Carbohydrate Research, 70 (1979) C9–C12

Preliminary communication

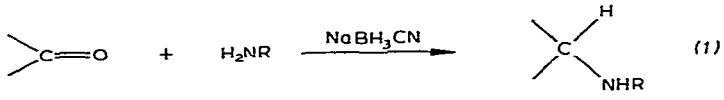
Selective activation and chemical derivatisation of the sialic acid and galactose components of glycoproteins: spin-labelling of fetuin, bovine submaxillary mucin, and erythrocyte surface glycoconjugates

JOHN D. APLIN*, MICHAEL A. BERNSTEIN*, CHARLES F. A. CULLING, LAURANCE D. HALL*, and PHILIP E. REID

Department of Chemistry and Department of Pathology, University of British Columbia, Vancouver, British Columbia, V6T 1W5 (Canada)*

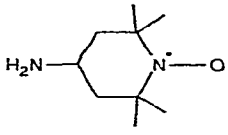
(Received November 21st, 1978; accepted for publication, January 8th, 1979)

As part of a general interest in the functional properties of the carbohydrate components of glycoproteins, we have been evaluating chemical methods whereby various spectroscopic probes can be attached to selected carbon atoms of specified sugar units. When a carbodiimide-mediated amidation procedure was used[1] to attach probe groups to the carboxyl group of sialic acid residues, derivatisation of carboxyl groups of the protein also occurred. We now report on the introduction of an aldehyde group at C-7 of sialic acid[2] C-6 of galactose[3], or C-3 (C-4) of galactose[4], and the subsequent attachment thereto of an amine-containing, spectroscopic probe by reductive amination[5] (Equation *1*).



(1)

These procedures are illustrated here in the context of nitroxide spin-labelling, using reagent **1**, of a typical serum glycoprotein (fetuin), a typical mucin (bovine submaxillary mucin, BSM), and human erythrocytes.



1

In the case of an intact glycoprotein, the sialic acid residues can be activated[2] as follows. The glycoprotein is dissolved in water, 10mM sodium metaperiodate (5 mol for each mol of sialic acid) is added, and the solution is kept at 0°C for 35 min. Excess of periodate is then decomposed by using an excess of potassium iodide and sodium thio-

sulphate*. After desalting [either by filtration through Centriflo membranes (retention limit, 25000 daltons), exhaustive dialysis, or Sephadex G-25 filtration], the oxidised glycoprotein is added to a 25-molar excess of the spin-label **1** (based on sialic acid) and a 5-molar excess of sodium cyanoborohydride ($NaBH_3CN$), and the solution is maintained at pH 8 for 2 h at ambient temperature. Following desalting, the aqueous solution is lyophilised. Such glycoproteins as BSM, which are *O*-acylated at C-7, C-8, or C-9 of sialic acid, are saponified prior to oxidation by using aqueous sodium hydroxide (M) for 30 min at ambient temperature, followed by neutralisation (M hydrochloric acid) and desalting.

Activation of HO-6 of the terminal D-galactose residues of an asialo-glycoprotein is conveniently combi ed with desialation of the glycoprotein, as follows. The glycoprotein is dissolved in acetate buffer (pH 6.9, containing 0.02% of $CaCl_2$), and 1 unit of *Vibrio cholera* neuraminidase and 2 units of D-galactose oxidase are added for each mg of glycoprotein. The solution is kept at ambient temperature for 48 h and then, following desalting, the coupling with **1** is performed as previously described.

Periodate oxidation, by the foregoing procedure, results in selective cleavage at C-3–C-4 of the terminal galactosyl** residues of asialo-fetuin[7] and of asialo-BSM; these residues can then be reductively aminated.

Derivatisation of the glycoconjugates of erythrocytes can be accomplished by the foregoing procedures, using cells suspended in physiological saline. Subsequent washing of the cells with phosphate-buffered saline is followed by exhaustive dialysis against the same buffer. Isolation of the erythrocyte membranes showed that the label had been attached to the membrane.

Typical electron paramagnetic resonance (e.p.r.) spectra of the spin-labelled materials are shown in Fig. 1; and motional correlation time, estimated[8] from these spectra, are as follows: periodate, fetuin (0.79 nsec); D-galactose oxidase, asialo-fetuin (0.52 nsec); periodate, asialo-fetuin (1.20 nsec); periodate, BSM (0.35 nsec); D-galactose oxidase, asialo-BSM (0.49 nsec); periodate, asialo-BSM (0.35 nsec); periodate, erythrocytes (1.0 nsec). In a control experiment (see Fig. 1D), the correlation time of non-specifically adsorbed material was 0.29 nsec. Spectral simulation[9,10] on a Nicolet 1180 computer, assuming isotropic tumbling, of the periodate–fetuin experiment (Fig. 1A) gave a correlation time of 0.45 nsec.

Although discussion of their significance is deferred, attention is drawn to the fact that all of the values are substantially faster than anticipated for molecules of these high molecular weights; this implies that, in these labelled systems, the nitroxide groups have additional degrees of motional freedom. This aspect is being further studied.

