mosaic virus (TMV) via diazonium coupling and the CuAAC reaction (Bruckman et al., 2008). This study also proved that the CuAAC reaction did not depend on the structure of the starting materials and will not affect the function of the azide (Bruckman et al., 2008). However, copper-catalyzed ligation between Cu(II) and 1,2,3-triazoles has cytotoxicity and might affect the viral structure (Liu et al., 2017; Wang et al., 2003). Moreover, Cu(II) can greatly decrease the fluorescence of the quantum dots (QDs) (Beaune et al., 2011; Bernardin et al., 2010). Therefore, a copper-free reaction was used to label the vaccinia virus and avian influenza A virus in live cells with QDs by linking azide-clicked virions to the dibenzocyclooctyne-derived QDs, resulting in intact, fluorescence-labeled and infectious virions for single-virion tracking, with an 80% labeling efficiency (Fig. 1a) (Hao et al., 2012). The fluorescence of labeled virions was strong enough for single-virion imaging and tracking (Hao et al., 2012).

Recently, metabolic incorporation of a clickable group into viral protein was reported as another strategy to label a virus or viral protein for investigating the behavior of viral proteins (Fernandez and Freed, 2017; Lin et al., 2013; Nikic et al., 2014; Plass et al., 2012; Sakin et al., 2017). One protocol is based on genetic code expansion. First, an amber stop codon (UAG) was introduced into the target protein by site-directed mutagenesis (Fernandez and Freed, 2017; Nikic et al., 2014). Then, a noncanonical amino acid (ncAA) could be incorporated into the amber stop codon when the engineered protein was coexpressed with the aminoacyl tRNA synthase/tRNA pair in the host cell, resulting in translation continuing through the amber stop codon, which was designated as amber suppression (Fernandez and Freed, 2017; Nikic et al., 2014). As the ncAA bears a ring-strained alkyne or alkene, the ncAA is clicked to azide- or tetrazine-containing dyes via SPAAC or SPIEDAC reactions, and thus, the mobility of the target viral protein, such as HIV-1 Env, can be measured in a real-time manner (Fig. 1b) (Fernandez and Freed, 2017; Nikic et al., 2014; Sakin et al., 2017). Moreover, HIV-1 particles can be generated by adding eGFP-tagged Gag to the system, and therefore, the Env accumulating sites of particle budding can be visualized by stimulated emission depletion (STED) nanoscopy (Fernandez and Freed, 2017; Sakin et al., 2017). However, the

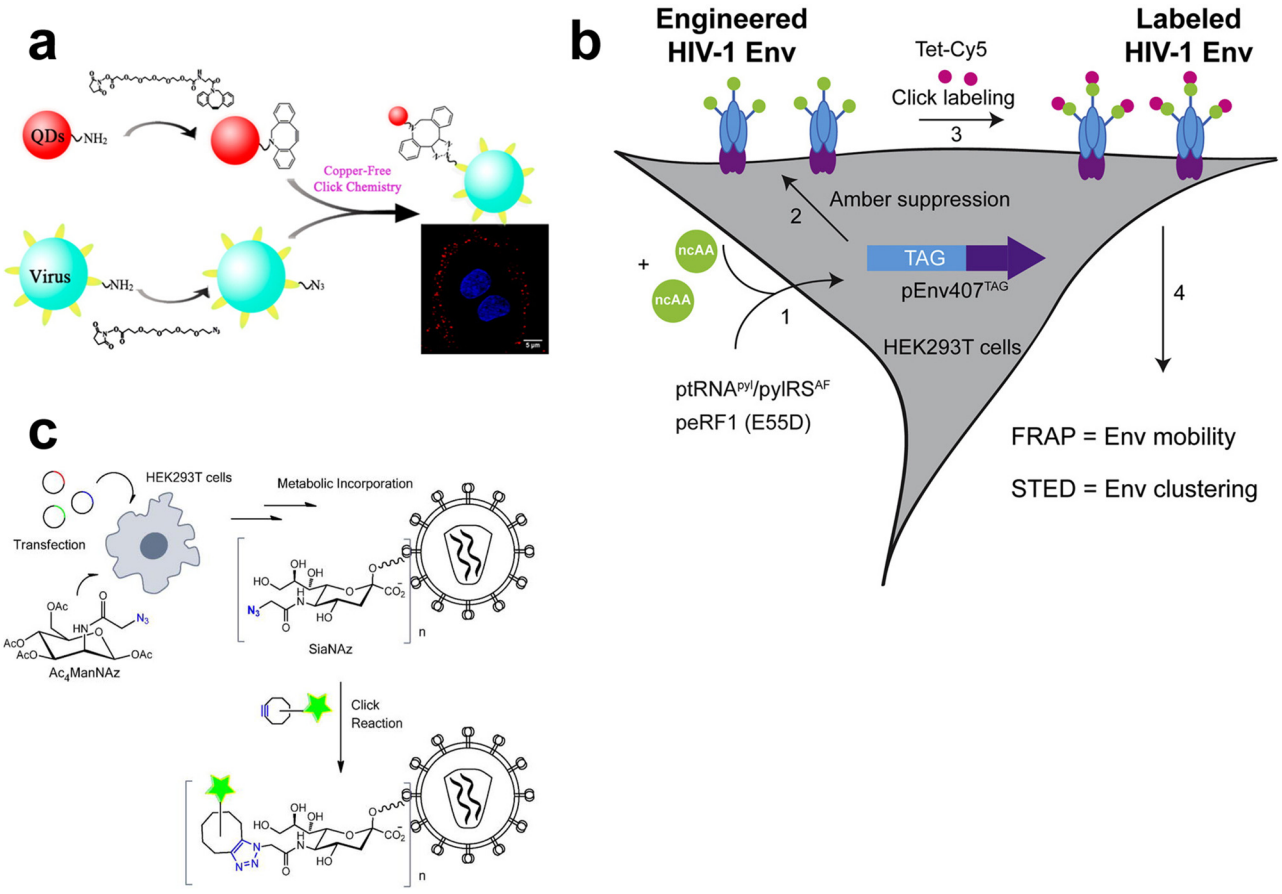

Fig. 1. Application of click chemistry in viral tracking. (a) Virions were labeled with quantum dots (QDs) via copper-free click chemistry by linking azide-clicked virions to the dibenzocyclooctyne-derived QDs, resulting in intact, fluorescence-labeled and infectious virions (Hao et al., 2012). (b) An amber (UAG) stop codon was inserted into the HIV Env protein by site-directed mutagenesis, followed by coexpression of the protein with an aminoacyl tRNA synthase/tRNA and noncanonical amino acid (ncAA) in the target cell (Fernandez and Freed, 2017; Sakin et al., 2017). Subsequently, the ncAA was incorporated at the amber stop codon, allowing the continuous translation through the amber stop codon (Fernandez and Freed, 2017; Sakin et al., 2017). When H-Tet-Cy5 was added, the Env was labeled between ncAA and tetrazine via a click reaction (Fernandez and Freed, 2017; Sakin et al., 2017). (c) Viral glycoprotein was labeled with a metabolically incorporated unnatural sugar (Ac4ManNAz), followed by a click reaction with organic fluorescent dyes, allowing virus-cell fusion to be visualized during the infection (Oum et al., 2016).