PRELIMINARY COMMUNICATION

Glycosylmethylamines as a New Tool in the Lectin Research

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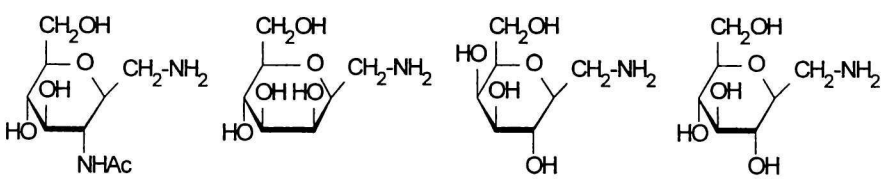
Glycopyranosylmethylamines (*e.g. I—IV*) are interesting, nonhydrolyzable glycopyranosylamine equivalents. Three-step synthesis from parent aldoses, especially after the introduction of the efficient methods of their preparation and isolation [1—3], has made them easily available *C*-glycosyl synthons. In spite of that they have been synthetically utilized for further biochemical applications rather rarely [4, 5]. Up to now, much more attention has been paid to their precursors, glycopyranosylnitromethanes which have been utilized for the synthesis of different glycomimetics [6—9]. Here we wish to report preliminary results on a new application of glycopyranosylmethylamines, namely for the recognition and affinity purposes in the lectin research.

For the isolation purposes of a new lectin from the crude extract of the *Pinus nigra* seed protein bodies, the poly(2-hydroxyethyl methacrylate) matrix of Spheron 300 (40—63 μm; Lachema, Brno) which fulfils practically all the requirements laid on the stationary phases for chromatography of proteins [10] was chosen. The absence of carbohydrate and aromatic moieties in the matrix excludes the most significant nonspecific interactions which might have competed with the selective recognition process proposed. In addition, a mild hydrophobic character of the polymer can be easily suppressed. The glycopyranosylmethylamine ligand, namely 2-acetamido-2-deoxy-β-D-glucopyranosylmethylamine (*I*) was linked to the matrix after its activation *via* the chlorination of the solvent exchange-dried Spheron with thionyl chloride in chloroform (Scheme 1) to a low

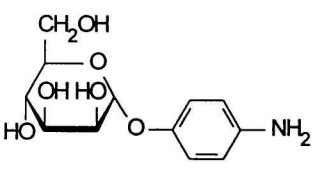


I

II

III

IV



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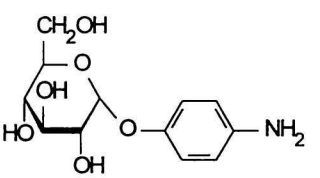

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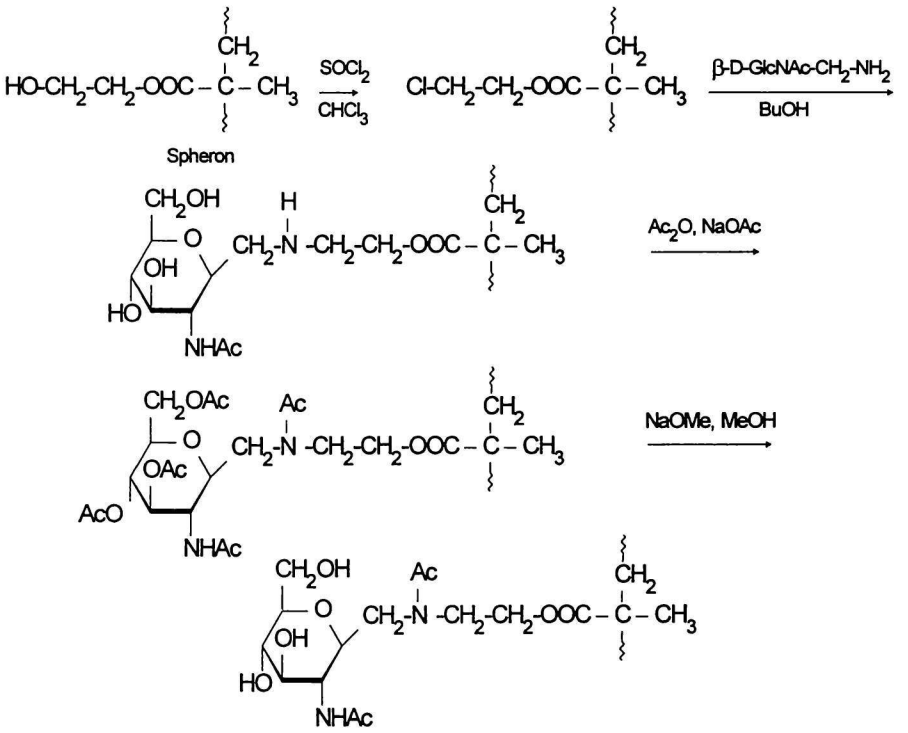