An important feature of our studies has been the development of assay procedures whereby the extent of covalent attachment to the sugar units can be compared with the level of randomly adsorbed material. The amounts of specified sugars were determined by

*The use of ethylene glycol to reduce excess of periodate is avoided, as liberated formaldehyde may react with, and modify, the protein.

**Other sugar residues having an unsubstituted *cis*-diol moiety could react similarly[4].

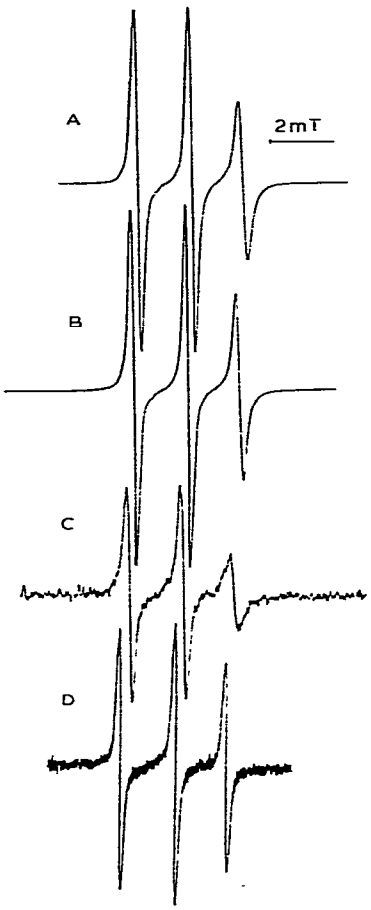


Fig. 1. E.p.r. spectra of nitroxide-labelled materials: Varian E-3 instrument at 9.5 GHz; power level, 10 mW; modulation amplitude, 0.1 mT; time constant, 0.1 sec (except for C); and ambient temperature. Aqueous samples were contained in a flat, quartz cell (Scanlon). Concentrations and relative gain are given in parentheses. A, periodate-activated fetuin (3.24 mg in 50 μl of H_2O; gain 1); B, D-galactose oxidase-treated asialo-BSM (2.67 mg in 50 μl of H_2O; gain 6.4); C, periodate-activated, intact erythrocytes (73 μl of packed cells in phosphate-buffered saline; gain 187.5); D, fetuin perfused with 1 and $NaBH_3CN$ for 2 h (3.00 mg in 50 μl of H_2O; gain 62.5).

a combination of methods[11,12]; using double integration of e.p.r. spectra, we have found, typically, that the foregoing derivatisations proceed to 20—30% for periodate oxidation and ~5% for neuraminidase and D-galactose oxidase. Less than 0.5% of that signal is attributable to adsorbed material. The spectra given in Figs. 1A and 1D clearly illustrate this latter point.

The procedures summarised here are believed to provide a general entry to spin-labelling of specified loci both of isolated glycoproteins and of cell-surface glycoconjugates, although, in the latter regard, the biological viability of the labelled cells remains to be

evaluated. Clearly, the same basic chemistry can be extended to include the incorporation of other classes of probes.

Since this work was initiated, two other groups have reported[13—15] the use of periodate oxidation–reductive amination for labelling sugar units of immunoglobulins.

ACKNOWLEDGMENT

This work was supported by operating grants from the National Research Council of Canada (A1905 to LDH).

REFERENCES

1 J. D. Aplin, D. E. Brooks, C. F. A. Culling, L. D. Hall, and P. E. Reid, unpublished data.
2 L. Van Lenten, and G. Ashwell, *J. Biol. Chem.*, 246 (1971) 1889–1894.
3 A. G. Morell, C. J. A. Van Den Hamer, I. H. Scheinberg, and G. Ashwell, *J. Biol. Chem.*, 241 (1966) 3745–3749.
4 R. D. Guthrie, *Adv. Carbohydr. Chem.*, 16 (1961) 105–158.
5 R. F. Borch, M. D. Bernstein, and H. D. Durst, *J. Am. Chem. Soc.*, 93 (1971) 2879–2904.
6 C. G. Gahmberg, *Methods Enzymol.*, 50 (1978) 204–206.
7 R. G. Spiro, *J. Biol. Chem.*, 239 (1964) 567–573.
8 T. J. Stone, T. Buckman, P. L. Nordio, and H. M. McConneli, *Proc. Natl. Acad. Sci. U.S.A.*, 54 (1965) 1010–1017.
9 P. Coffey, B. H. Robinson, and L. R. Dalton, *Chem. Phys. Lett.*, 35 (1975) 360–366.
10 P. Coffey, B. H. Robinson, and L. R. Dalton, *Mol. Phys.*, 31 (1976) 1703–1715.
11 L. Warren, *J. Biol. Chem.*, 234 (1959) 1971–1975.
12 G. W. Jourdian, L. Dean, and S. Roseman, *J. Biol. Chem.*, 246 (1971) 430–435.
13 K. J. Willan, B. Golding, D. Givol, and R. A. Dwek, *FEBS Lett.*, 80 (1977) 133–136.
14 R. S. Nezlin, V. P. Timofeev, Yu. K. Sykulev, and S. E. Zurabyan, *Immunochemistry*, 15 (1978) 143–144.
15 V. P. Timofeev, I. V. Dudich, Yu. K. Sykulev, and R. S. Nezlin, *FEBS Lett.*, 89 (1978) 191–195.