



Short communication

A novel carbohydrate labeling method utilizing transfer hydrogenation-mediated reductive amination

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ABSTRACT

One of the most frequently used high-resolution glycan analysis methods in the biopharmaceutical and biomedical fields is capillary electrophoresis with laser-induced fluorescence (CE-LIF) detection. Glycans are usually labeled by reductive amination with a charged fluorophore containing a primary amine, which reacts with the aldehyde group at the reducing end of the glycan structures. In this reaction, first a Schiff base is formed that is reduced to form a stable conjugate by a hydrogenation reagent, such as sodium cyanoborohydride. In large scale biopharmaceutical applications, such as clone selection for glycoprotein therapeutics, hundreds of reactions are accomplished simultaneously, so the HCN generated in the process poses a safety concern. To alleviate this issue, here we propose catalytic hydrogen transfer from formic acid catalyzed by water-soluble iridium(III)- and ruthenium(II)-phosphine complexes as a novel alternative to hydrogenation. The easily synthesized water-soluble iridium(III) and the ruthenium(II) hydrido complexes showed high catalytic activity in carbohydrate labeling. This procedure is environmentally friendly and reduces the health risks for the industry. Using carbohydrate standards, oligosaccharides released from glycoproteins with highly sialylated (fetuin), high mannose (ribonuclease B) and mixed sialo and neutral (human plasma) N-glycans, we demonstrated similar labeling efficiencies for iridium(III) dihydride to that of the conventionally used sodium cyanoborohydride based reaction. The derivatization reaction time was less than 20 min with no bias towards the above mentioned specific glycan structures.

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1. Introduction

Protein-linked glycans are participating in important biological processes such as cell-cell interactions, receptor activation and cell attachment to the extracellular matrix, therefore represent an important field for the biopharmaceutical industry [1]. Recent advances in understanding the role of glycosylation of therapeutic proteins and the search for glycobiomarkers in specific diseases have created the need for fast and sensitive high-throughput glycoanalytical methods [2,3]. To separate and analyze released and subsequently derivatized oligosaccharides, liquid phase bioanalytical methods (liquid chromatography – LC and capillary electrophoresis – CE) are mostly used, in addition to mass spec-

trometry and NMR based structural elucidation techniques. Since the application of liquid phase separation methods with optical or mass spectrometric detection for the characterization of complex carbohydrates is hindered by the lack of chromophore/fluorophore moieties and their poor ionization properties, a wide variety of derivatization strategies with a large number of different labels have been introduced [4].

8-Aminopyrene-1,3,6-trisulfonate (APTS) is one of the most frequently used reductive amination based derivatization agent in capillary electrophoresis, possessing three strong negative charges and great fluorescent properties, therefore very suitable for CE-LIF applications [5,6]. This label facilitated sensitive detection by excitation with the stable argon-ion or solid state 488 nm lasers commonly used in commercial CE equipments, also providing good separation characteristics [7]. The detection limit of laser-induced fluorescence (LIF) of APTS labeled sugars in CE is in the low femtomolar range [8]. The most widely applied reducing agents for these reductive amination reactions are sodium cyanoborohydride and 2-picoline borane. Very high yields of oligosaccharide labeling

Abbreviations: LIF, laser induced fluorescence; APTS, 8-Aminopyrene-1,3,6-trisulfonate; mtppms, monosulfonated triphenylphosphine Na salt.

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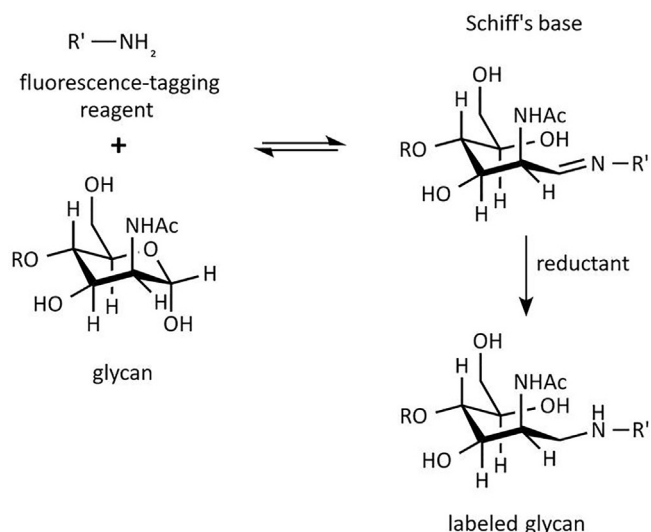


Fig. 1. Sugar labeling reaction with an amino-fluorescent tagging. (R: rest of the sugar molecule; R': fluorophore).

have been reported using these reductive agents [9]. During the first step of the derivatization reaction, the open-ring form of the carbohydrate reversibly reacts with the primary amine group of the dye with the loss of a water molecule, forming a Schiff-base. In a second, irreversible step, the Schiff-base is reduced to stable conjugate as delineated in Fig. 1.

Due to the HCN release associated with the use of sodium cyanoborohydride, alternative reducing agents, such as sodium triacetoxyborohydride ($\text{NaBH}(\text{OAc})_3$) [10,11], borane-diethylamine and 2-picoline borane have been suggested [9].

Our group has recently developed several high-throughput sample preparation methods for N-glycan labeling and analysis, which required reductive amination-based fluorophore tagging of sugars with APTS. To avoid the generation of the acutely toxic hydrogen cyanide, several substitutes were evaluated to replace the sodium cyanoborohydride as reductive agent. Here, we report on the use of water-soluble $[\text{IrH}_2\text{Cl}(\text{mtppps})_3]$ and $[\text{RuCl}_2(\text{mtppps})_2]$ complexes, applied as catalysts for equally efficient hydrogenation by H-transfer from formic acid as an alternative to NaBH_3CN for oligosaccharide labeling by reductive amination.

