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t-Butyloxycarbonyl: An ordinary but promising group for protecting peptides from deiodination

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Abstract

In order to protect directly radioiodinated peptides from in vivo deiodination, a novel procedure was explored. Two peptides, Try-Gly-Gly-Gly-Gly-Gly-Cys-Asn-Gly-Arg-Cys (YG5) and t-Boc-Try-Gly-Gly-Gly-Gly-Gly-Cys-Asn-Gly-Arg-Cys (t-BOC-YG5) were synthesized and radiolabeled. A paired-label biodistribution study using [^{131}I]t-BOC-YG5 and [^{125}I]YG5 was undertaken in normal mice. Compared to [^{125}I]YG5, [^{131}I]t-BOC-YG5 was quite resistant to in vivo deiodination, resulting in rapid reduction of the radioactive background and negligible radioactivity accumulation in both thyroid and stomach. [^{131}I]t-BOC-YG5 was also stable in human serum even after 24 h. In conclusion, the t-BOC group has the potential to protect peptide from deiodination.
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1. Introduction

During recent decades, considerable interest has been focused on the use of radiolabeled peptides as imaging agents or in vivo chemical probes. Iodine radionuclides, are especially attractive for various applications because the type of radiation is wide-ranging and well-suited for single photon emission tomography (^{123}I, ^{124}I), β-particle therapy (^{131}I), and positron emission tomography (^{124}I) (Larson and Carrasquillo, 1988).

The direct radioiodine labeling of peptides or proteins is, in fact, the radioiodination of tyrosine residues via electrophilic substitution. Unfortunately, such procedures often yield a product, which is susceptible to deiodination in vivo (Bakker et al., 1990, 1991). Internalization of peptides, such as anti-EGFRvIII antibody (Wikstrand et al., 1995), anti-μ mAb DA4-4 (Geissler et al., 1992), and cyclic RGD (Hart et al., 1994), after binding to the receptor expressed on the cell membrane is an important phenomenon. The internalization results in deiodination of directly radioiodinated peptides. The low molecular weight radi-

olabeled catabolites formed, mainly monoiodotyrosine, are quickly removed from tumors, and significant uptake of radioiodide in thyroid gland results (Geissler et al., 1992; Wikstrand et al., 1995).

Alternative approaches for the prevention of in vivo deiodination of radioiodinated peptides have been evaluated. The use of an acylation agent derived from the prototypical N-succinimidyl 3-iodobenzoate (SIB) for labeling proteins decreases deiodination in mice compared to proteins labeled by direct radioiodination methods (Pradeep et al., 1996; Reist et al., 1996; Shankar et al., 2004). However, the synthesis of such a prosthetic group and its subsequent binding to a peptide could be labor-intensive and time-consuming. In addition, the acylation agents that have to be conjugated to the ε-amino group of a lysine moiety are not applicable when lysine is the functional group or when no lysine moiety exists in a peptide. Modification of tyrosine has also been reported to increase the in vivo stability of a radioiodinated product (Krummeich et al., 1994). However, O-methylation of tyrosine reduces the reactivity of the aromatic nucleus and requires a severe labeling reaction, which may alter the peptide structure. Another approach to tyrosine modification, the methylation of tyrosine's α-position, has not yet

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G5:   NH$_2$-Gly-Gly-Gly-Gly-Gly-Cys-Asn-Gly-Arg-Cys-OH

t-BOC-YG5:   NH-Tyr-Gly-Gly-Gly-Gly-Gly-Cys-Asn-Gly-Arg-Cys-OH

YG5:   NH$_2$-Tyr-Gly-Gly-Gly-Gly-Gly-Cys-Asn-Gly-Arg-Cys-OH
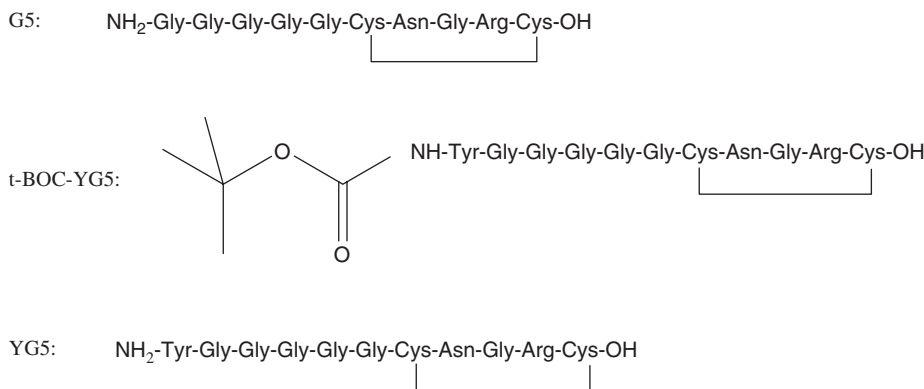
Fig. 1. The amino acid sequences for G5, *t*-BOC-YG5, and YG5.

been applied to protect peptide from deiodination for some reason.

