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Short communication

A low-toxic artificial fluorescent glycoprotein can serve as an efficient cytoplasmic labeling in living cell

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ABSTRACT

To maintain the virtue of good optical property and discard the dross of conventional fluorescent staining dyes, we provide a strategy for designing new fluorescent scaffolds. In this study, a novel fluorescent labeling glycoprotein (chitosan-poly-L-cysteine, CPC) was synthesized through graft copolymerization. CPC gives emission peak at 465–470 nm when excited at 386 nm. The submicro-scale CPC microspheres could be localized and persisted specifically in the cytoplasm of living cells, with strong blue fluorescence. Moreover, CPC was highly resistant to photo bleaching, the fluorescence was remained stable for up to 72 h as the cells grew and developed. The glycoprotein CPC was bio-compatible and in zero grade cytotoxicity as quantified by MTT assay. The fluorescent labeling process with our newly designed glycoprotein CPC is exceptionally efficient.

1. Introduction

Fluorescent labeling technique has attracted much attention for its wide application in biological and medical science. It serves as a versatile and indispensable tool for flow cytometry, molecular and cellular imaging, etc. (Boonacker & Van Noorden, 2001; Goncalves, 2009; Johnson, 1988; Kobayashi, Ogawa, Alford, Choyke, & Urano, 2010; Lavis & Raines, 2008; Petit, Denis-Gay, & Ratinaud, 1993; Tinnefeld & Sauer, 2005; Walter, Huang, Manzo, & Sobhy, 2008; Zhang, Campbell, Ting, & Tsien, 2002). The interests of structurally diverse small-molecular fluorescent dyes and genetically encoded fluorescent proteins such as "green fluorescent protein (GFP) family" with different spectroscopic properties, realize the discovery of the first biological single cell lasers (Gather & Yun, 2011) and the monitoring in time and space of various life phenomenon in living cells and organisms like gene expression, protein localization and dynamics, chromosome replication and cell division (Cha, Snyder, Selvin, & Bezanilla, 1999; Chalfie, Tu, Euskirchen, Ward, & Prasher, 1994; Giepmans, Adams, Ellisman, & Tsien, 2006; Goddard & Reymond, 2004; Lampert & Westermann, 2011; Topf, Theis, & Stockem, 1996; Willoughby & Cooper, 2008). So far, most of the fluorescent dyes are used for staining nucleic acid, bilayer lipid and cellular organelle. Few attentions have been paid to the specific cytoplasmic labeling. Cytoplasmic fluorescent labeling is an effective and powerful tool for the exploration of cell membrane integrity, long-term cell tracing, drug transport and distribution (Brisson et al., 2011; Hawkins et al., 2010; Wang et al., 2011). Whereas, at present, the specific cytoplasmic fluorescent labeling compounds are quite few and are mainly limited to rigid polycyclic aromatic dyes, such as Calcein-AM, BCECF-AM and CFSE, and most of them are restricted in staining living cell for their high toxicity. Thus, it is necessary for further development of fluorescent material for cytoplasmic labeling with good biocompatibility.

To maintain the virtue of good optical property and discard the dross of conventional fluorescent staining dyes, we provide

Abbreviations: GFP, green fluorescent protein; CPC, chitosan-poly-L-cysteine; DP, the average polymerization degree; S-benzyl-L-cysteine-NCA, S-benzyl-L-cysteine-N-carboxy-anhydride; CHL cells, Chinese hamster lung cells; PBS, phosphate-buffered saline; CLSM, confocal laser scanning microscopy; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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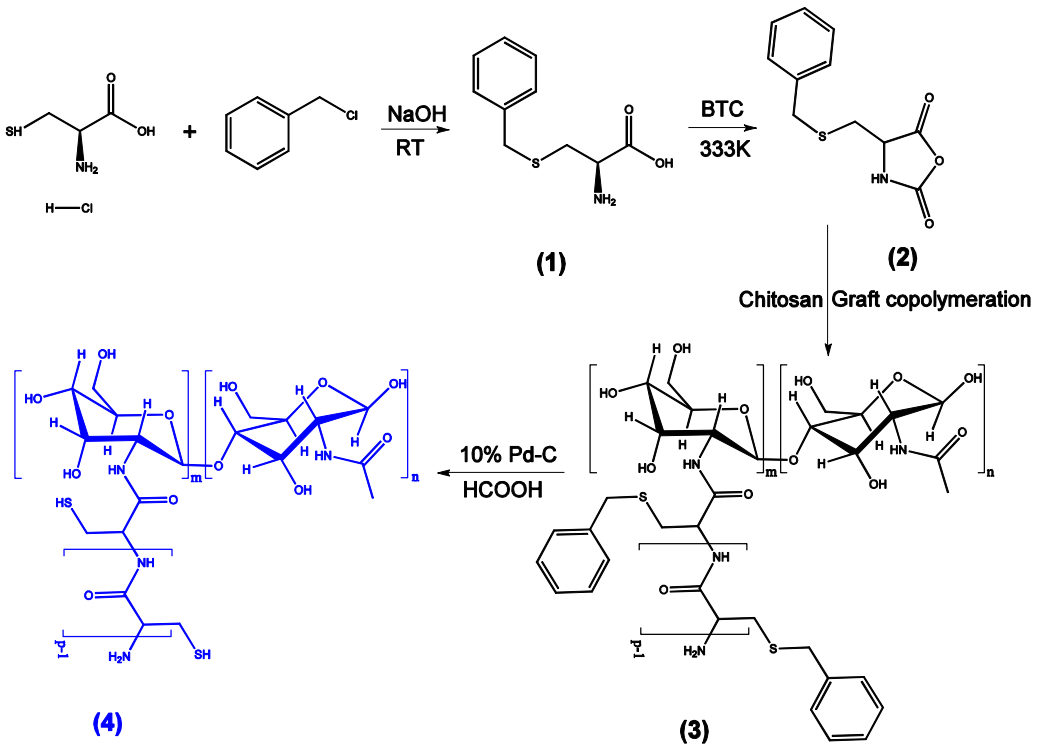

