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Optimized protocol for the radioiodination of hydrazone-type polymer drug delivery systems

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HIGHLIGHTS

- Hydrazone-type drug delivery systems with doxorubicin were radioiodinated.
- Radioiodination was performed *via* polymer-bound phenolic moiety.
- Radioiodination step must be performed before deprotection and drug binding.

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ABSTRACT

Hydrazone conjugates of polymers with doxorubicin represent a very promising tool for cancer chemotherapy. However, these conjugates are very difficult to radiolabel with iodine radionuclides, which possess otherwise very advantageous nuclear properties to, *e.g.*, follow biodistribution. In this study, we developed a robust protocol for the high-yield radioiodination of hydrazone-type drug delivery systems with doxorubicin. In particular, it is crucial that the polymer radioiodination step be performed before the deprotection of the hydrazide and doxorubicin binding.

1. Introduction

Polymer drug delivery systems represent an efficient way of suppressing the disadvantageous properties of drugs, such as poor water solubility, short half-life in the blood plasma or unfavorable biodistribution (Allen and Cullis, 2004; Langer, 1998; Moses et al., 2003). These systems are based on bioactive components attached to a biocompatible polymer carrier through a degradable bond (Binauld and Stenzel, 2013; Schmaljohann, 2006). This polymer prodrug is then delivered to the target tissue in an inactive form, and the drug in its fully active form is released from the polymer into the target tissue (Omelyanenko et al., 1998). This technique enables antiproliferative drugs such as doxorubicin (DOX) to be delivered preferentially to the cancer tissue due to the Enhanced Permeability and Retention (EPR) passive effect, possibly further enhanced by ligand targeting, resulting in increased efficacy and reduced side effects (Kopeček et al., 2001). In addition to targeting, the key step is site-specific activation. Hydrazone bonds are especially advantageous for the delivery of oxo group-containing anticancer drugs, such as DOX or ellipticinium derivatives, to solid tumors due to the pH-dependence of their hydrolysis (Willner et al., 1993). The hydrazone bond is relatively stable in the blood plasma (pH 7.4) and is hydrolyzed rapidly in the mildly acidic milieu of the tumor interstitial space or in endosomes after internalization into cancer cells (the pH value of late endosomes can drop as low as 5.0) (Lee et al., 1996; Sedláček et al., 2013). This results in cancer tissue-specific drug activation. Not surprisingly, modern hydrazone-based polymer anticancer drug delivery systems are very popular, accounting for 126 entries in the Web of Knowledge (query: hydrazone AND polymer AND drug delivery), including 106 DOX (query: hydrazone AND polymer AND doxorubicin) conjugates as of the submission date of this paper.

A favorable biodistribution is critical for the therapeutic effect of any drug (Owens III and Peppas, 2006). The easiest and most accurate way to study the biodistribution of compounds (including polymer drug conjugates) *in vivo* in real time is the use of radiolabeled systems (Rosebrough, 1993). Depending on the radionuclide decay mode, the biodistribution of a compound of interest may be followed with single photon emission computed spectroscopy (SPECT, γ-emitters)

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(Brinkhuis et al., 2012) or with positron emission tomography (PET, β^+-emitters) (Welch et al., 2009). However, for polymer prodrugs, it is especially important to note that radioimaging essentially allows the fate of the radiotracer, which may be attached to the drug, the carrier or both, to be followed; double-labeled systems using radionuclides with different nuclear properties allow the fates of the drug and carrier to be simultaneously followed (Nori and Kopeček, 2005). Radiolabeling also allows theranostic possibilities (*thera*py + dia*gnostic*) for the individual customization of therapy (Lammers et al., 2011). The use of radionuclides provides benefits for those anticancer drugs that have synergic effects with radiation, which is especially the case for DOX (Chenoufi et al., 1998). Radiation potentiates the therapeutic effects of DOX due to the *in situ* production of a DNA-intercalating alkylating agent from DOX (Sun et al., 2009). Such a system has been proposed for local chemoradiotherapy with a thermoresponsive system (Hrubý et al., 2008).

Radiolabeling may be performed through either chelating approaches (*e.g.*, metal radionuclides, *e.g.*, ^{99m}Tc and ^{177}Lu) (Liu, 2004) or covalent approaches (*e.g.*, ^{18}F and iodine radionuclides) (Stockhofe et al., 2014). The chelating approach has certain advantages, such as technical ease, but some chelating approaches also have some drawbacks, such as sensitivity to metal impurities, significant changes in the system properties due to the necessary introduction of the chelators, kidney-targeting properties (*e.g.*, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-DOTA or 6-hydrazinonicotinic acid) (Froidevaux et al., 2005) and the need to heat up the system during radiolabeling (*e.g.*, radiolabeling *via* DOTA), which may be incompatible with some drugs or targeting moieties, such as antibodies (Breeman et al., 2003). However, covalent radiolabeling does not have these disadvantages. In particular, iodine radionuclides are advantageous due to the wide selection of available radionuclides with various half-lives and decay modes (Seevers and Counsell, 1982). Namely, ^{131}I ($T_{1/2}$ = 8.04 days) is a theranostic β- and γ-emitter that is suitable for both therapy and SPECT, (Moderegger, 2012) ^{125}I ($T_{1/2}$ = 60.14 days) decays by electron-capture and can be utilized in biological assays, SPECT and endoradionuclide therapy, (Sedlacek et al., 2014a) ^{124}I ($T_{1/2}$ = 4.18 days) is a relatively long-lived positron emitter that is suitable for PET, (Simone et al., 2012) and ^{123}I ($T_{1/2}$ = 13.13 h) is an electron capture emitter well suited for low-radiation-burden SPECT diagnostics (Culbert and Hunter, 1993).