Scheme 1

chlorine content (0.5 mass %). Subsequent alkylation of *I* with 2-chloroethyl moieties of the activated matrix in boiling butan-1-ol afforded a glycosylated Spheron 300 material containing basic, secondary amino groups which were conveniently used for the determination of the degree of glycosylation by a simple acid-base titration. The glycosylation procedure afforded the modified Spheron 300 packing material containing 5.6 mass ‰ of the 2-acetamido-2-deoxy-β-D-glucopyranosylmethylamine moieties. In order to avoid ionic interactions which could have occurred during the matrix applications, its basic, secondary amino groups were *N*-acetylated in two steps (Scheme 1); first by its per-*N*,*O*-acetylation with acetic anhydride and sodium acetate followed by de-*O*-acetylation with sodium methoxide in methanol. The control titration of the finally modified chromatographic matrix has shown no presence of basic groups thus proving the efficiency of the *N*-acetylation. A similar modification of the same polymer matrix of HEMA BIO 1000 (10 μm; Tessek, Prague) used for the agglutination assays afforded the material containing 47 mass ‰ of the 2-acetamido-2-deoxy-β-D-glucopyranosylmethylamine moieties. Thus, the glycosylmethylamine glycosylation procedure is a substantially simpler method than the commonly used thioglycoside procedure [11, 12].

Affinity chromatography of the crude extract from the protein bodies of the *Pinus nigra* seeds on this GlcNAc-*C*-glycosylated Spheron 300 eluted with phosphate buffer (pH 7.2 and c = 0.05 mol dm^{-3}) containing 20 vol. % of ethylene glycol allowed to purify by a one-step isolation procedure the *Pinus nigra* lectin (PNL) homogeneous in sodium dodecyl sulfate—poly(acrylamide) gel electrophoresis (SDS—PAGE) (Fig. 1) and reverse phase chromatography (not shown here) and without any trace of β-*N*-acetyl-D-glucosaminidase. The isolated PNL exhibited the agglutination activity[1] equal to 2048 with both *Trichoderma viride* spores and GlcNAc-*C*-glycosylated HEMA BIO 1000 microparticles and interacted with fungal cell walls. The attempts to isolate PNL by standard purification procedures using *e.g.* powdered chitin of crab shells or poly(acrylamide-*co*-allyl 2-acetamido-2-deoxy-β-D-glucopyranoside) were not successful.

The GlcNAc-*C*-glycosylated HEMA BIO 1000 microparticles were used for the agglutination assays also with other known lectins. Thus, wheat germ agglutinin (WGA), *Datura stramonium* agglutinin (DSA), and *Lens culinaris* lectin (LCH) exhibited respective agglutination activities equal to 1024, 512, and 128. The lectin activities thus determined are in accordance with their generally known specificities described earlier. Moreover, the advantage of the HEMA BIO 1000 microparticles is in their stability and reproducibility what is not a case of generally used biological agglu-

[1] The value of this quantity (2^n) gives the number of dilutions of the original lectin concentration 1 mg cm^{-3} by n times repeated dilution of the volume of the solution to its double.