Scheme 1. Synthesis of fluorescent glycoprotein (chitosan-poly-L-cysteine, CPC) [*m*, *n* represent the number of deacetylated and acetylated units respectively which were randomly distributed in the chitosan molecular chains. p represents the number of the poly-L-cysteine grafted on the chitosan].

here a strategy for designing a new fluorescent compound as a specific cytoplasmic labeling material with low-toxicity, by employing low molecular weight (M_W) chitosan as a scaffold and poly-L-cysteine as side chains, to form a well-defined fluorescent glycoprotein (chitosan-poly-L-cysteine, CPC) (Xiang, Si, Zhang, Liu, & Guo, 2009). Here, thiol groups on the side chains were used as the important chromophores besides pyranose rings of chitosan scaffold.

2. Experimental

2.1. Synthesis of CPC

The whole synthesis process of CPC includes the following four steps: the protection of thiol by benzyl group, S-benzyl-L-cysteine-N-carboxy-anhydride (NCA), graft copolymerization between chitosan and NCA and removing of the protective group.

2.2. The culturing of Chinese hamster lung (CHL) cells

The detailed culturing process is shown in the supporting information.

3. Results and discussion

The glycoprotein CPC was synthesized according to the route as shown in Scheme 1. The average polymerization degree (DP) of poly-L-cysteine side chains and the average M_W of CPC were 7 and 46.66 kDa, respectively, which were adjustable by controlling the different ratio between chitosan and S-benzyl-L-cysteine-N-carboxy-anhydride (S-benzyl-L-cysteine-NCA).

In this study, glycoprotein CPC exhibited a unique photophysical property. When excited at 380 nm, glycoprotein CPC gives a maximum emission peak at 465–470 nm with a large Stokes shift (85–90 nm), which is benefiting for signal detection and imaging applications (shown in Supporting Information Fig. S2). The submicro-scale glycoprotein CPC microspheres dispersing in water showed strong blue fluorescent signal (Fig. S3).

CHL cells, with low phagocytic ability and reproduction rate, were selected to study the fluorescent properties of CPC in the living cells. During 48 h of co-incubation, the glycoprotein CPC undergone the following three steps to be internalized in CHL cells: (i) the aggregation of CPC microsphere around the living CHL cells (Fig. S4); (ii) small microspheres entered the living cells, exhibited by the appearance of a few bright fluorescent spots in the living cells (Fig. 1a); (iii) a great number of microspheres entered and aggregated in the living cells, resulting in the appearance of stronger fluorescent bulks (Fig. 1b). The fluorescent intensity of CHL cells increased correspondingly with the time of co-incubation, as shown in Fig. 1c. Notably, after 48 h of co-incubation, CHL cells regained their original peculiar spindle shape from morbid round shape, indicating glycoprotein CPC was low-toxic and the CHL cells could gradually adapt to the environment with the addition of glycoprotein CPC. The cytotoxicity of glycoprotein CPC was further quantified by MTT assay (Magrez et al., 2006) with the result of zero grade (Fig. S6), much lower than that of the traditional fluorescent dyes. On the other hand, the CHL cells remained stably labeled for up to 72 h as they grew and developed, indicating the glycoprotein CPC also have high photostability.

