Preparation of fluorescein-labelled and biotinylated derivatives of polysaccharides for lectin-saccharide binding studies

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Fluorescein- and biotin-labelled polysaccharides were prepared using ethylene diamine coupled to a polysaccharide either by carbodiimide reaction to carboxyl group or after periodate oxidation of saccharide residue and the derivative was used for labelling. Labelled hyaluronic acid, chondroitin sulfate and dextran sulfate were prepared.

Introduction

Labelled saccharide derivatives are needed in the detection and the characterization of a biorecognition process involving protein–saccharide interactions. For the labelling of glycoconjugates, such as glycoproteins or glycopeptides, the protein moiety is employed. In the case of simple saccharides and their derivatives, macromolecular derivatives are usually synthesized, e.g. polyacrylamide derivative (Chadli et al., 1992, Klein et al., 1995), making possible to link a label. Preparation of low-molecular biotinylated derivatives of monosaccharides was described by Lauc et al. (1994). In the case of polysaccharide ligands, only few examples of labelling have been described (Debbage et al., 1988, Yang et al., 1995).

The preparation of polyacrylamide derivatives of different polysaccharides has been described in our previous communication (Novotná et al., 1996); the presence of free unsubstituted amino groups in the polyacrylamide part of the glycoconjugate enabled biotin labelling (Novotná et al., 1996). An increased molecular mass of the resulting product might be a drawback in an application of such derivatives in some cases.

A simple technique for preparing labelled polysaccharides without a significant change in their molecular weight and without the presence of non-polysaccharide part is described in our present communication.

Materials and methods

Materials

1-(3-dimethyl-aminopropyl)-3-ethyl carbodiimide was purchased from Fluka, dextran sulfate, chondroitin sulfate (bovine trachea), hyaluronic acid (from bovine trachea), fluorescein isothiocyanate and N-hydroxy-succinimido-biotin from Sigma. Water soluble poly(acrylamide-allylamine) copolymers were prepared by copolymerization of acrylamide and allylamine as described previously (Klein et al., 1995). Heparin binding proteins (HB proteins) were isolated from boar seminal plasma by affinity chromatography on heparin-polyacrylamide gel (Tichá et al., 1994) and by RP HPLC. Fractions B6–B8 from the last step of purification were used as spermadhesins of AWN subfamily (publication in preparation). Using affinity chromatography on Heparin Sepharose and RP HPLC, 10 fractions of heparin binding proteins was obtained from human seminal plasma (publication in preparation).

Coupling of ethylene diamine to acidic polysaccharides

Aqueous solution of an acid polysaccharide (50 mg in 5 ml) was mixed with ethylene diamine (20 Yl) and then N-ethyl-N’-(3-dimethyl-aminopropyl) carbodiimide (40 mg) was added. The mixture was shaken for 2 hrs. at room temperature and then dialyzed against distilled water and lyophilized.
Coupling of ethylene diamine to periodate oxidized polysaccharides

Aqueous solution of a polysaccharide (50 mg in 5 ml) was mixed with 1% periodic acid (1 ml) and ethylene diamine (20 μl). The mixture was shaken for 2 hrs. and the reaction was stopped by the addition of ethylene glycol (4 ml). After 15 min. standing at room temperature, natrium cyanoborohydride (80 mg) was added. The reaction mixture was dialyzed against distilled water and lyophilized.

Polyacrylamide derivatives of polysaccharides

These were prepared as described previously (Novotná et al., 1996). Chondroitin sulfate and hyaluronic acid were coupled to poly(acrylamide-allyl amine) copolymer using carbodiimide reaction; for coupling dextran sulfate, its periodate oxidized derivative was used.

Biotinylation of polysaccharide derivatives

N-hydroxy-succinimido-biotin (10 mg) dissolved in dimethylformamide (25 μl) was added to the solution of the polysaccharide derivative (20 mg) in 0.1 M borate buffer pH 8.5. The mixture was stirred for 30 min. at room temperature and then 0.2 M NH₄Cl was added to adjust to pH 6.0. After dialysis against distilled water, the solution was lyophilized.

