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Glycan-specific whole cell affinity chromatography: A versatile microbial adhesion platform

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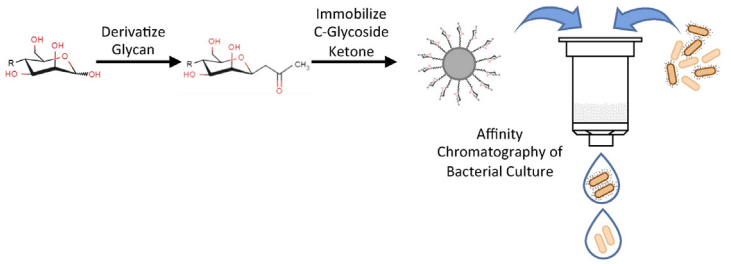
GRAPHICAL ABSTRACT



ABSTRACT

We have sought a universal platform for elucidating and exploiting specificity of glycan-mediated adhesion by potentially uncharacterized microorganisms. Several techniques exist to explore microbial interactions with carbohydrate structures. Many are unsuitable for investigating specific mechanisms or uncharacterized organisms, requiring pure cultures, labeling techniques, expensive equipment, or other limitations such as questionable stability, stereospecificity, or scalability. We have adapted an affinity chromatography resin as a model to overcome these drawbacks, among others. It readily allows for the quantification, selection, and manipulation of target organisms based on interactions with glycan ligands. To maximize its utility as a selective screening method, we have constructed the tool such that it:

- Promotes whole-cell interactions using viable, unaltered cells.
- Provides robust spatial interactions with target glycans, presented with controlled stereo-specificity, for high affinity/avidity interactions that reflect a complex *in vivo* matrix.
- Has the ability to utilize any reducing glycan, is quick, efficient, safe, and affordable to construct, and is scalable and reusable for multiple applications.

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Method details

Part 1: Formation of C-glycoside ketones and their immobilization

In brief, the affinity chromatography model construction consists of derivatizing a target glycan with a ketone modification at the reducing end to facilitate covalent attachment to a hydrazide-functionalized resin. Quantitative conversion of a glycan to its *C*-glycoside ketone derivative can be accomplished as described previously [1] via a straightforward, one-pot reaction requiring no activating or protecting groups. Ketone derivatization makes the glycoside highly reactive toward hydrazines, facilitating rapid immobilization onto hydrazide-modified chromatographic resin. Furthermore, formation of a *C*-glycoside ketone derivative ensures a stereospecific modification that maintains the ring integrity of the glycoside, which may be essential for proper presentation to cell adhesins. For more information on the derivatization chemistry, see [2].

The resulting *C*-glycoside ketohydrazide resin could be constructed with any carbohydrate with an aldose residue at its reducing end. The reactions detailed below are scalable as long as the molar ratio of the components is equivalent; we have successfully derivatized over 5 mmol of carbohydrate and made batches of resin up to 5 mL. While we have found that the specified hydrazide-functionalized resin works well, any other hydrazide-functional substrate should also work, but may require an altered ratio of *C*-glycoside ketone.

Materials

- Glycan(s) of interest
- Sodium bicarbonate buffer: 38 g L^{-1} $NaHCO_3$, pH 8.5
- Acetyl acetone
- Ethyl acetate
- Dowex 50WX8 strong acid ion exchange resin (Sigma–Aldrich, cat. 217492)
- Ultralink hydrazide resin (Thermo Scientific, cat. 53149)
- Pyridine
- Sodium azide

Note: Materials lists include only non-standard items. Common laboratory ware and equipment such as water baths are assumed to be available.

Procedure

A1 In a glass screw-cap tube, dissolve 0.3 mmol of a desired glycan in 1 mL of sodium bicarbonate buffer.
A2 Add 35 μL of acetyl acetone (approx. 1.1:1 molar ratio with glycan) and vortex to dissolve.
A3 Incubate sealed glass tube in a water bath or heating block at 80–90 °C for at least 4 h.
A4 Remove tube and cool to ambient temperature. A slight yellow-brown discoloration may develop, particularly with larger or more complex glycans.
  a. A small amount of pressure from CO_2 release may build within the tube during incubation. Vent the tube slowly prior to opening and it should not be problematic.
A5 Add 3 mL of ethyl acetate and shake to mix. Allow ethyl acetate to separate from the aqueous phase and discard this upper layer.
  a. If the ethyl acetate is slow to separate, place the reaction in a freezer for 10–15 min to help.
A6 Add acid ion exchange resin to the aqueous phase, 10–20 mg at a time in order to neutralize. Shake gently to release CO_2 and add more resin until no more gas is generated.
A7 Remove the aqueous phase, containing the newly formed *C*-glycoside ketone, from the ion exchange resin and retain.
  a. *C*-glycoside ketone formation may be verified by mass spectrometry at this stage if desired.
A8 Prepare 1 mL (bed volume) of hydrazide resin in a glass vial and wash with water to remove storage buffer.
A9 Remove excess water from the resin and apply 200 μL of the *C*-glycoside ketone solution.
A10 Evaporate the majority of the aqueous phase from the resin under an air stream, carefully so as to not displace the resin from the vial.
A11 Dilute the resin in 2 mL pyridine, vortex to mix, and incubate at ambient temperature for at least 1 h.
A12 Carefully remove pyridine with a Pasteur pipette and wash the resin in 3–4 volumes of water. Wash 5–6 times to remove all pyridine.
A13 Retain the resin for use or suspend in 0.02% sodium azide for 4 °C storage. We have found that the resin remains functional for longer than two months.

Part 2: C-Glycoside resin verification

Once the above procedure has been completed, validation that the target glycan has been immobilized may be desired. We have found that if the glycan is a disaccharide or larger, glycosidic linkages can be acid-hydrolyzed to release constituent