However, direct electrophilic radioiodination may be tricky for polymer systems because it is not fully compatible with several groups, including hydrazones, (Pross and Sternhell, 1970) hydrazides, (Curtius, 1894; Yang and Tian, 2013) other reducing groups (Knight and Welch, 1978) and DOX (Wolf et al., 1997).

To the best of our knowledge, there are no reports of efficient methods for the radioiodination of hydrazone-type DOX conjugates. In this paper, we describe for the first time a robust radioiodination protocol for the high-yield chemically stable radiolabeling of a hydrazone-type drug delivery system using DOX (which has synergistic biological effects with ionizing radiation) as a model drug. This system also accounts for all structural and chemical obstacles to radioiodination that are common in such polymer drug delivery systems, making it an ideal model for the development of a universal protocol for the radioiodination of hydrazone-containing polymer conjugates.

2. Materials and Methods

2.1. Materials

Carrier-free iodine-125 (as [^{125}I]-NaI) was purchased from Lacomed Ltd. (Rez, Czech Republic). *N*-methacryloyl-L-tyrosinamide (MATA), (Hrubý et al., 2005b) *N*-(2-hydroxypropyl) methacrylamide (HPMA) and *N*-boc-methacryloyl-6-aminocaproic hydrazide were synthesized as described previously (Chytil et al., 2010). All other chemicals, including phosphate-buffered saline pH 7.4 (PBS) in tablet form, were purchased from Sigma-Aldrich Ltd. (Prague, Czech Republic).

2.2. General methods

NMR spectra were measured on a Bruker Avance MSL 300 MHz NMR spectrometer (Bruker Daltonik GmbH., Germany). The molecular weights of the polymers were determined by size exclusion chromatography (SEC) using an HPLC Dionex Ultimate 3000 system (Pragolab Ltd., Prague, Czech Republic) equipped with a SEC column (TSKgel G3000SWxl 300 × 7.8 mm^2; 5 μm) and the following detectors: UV/VIS, Flow Star LB 513 radiodetector (Berthold Technologies GmbH & Co. KG, Germany), RI Optilab®-rEX and MALS DAWN EOS (both Wyatt Technology Co., USA). A methanol-sodium acetate buffer (0.3 M; pH 6.5) mixture (80:20 v-v) was used as the mobile phase at a flow rate of 0.5 mL/min. The applied refraction index increment was $dn/dc = 0.168$, as determined by differential refractometry. UV/VIS spectra were measured on a SPECORD 205 Spectrometer (Analytik Jena AG, Germany). The radioactivity of the samples was measured using a Bqmetr 4 ionization chamber (EMPOS Ltd., Prague, Czech Republic).

2.3. RAFT copolymerization

Polymers were synthesized in a similar way to the procedure described before (Chytil et al., 2010) with a molar ratio of monomers: chain transfer agent:initiator of 500:2:1. Different ratios of monomers were used for the synthesis of polymers **1a–d**; see Table 1 for details. Briefly, for **1b**, HPMA (159 mg, 1.11 mmol), *N*-Boc-methacryloyl-6-aminocaproic hydrazide (39 mg, 0.125 mmol), MATA (3.1 mg, 12.5 μmol), 2-cyano-2-propyl benzodithioate (0.82 mg, 5.0 μmol) and 2,2′-azobis(2-methylpropionitrile) (AIBN, 0.55 mg, 2.5 μg) were dissolved in *tert*-butanol (1.4 mL), and the solution was purged with argon. The ampoule was then sealed and stirred at 70 °C for 7 h. The polymer was subsequently precipitated in ethyl acetate, filtered and dried, yielding 171 mg of product (84%). The resulting polymer was dissolved in dimethyl sulfoxide (2 mL); AIBN (22 mg, 0.1 mmol) was subsequently added, and the mixture was stirred at 60 °C for 3 h. The reaction mixture was diluted with methanol (2 mL) and purified on a Sephadex LH-20 column with methanol as the eluent. The polymer-containing fractions were collected, concentrated under reduced pressure and precipitated into diethyl ether. The precipitate was collected and dried, resulting in **1b** (156 mg, 91%) as a white powder.

The content of MATA was determined by ^{1}H NMR from the ratio of signal integrals at δ = 6.79 ppm (2-Ar-**H** of MATA) and at δ = 3.87 ppm (C**H**-OH of HPMA).

Table 1
Characteristics of ternary copolymers of MATA with HPMA and *N*-Boc-methacryloylaminocaproic hydrazide.

Polymer	[a]Theoretical content of MATA (mol%)	[b]Content of MATA (mol%)	[c]M_w (kDa)	[c]M_w/M_n
1a	0	–	24.8	1.18
1b	1	0.93	24.3	1.21
1c	2	1.97	25.6	1.16
1d	5	4.82	25.1	1.19

[a] As present in the monomer reaction mixture.
[b] Calculated from ^{1}H NMR.
[c] Measured by SEC chromatography.