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Fluorescent glycopolymers for probing plant glycan-binding proteins

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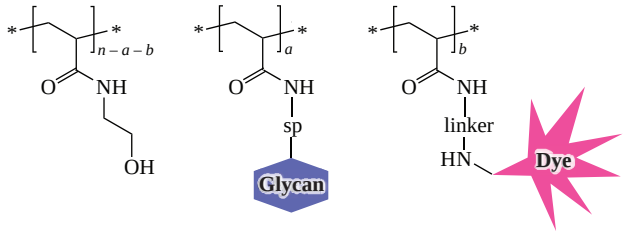
The synthesis of glycoprobes based on water-soluble polyacrylamide with a custom content of any low molecular weight fluorescent label is described. The synthesis is carried out in a stepwise manner: in the first step, the polymer is strictly dose-modified with a side substituent-linker containing an amino group; in the second step, the amino group is quantitatively acylated with an activated ester of the dye. The probes are designed to identify and study carbohydrate-binding proteins of plant cells.



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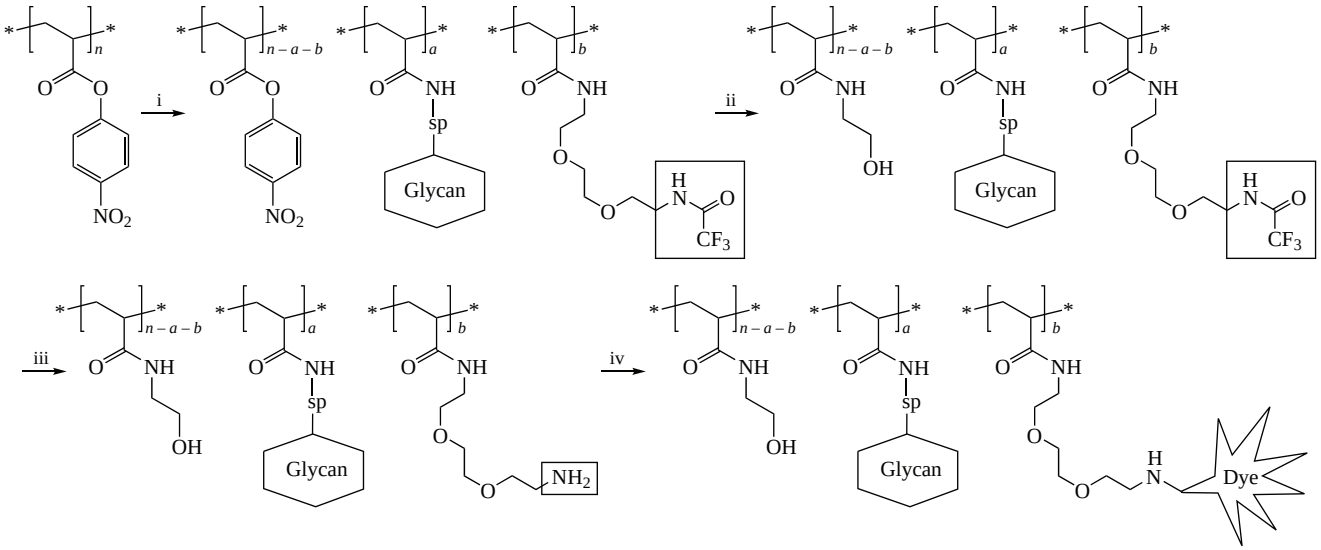
Polyacrylamide (PAA) probes with glyco substituents and fluorescein label[1] are widely used as tools for studying endogenous lectins of mammalian cells using flow cytometry and confocal microscopy.[2] An attempt to employ the same molecules in the studies of plant glycan-binding proteins[3,4] was unsuccessful due to high non-specific binding level to various plant cell structures, which was caused by the fluorescein label. To solve this problem, we attempted to synthesize probes with a different fluorescent label in a similar way, however, under the conventional synthesis conditions[1] which included conjugation of the amino spacer-derived label with activated polyacrylic acid and subsequent quenching of excess active groups with ethanolamine, the partial or almost complete destruction of fluorescent labels such as rhodamine (Rho), Cyanine 5 (Cy5), sulfo-Cyanine 5 (SuCy5), sulfo-Cyanine 3 (SuCy3) and BODIPY_FL occurred. In this work, we changed the synthetic route, first carrying out a conjugation with amino-spacered glycan and strictly 'dosed' coupling of a monoprotected diamine, then quenching the excessive active groups with ethanolamine (as above), removing the protecting group, and, in the last step, under mild non-destructive conditions, coupling of a dye equipped with an activated carboxyl group (Scheme 1), Dye NHS ester (NHS – *N*-hydroxysuccinimide). The final PAA-based glycoprobes are characterized by 'bespoke' content of a fluorescent label.