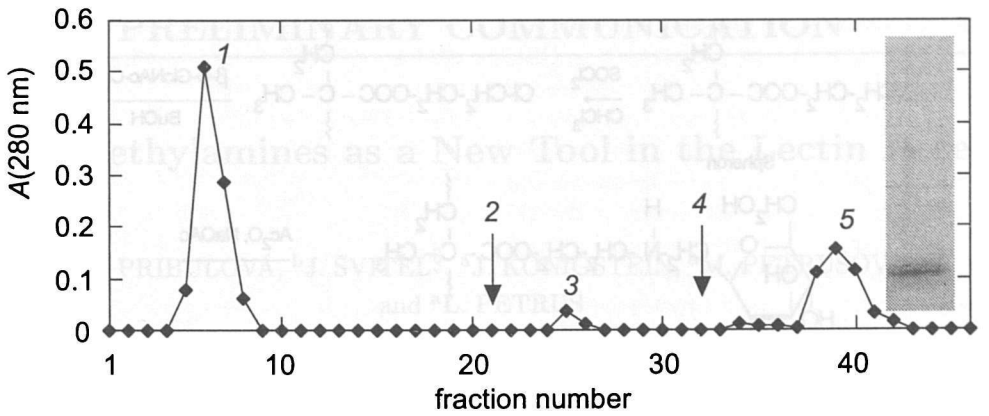

Fig. 1. Affinity chromatography of the crude protein extract from *Pinus nigra* protein bodies on the GlcNAc-*C*-glycosylated Spheron 300 using elution with a phosphate buffer (pH 7.2 and $c = 0.05$ mol dm^{-3}) containing 20 vol. % of ethylene glycol (EG). *1.* Protein portion not adsorbed, *2.* the arrow indicates the change of the eluant to a solution of acetic acid ($c = 0.1$ mol dm^{-3}) containing 20 vol. % EG resulting in the desorption of the protein portion (*3*), *4.* the arrow indicates the change of the eluant to a carbonate buffer (pH 10.0 and $c = 0.05$ mol dm^{-3}) containing 20 vol. % EG releasing the *Pinus nigra* lectin (*5*) homogeneous in SDS—PAGE (shown in the insert).

tination preparations (*e.g.* erythrocytes).

Surface plasmon resonance (SPR) being used for analysis of sugar binding specificities represents the last new application of glycosylmethylamines we wish to present here. Compounds *I*—*IV* were linked to the *O*-carboxymethyl dextran sensor surface using the standard BIACore procedure [13, 14]. The remaining active carboxyl groups were deactivated with ethanolamine which was used also for a control experiment. Several lectins were injected to the corresponding sensor chip surfaces according to their specificities. The regeneration of every chip was accomplished by washing the lectin linked to its surface with the pertinent glycosylmethylamine. Thus, in accordance with the known specificities, the respective chip-linked glycosylmethylamine *I* interacted with WGA and DSA and glycosylmethylamine *III* with *Ricinus communis* lectin. Surprisingly, LCH and *Vicia faba* lectin, in addition to their expected interactions with chip-linked 4-aminophenyl-α-glycosides of the configurations D-*manno* (*V*) and D-*gluco* (*VI*), exhibited the interaction also with β-D-mannopyranosylmethylamine (*II*). The chip-linked compound *IV* did not show any specificity with the lectins applied.

The SPR interactions of WGA and DSA with the sensor-immobilized compound *I* were used also for kinetic measurements (Fig. 2). The ascending parts of the curves *1* (WGA) and *2* (DSA) represent the association process of the interactions while the descending bends reflect the respective dissociations. From the shape of the SPR signals the corresponding rate association constants k_a and rate dissociation constants k_d can be determined [15]. Thus, for WGA and DSA the respective SPR interaction rate constants are $k_a = 4.67 \times 10^{10}$ mol^{-1} s^{-1}, $k_d = 4.96 \times 10^{-4}$ s^{-1} and $k_a = 1.19 \times 10^{9}$ mol^{-1} s^{-1}, $k_d = 3.84 \times 10^{-4}$ s^{-1}.

In summary, the preliminary results clearly demonstrate the usefulness of the easily available glycosylmethylamines for the lectin study by several different methods. All the promising methods are being further developed and will be published in detail elsewhere.

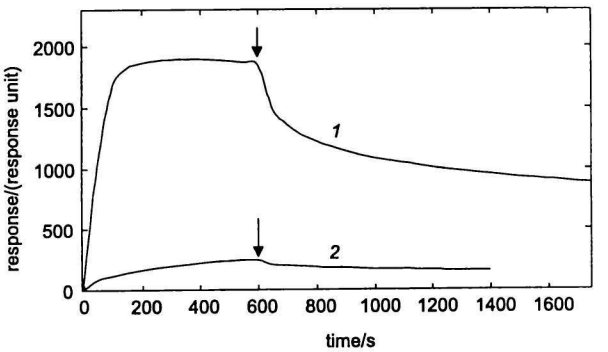

Fig. 2. Surface plasmon resonance (SPR) sensograms showing the interaction of wheat germ agglutinin (WGA, *1*) and *Datura stramonium* agglutinin (DSA, *2*) with immobilized 2-acetamido-2-deoxy-β-D-glucopyranosylmethylamine (*I*). Injected concentrations of lectins were $\rho_{WGA} = 0.01$ mg cm^{-3} and $\rho_{DSA} = 0.2$ mg cm^{-3}. The arrows indicate the beginning of the chip-regeneration phase using in both cases concentration of *I* 0.2 mol dm^{-3}.

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