The *t*-butyloxycarbonyl (*t*-BOC) group is a widely used α-amino-protecting group. The synthesis of *t*-BOC-protected peptide has been thoroughly studied and now is a routine technique. In addition, because the *t*-BOC group is stable over the pH range of 4–12, *t*-BOC-protected peptides can survive at the low lysosomal pH of 5.

The NGR sequence motif (Asn-Gly-Arg) is an aminopeptidase N (CD13) ligand that targets activated blood vessels in tumors (Pasqualini et al., 2000). In the present study, two NGR—containing cyclic peptide analogues, Try-Gly-Gly-Gly-Gly-Gly-Cys-Asn-Gly-Arg-Cys (YG5) and *t*-Boc-Try-Gly-Gly-Gly-Gly-Gly-Cys-Asn-Gly-Arg-Cys (*t*-BOC-YG5) (Fig. 1), were synthesized and labeled with ^{125}I or ^{131}I, respectively. A paired-label biodistribution study of [^{131}I]*t*-BOC-YG5 and [^{125}I]YG5 was undertaken in normal mice in order to determine whether *t*-BOC could protect the peptide from in vivo deiodination.

2. Materials and methods

The cyclic peptide G5 (Fig. 1) was purchased from GL Biochem Ltd. (Shanghai, China). All other reagents were of A. R. grade and used as supplied without further purification. A Symmetry C18 column (5 μm, 3.9 × 150 mm, Waters, Massachusetts, USA) was used for sample analysis and separation. All reverse-phase high-performance liquid chromatography (RP-HPLC) analyses were performed with a Waters 600E multisolvent delivery system (Massachusetts, USA). The elution was also monitored with a Packard 500 TR flow scintillation radioactivity detector (Connecticut, USA) in addition to the UV detector. The radiochemical recovery was obtained from the added and recovered radioactivity measured with a Capintec CRC-15R Dose Calibrator (New Jersey, USA). A Packard Cobra II Series Counting System (Connecticut, USA) was used to count the radioactivity of tissue samples. A Waters C18 Sep-Pak mini cartridge (Massachusetts, USA) was employed for desalination. Na^{131}I and Na^{125}I were purchased from the China Institute of Atomic Energy and Beijing Atom Hightech Co. Ltd., respectively. The

matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectra were provided by the Beijing Mass Spectrometry Center, Chinese Academy of Sciences (CAS). Kunming mice (weighing 20–25 g) were purchased from Institute of Zoology, CAS.

2.1. Synthesis of t-BOC-YG5

t-BOC-tyrosine-NHS was synthesized and purified by our laboratory according to the method of Iuchi et al. (1988). The cyclic peptide G5 (1 μmol in 16 μL DMF) was added to 400 μL DMF. *N,N*-diisopropylethylamine (2 μmol in 10 μL DMF) and *t*-BOC-tyrosine-NHS (5 μmol in 100 μL DMF) were added to that solution. The mixture was shaken at room temperature for 1 h. Hydrochloric acid (4 μmol in 40 μL DMF) was added to stop the reaction. The mixture was dried under vacuum and washed with acetonitrile (3 × 1 mL). Water (500 μL) was added to dissolve the sample and ethyl acetate (5 × 2 mL) was added to remove the *t*-BOC-tyrosine-NHS completely. The MALDI-TOF-MS *m/z* calculated for *t*-BOC-YG5 ([M + H]$^+$) was 1097.4, found: 1098.3, Fig. 2.

2.2. Synthesis of YG5

The YG5 was synthesized by the method of Huang and Chen (1985). Briefly, the *t*-BOC-YG5 synthesized above (25 μL, 2 × 10^{-3} M) was dried under vacuum and then 100 μL of formic acid (>90%) was added to remove the *t*-BOC group at room temperature. After standing for 2 h, the formic acid was removed under vacuum. MALDI-TOF-MS *m/z* calculated for YG5 ([M + H]$^+$) was 998.4, found: 998.3, depicted in Fig. 2.

2.3. Radioiodine labeling

Peptides were labeled with ^{125}I or ^{131}I by using the iodogen method (Wang et al., 1985). A solution of *t*-BOC-YG5 (25 μg, 50 μL in PBS, pH 7.4) was added to 300 μCi of Na^{131}I in a plastic vessel coated with 100 μg of iodogen. After incubation at room temperature for 10 min, the reaction was stopped by removing the solution from the