Since conjugation with the desired label or effector in the proposed strategy is performed in the last step, it may be



Scheme 1 *Reagents and conditions*: i, Glycan-O$(CH_2)_3NH_2$ (as the Glycan-spacer amine), $CF_3(CO)NH–(CH_2CH_2O)_2–CH_2CH_2NH_2 \cdot CF_3COOH$, Et_3N, dry DMSO, 40 °C, 4 h; ii, $H_2N(CH_2)_2OH$, 40 °C, 2 h; iii, Et_3N, H_2O/DMSO (2 : 1), 40 °C, 18 h, isolation on Sephadex LH-20 in MeCN/H_2O (1 : 2) + 0.2% AcOH, freeze-drying; iv, Dye NHS ester (3–4 equiv. per amine), Pr^i_2EtN (1 equiv. per amine + 1 equiv. per Dye NHS ester), dry DMSO, 40 °C, 1 h, isolation on Sephadex LH-20 in MeCN/H_2O (1 : 2) + 0.1% AcOH, freeze-drying.

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applicable to other tags unstable or able to interact ambiguously under routine conditions of ligand-to-PAA conjugation (see above). In particular, this two-step approach has been used to bind peptides through their end cysteine to a maleimide-substituted PAA carrier.[5]

Molecular probes with the general formula Glyc$^{(20)}$–PAA–Dye and Glyc$^{(40)}$–PAA–Dye containing 1–2 mol% of the Dye substituent (Glyc is oligosaccharide, superscript indices (20) and (40) relate to its molar percentage) ~20 kDa were designed due to the following considerations. The relatively small size of the polymer allows for easy diffusion into cellular structures. The high content (20 or 40 mol%, that is, 20 or 40 of the 100 acrylamide units of the polymer carry the substituent) of the Glyc moiety provides multipoint binding of the probe to the glycan-recognizing target proteins. The 1 or 2 mol% label content ensures that each polymer chain carries at least one (but not many, leading to the risk of non-specific interaction) of the Dye residues.

Note that the attachment of the monoprotected diamine linker to the activated polymer in the first step occurs quantitatively,[1] which guarantees the modification dosage, and in the second step it allows one to use a certain excess of the label-NHS ester reagent; the glycopolymer containing no added amino groups does not bind the Dye, *i.e.*, after 20 h at 40 °C, only traces of attached Dye appear on the polymer (TLC data), apparently due to the reaction of the dye NHS ester with the hydroxy groups of the glycan or ethanolamide. The coupling of a monoprotected diamine linker into the polymer chain was tested in two variants using $CF_3CONH–(CH_2CH_2O)_2–CH_2CH_2NH_2$ and $N_3–(CH_2CH_2O)_3–CH_2CH_2NH_2$ linkers, both having shown equally good results and preparative convenience.

The described method allows the insertion of a variable amount of a fluorescent dye into PAA glycoconjugates, we consider 1 mol% (maximum 2 mol%) to be optimal based on the considerations below. Amount of 1 mol% in Glyc$^{(20)}$–PAA–Dye corresponds to approximately one Dye molecule per macromolecule; this, according to our practice, is sufficient for detection in both flow cytometry and confocal microscopy. An increase in the label content to 2 mol% gives an increase in the signal intensity, but at the same time, partial quenching of the Dye is already observed due to the formation of its H-dimers (homodimers) which absorb at a shorter wavelength.[6] Indeed, Figure 1 shows the absorption spectra of the free Cy5 (λ_{abs} = 646 nm)[8] and its four PAA glycoconjugates Galα1–3Galβ1–4GlcNAc$^{(40)}$–PAA–Cy5, where the probes are contained 1, 2, 3 or 5 mol% of Cy5. The spectrum of the conjugate with 1 mol% of Cy5 differs a little from the spectrum

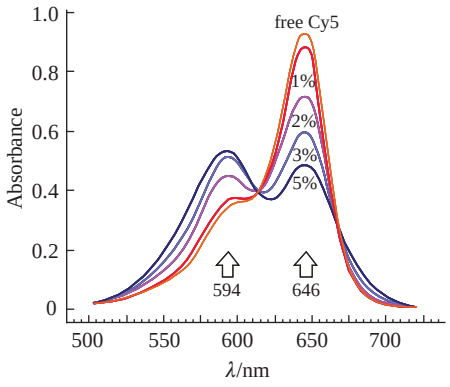

Figure 1 Absorbance spectra in water of free Cy5 dye and Galα1–3Galβ1–4GlcNAc$^{(40)}$–PAA–Cy5 conjugates with 1, 2, 3 or 5 mol% of Cy5. The maximum 646 nm corresponds to the λ_{abs} of Cy5, the maximum 594 nm corresponds to the λ_{abs} of Cy5 H-dimer. Concentration of free Cy5 was 4×10^{-6} mol dm^{-3}, concentrations of glycoconjugates were 0.03–0.07 mg ml^{-1}. The spectra of glycoconjugates were scaled to the peaks area equal the peak for the free dye.