Coupling of fluorescein label

Polysaccharide derivative (20 mg) was dissolved in 0.1 M carbonate buffer pH 9.2 (3 ml) and mixed with the fluorescein isothiocyanate solution (A) (200 μl); a solution was prepared by dissolving fluorescein isothiocyanate (20 mg) in a mixture of dimethylformamide (1 ml) and ethylene glycol (8 ml). The reaction mixture was stirred in the dark at 4 °C for 1 hr. Then the next portion of the A solution (200 μl) was added and the step was repeated 3 times. An excess of the labelling reagent was separated from the labelled polysaccharide using gel chromatography on Sephadex G-25 equilibrated with 0.05 M NH₄HCO₃. Fractions corresponding to the labelled polysaccharide were collected and lyophilized.

Analytical methods

The content of coupled biotin was determined using HABA (2-hydroxyazobenzene-2′-carboxylic acid)-avidin reagent (Green, 1970). For the determination of the fluorescein label content the measurement of absorbance at 495 nm was used. Polysaccharide content in polyacrylamide derivatives was determined by the phenol-sulfuric acid method (Duboise et al., 1956) using the corresponding polysaccharide as a standard.

Enzyme-linked binding assay (ELBA)

This was performed as described previously (Novotná et al., 1996).

Results

Preparation of FITC-labelled and biotinylated derivatives of polysaccharides

In the first step, ethylene diamine was coupled to polysaccharides by two different reactions. In the case of acidic polysaccharides (containing carboxyl groups), carbodiimide reaction was used and a stable amide bond was formed. Neutral polysaccharides were oxidized using periodate; the formed aldehyde groups reacted with ethylene diamine. After the reduction with natrium cyanoborohydride, a stable secondary amine was obtained.

For comparison, the same polysaccharides were coupled to polyacrylamide copolymer containing covalently-bound amino groups (Novotná et al., 1996); the same reactions were used as described above.

Labelling of polysaccharide derivatives

Both types of the prepared polysaccharide derivatives were labelled either using fluorescein isothiocyanate or by N-hydroxy succinimido-biotin. In the reaction either free unsubstituted amino groups of polyacrylamide copolymer or the amino group of ethylene diamine (incorporated into polysaccharide) participate. The label content in the prepared polysaccharide derivatives is summarized in Table 1.

Application of the prepared labelled polysaccharide derivatives

Both types of biotinylated derivatives of polysaccharides (polyacrylamide based or those containing ethylene diamine) were used to study the saccharide binding properties of heparin binding proteins B6–B8 belonging

<table>
<thead>
<tr>
<th>Polysaccharide derivative</th>
<th>Polysaccharide content [g/100 g]</th>
<th>Biotin content [mmole/100 g]</th>
<th>Fluorescein content [mmole/100 g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran sulfate-PAA</td>
<td>6.3</td>
<td>0.35</td>
<td>1.02</td>
</tr>
<tr>
<td>Dextran sulfate-ED</td>
<td>–</td>
<td>0.40</td>
<td>7.00</td>
</tr>
<tr>
<td>Hyaluronic acid-PAA</td>
<td>4.4</td>
<td>0.34</td>
<td>3.84</td>
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<tr>
<td>Hyaluronic acid-ED</td>
<td>–</td>
<td>0.88</td>
<td>1.50</td>
</tr>
<tr>
<td>Chondroitin sulfate-PAA</td>
<td>6.2</td>
<td>0.39</td>
<td>2.02</td>
</tr>
<tr>
<td>Chondroitin sulfate-ED</td>
<td>–</td>
<td>0.31</td>
<td>1.10</td>
</tr>
</tbody>
</table>
to spermadhesins AWN subfamily from boar seminal plasma (Fig. 1A) and heparin binding proteins from human seminal plasma (Fig. 1B). The method of ELBA (enzyme linked binding assay) was used: proteins immobilized onto plastic microtiter wells interacted with biotinylated polysaccharide derivatives. Results obtained showed that both types of biotinylated derivatives could be applied in ELBA tests and in the case of studied heparin binding proteins similar results were obtained.

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References

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