2. Materials and methods

2.1. Chemicals and reagents

Sodium hydroxide, formic acid, sodium formate, sodium cyanoborohydride (1 M in THF), fetuin (fetal calf serum), ribonuclease B (bovine pancreas) and human plasma were from Sigma-Aldrich (St. Louis, MO). PNGase F was from ProZyme (Hayward, CA). The 8-aminopyrene-1,3,6-trisulfonate (APTS), the standard maltooligosaccharide ladder and the NCHO Carbohydrate Labeling and Analysis kit were from SCIEX (Brea, CA). $[\text{IrH}_2\text{Cl}(\text{mtppps})_3]$ (trisodium *cis-mer*-chloro-dihydrido-tris(diphenylphosphinobenzene-*m*-sulfonato)iridium(III)) and $[\text{RuCl}_2(\text{mtppps})_2]$ (disodium dichloro-bis(diphenylphosphinobenzene-*m*-sulfonato)ruthenium(II)) (see structures in Fig. 2) were made in house as described earlier in [12] and [13], respectively. (Please note that the Ru(II)-complex has a dimeric structure in solid state as shown in Fig. 2, however, for the sake of simplicity we refer to here as a monomer). Both complexes are highly water-soluble and can be synthesized easily from their halide precursors of

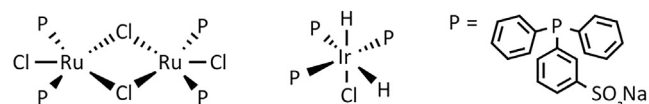


Fig. 2. The water-soluble $[\text{RuCl}_2(\text{mtppps})_2]$ and $[\text{IrH}_2\text{Cl}(\text{mtppps})_3]$ complexes used in the reductive amination reactions.

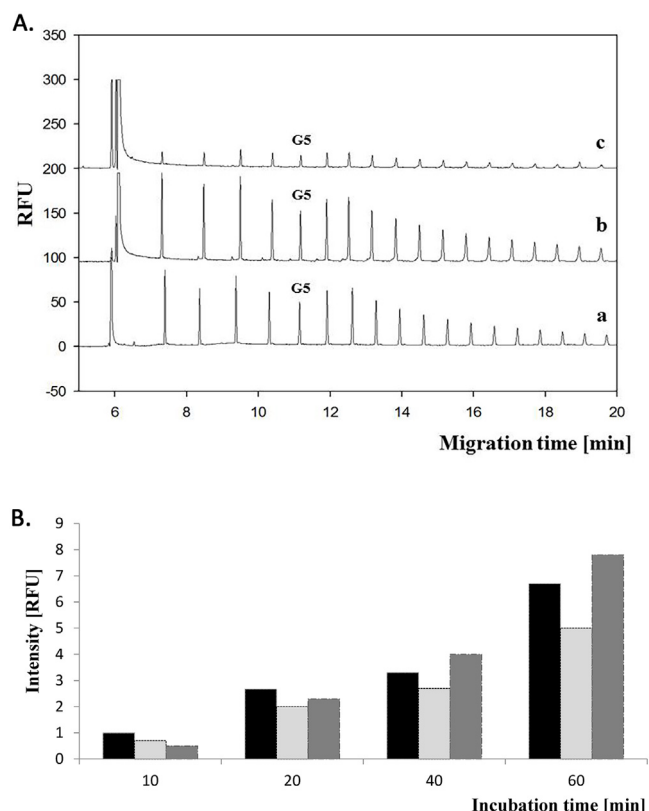


Fig. 3. A. CE-LIF separations of APTS labeled maltooligosaccharides using NaBH_3CN (a), and HCOOH as hydrogen donor with $[\text{IrH}_2\text{Cl}(\text{mtppps})_3]$ (b) and $[\text{RuCl}_2(\text{mtppps})_2]$ (c) catalysts. G5 denotes the maltopentaose peak. Conditions: BFS capillary (50 cm effective/60 cm total length), 50 μm i.d., 30 kV separation voltage, reversed polarity, NCHO separation gel buffer, 20 °C temperature, 1 psi/5 s injection. B. Labeling intensities for maltopentaose in reductive amination reactions with NaBH_3CN (dark gray), $\text{HCOOH}/[\text{IrH}_2\text{Cl}(\text{mtppps})_3]$ (black) and $\text{HCOOH}/[\text{RuCl}_2(\text{mtppps})_2]$ (gray) as a function of time. Reaction temperatures were 55 °C in all instances.

$\text{IrCl}_3 \cdot 3\text{H}_2\text{O}$ [12] and $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ [13], respectively, in reaction with monosulfonated triphenylphosphine (*mtppps* [13]).

2.2. Sample preparation

The following reaction mixture was used as derivatization control: 6 μL of 40 mM 8-aminopyrene-1,3,6-trisulfonic acid in 20% acetic acid and 1.5 μL of 1 M sodium cyanoborohydride (in THF) were added to the dried sugars [14] and incubated at 55 °C for 60 min. For Ir- or Ru-complex based transfer hydrogenation-mediated reductive amination, 6 μL of 40 mM 8-aminopyrene-1,3,6-trisulfonic acid in 20% acetic acid and 1.5 μL of concentrated (98%) formic acid, 1.5 μL of water-soluble iridium(III) dihydride complex (5 mg/mL) or 1.5 μL of water-soluble ruthenium complex (5 mg/mL) were added to the dried sugar samples followed by incubation at 55 °C, as specified under the actual application descriptions. The labeled samples were partitioned from the excess labeling dye with the magnetic beads provided in the Fast Glycan Sample Preparation and Analysis kit (SCIEX) and immediately used for CE-LIF analysis or stored at –20 °C.

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