of free dye. Raising the Cy5 content in the glycoconjugate leads to an increase in the formation of H-dimers (λ_{abs} = 594 nm). Similar effect is observed (not shown) for PAA glycoconjugates containing SuCy5 or BODIPY_FL dyes.

The glycoconjugates with fluorescent labels Glyc–PAA–Dye synthesized in this work are as follows (the superscript figures in parentheses placed after the oligosaccharide and the dye designate their molar percentages, the structures of the fluorescent dyes are given in Online Supplementary Materials, Figure S1):

D-Xylβ1–2Manα$^{(20)}$–PAA–SuCy3$^{(2)}$,

Galα1–3Galβ1–4GlcNAcβ$^{(40)}$–PAA–BODIPY_FL$^{(3)}$,

Galα1–3Galβ1–4GlcNAcβ$^{(40)}$–PAA–Cy5$^{(3)}$,

Galα1–6Glcβ$^{(20)}$–PAA–SuCy5$^{(2)}$,

GlcNAcβ1–4GlcNAcβ$^{(40)}$–PAA–BODIPY_FL$^{(3)}$,

GlcNAcβ1–4GlcNAcβ$^{(40)}$–PAA–SuCy5$^{(3)}$,

GlcNAcβ1–4GlcNAcβ$^{(40)}$–PAA–Cy5$^{(3)}$.

The synthesized glycopolymers containing the fluorescent dyes[7] BODIPY_FL, Cy5, SuCy5 and SuCy3[9] did solve the problem of avoiding nonspecific interaction of fluorescein residue with plant sections (see Online Supplementary Materials, Figure S3). These glycopolymers contain 2, 3, or 5 mol% fluorescent dye, which is expected to be excessive for staining plant sections, but makes it possible to identify nonspecific interactions at the stage of optimizing dye content.

In summary, a method for attaching a fluorescent label of any nature in a precisely programmed amount to a polymer matrix has been proposed. It allows one to synthesize molecular probes optimal for specific biological objects.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.01.004.

References

1 A. Tuzikov, A. Chinarev, N. Shilova, E. Gordeeva, O. Galanina, T. Ovchinnikova, M. Schaefer and N. Bovin, *Glycoconjugate J.*, 2021, **38**, 89.
2 O. Kurmyshkina, E. Rapoport, E. Moiseeva, E. Korchagina, T. Ovchinnikova, G. Pazynina, I. Belyanchikov and N. Bovin, *Acta Histochem.*, 2010, **112**, 118.
3 E. H. E. Konozy and M. E. M. Osman, *Biochimie*, 2022, **202**, 136.
4 R. D. Cummings, M. E. Etzler, T. N. C. Ramya, K. Kato, G. A. Rabinovich and A. Surolia, in *Essentials of Glycobiology*, 4th edn., eds. A. Varki, R. D. Cummings, J. D. Esko, P. Stanley, G. W. Hart, M. Aebi, D. Mohnen, T. Kinoshita, N. H. Packer, J. H. Prestegard, R. L. Schnaar and P. H. Seeberger, Cold Spring Harbor Laboratory Press, New York, 2022, ch. 32.
5 I. M. Ryzhov, A. B. Tuzikov, A. V. Nizovtsev, L. K. Baidakova, O. E. Galanina, N. V. Shilova, M. M. Ziganshina, N. V. Dolgushina, G. R. Bayramova, G. T. Sukhikh, E. C. Williams, R. Nagappan, S. M. Henry and N. V. Bovin, *Bioconjugate Chem.*, 2021, **32**, 1606.
6 M. Ogawa, N. Kosaka, P. L. Choyke and H. Kobayashi, *ACS Chem. Biol.*, 2009, **4**, 535.
7 R. P. Haugland, *Methods Cell Biol.*, 1994, **42 B**, 641.
8 B. Mujumdar, A. Ernst, S. R. Mujumdar, C. J. Lewis and A. S. Waggoner, *Bioconjugate Chem.*, 1993, **4**, 105.
9 I. O. Aparin, O. V. Sergeeva, A. S. Mishin, E. V. Khaydukov, V. A. Korshun and T. S. Zatsepin, *Anal. Chem.*, 2020, **92**, 7028.

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