Fig. 2 illustrates the localization of glycoprotein CPC microspheres in the living CHL using confocal laser scanning microscopy (CLSM). Results show the main regions of CHL cell were well partitioned. The glycoprotein CPC, represented with strong blue fluorescence (Fig. 2a), was specifically localized in cytoplasmic region of living CHL cells. On the other hand, the central area, labeled in green (Fig. 2b) and red (Fig. 2c), represented nucleic zone stained with Acridine Orange (AO), a nucleic acid-specific dye; the limited red spots (Fig. 2d) were RNA in cytoplasm of CHL cells. The CLSM

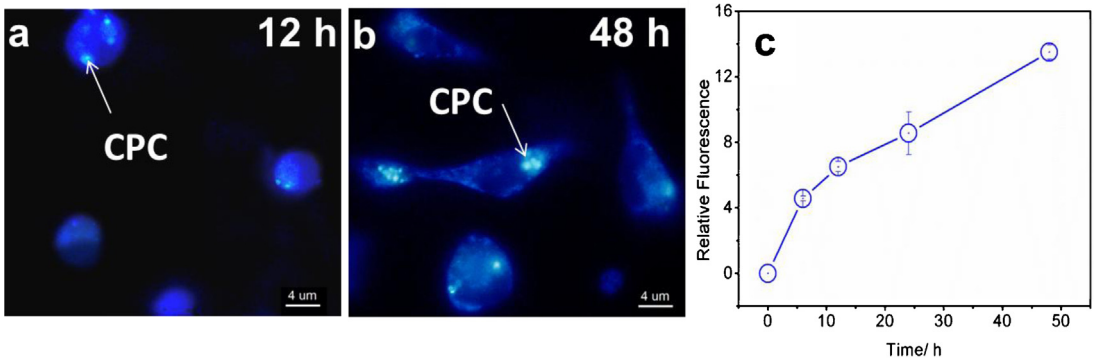

Fig. 1. Fluorescent images of living CHL cells co-incubated with glycoprotein CPC microspheres for (a) 12 h, (b) 48 h and (c) the quantitative fluorescent intensity of living CHL cells increased along with the increasing co-incubation time.

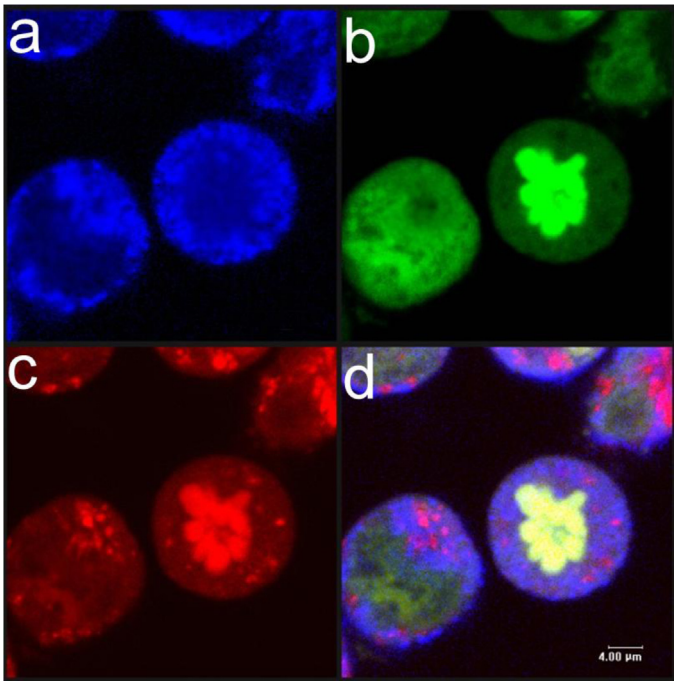

Fig. 2. CLSM images of living CHL cells, which were co-incubated with glycoprotein CPC microspheres for 24 h and then counterstained with AO. The fluorescence images are acquired under the excitation at (a) 351–364 nm (Blue), (b) 460 nm (Green), (c) 510 nm (Red) and (d) Merge image of (a), (b) and (c). The blue signal represents the glycoprotein CPC in the cytoplasmic region of CHL cells; the green signal represents the DNA (nucleic region); the red spots represent the RNA; Scale bar is 4 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

scanning results revealed CPC was highly chemical-stable and could be utilized for cytoplasmic labeling process, not affected by the counterstaining with AO.

4. Conclusion

In conclusion, we successfully designed and synthesized homogenous fluorescent glycoprotein CPC and realized the efficient and specific cytoplasmic labeling in living cells. The low-cost, low-toxic and photo-stable cytoplasmic fluorescent labels material would offer an extremely important and valuable tool for multi-subject, such as analyzing the dynamic behavior of living cells, monitoring drug transport and distribution in cell over long periods of time, and the potential applications in single-cell biological laser devices.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.08.118.

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