METHOD OF SELECTING HISTOCHEMICAL DETECTION OF SIALOGLYCANS USING ELDERBERRY (*Sambucus nigra* L.) LECTINS

A. N. Yatskovskii and A. D. Lutsik

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Despite the known progress in carbohydrate histochemistry, selective detection of sialoglycans in histological sections still remains an urgent unsolved problem in morphology. This is due, on the one hand, to the important role of sialated glycoproteins in the regulation of the adhesion-migration properties of cells, and maintenance of their surface charge [1, 5], and on the other hand, to the absence of reliable, strictly specific and highly sensitive methods of sialic acid identification in histological preparations [5, 9, 13]. Investigation of sialoglycans is further complicated by the fact that in the sets of lectins currently in use, the most selective and sensitive histochemical reagents for the detection of individual types of terminal monosaccharide residues in glycopolymer molecules, there are no purely sialospecific lectins which can be readily obtained from Soviet sources of raw materials [3, 13]. In this context the aim of the present investigation was to study the possibility of using lectin from elderberry (*Sambucus nigra* L.) bark for the selective detection of sialic acids in the composition of complex carbohydrates and carbohydrate-containing macromolecules in cells and tissue structures.

EXPERIMENTAL METHOD

The submandibular salivary gland of a sheep, the secretion of which consists mainly of sialated glycoproteins with a terminal disaccharide residue with the structure Neu5Ac(α2, 6) GalNAc, was used as the test object [4, 6]. Pieces of the gland were fixed in a 10% solution of neutral formalin and embedded in paraffin wax. Sections 5 μ thick were glued to slides, previously gelatinized by the method in [8]. The histological preparations were obtained by the use of elderberry lectin (SNA, specific for the disaccharide Neu5Ac (α, 2, 6) GalNAc, and to a lesser degree, to D-galactose and N-acetyl-D-galactosamine residues) [10, 13]; peanut lectins (PNA, specific for D-galactose), and soy lectin (SBA, specific for N-acetyl-D-galactosamine) [1, 3]. The lectins were labeled with horseradish peroxidase. (Peanut and soy lectins, native and conjugated with peroxidase, and elderberry lectin were obtained from the "Lektinotest" Research-Production Cooperative attached to L'vov Medical Institute). Treatment of the sections with lectin followed by visualization of their binding sites with diaminobenzidine tetrahydrochloride, was carried out as described previously [2]. Sialic acid residues in some sections were removed by preincubation of the sections in a solution of Vibrio cholerae neuraminidase ("Boehring," West Germany). The neuraminidase solution was generously provided by Dr. M. Witte (Tübingen, Germany), and was used in a dose of 0.3 U/ml for 24 h at 37°C by the method in [14]. Control sections were incubated under the same conditions in acetate buffer, pH 5.5. In a separate series of experiments, some of the sections, before application of the solution of labeled elderberry lectin, were methylated at 37°C (mild methylation) or at 60°C (rigorous methylation) for 4 h, followed by saponification for 30 min [12]. To block the terminal, and also to unmask subterminal residues of D-galactose and N-acetyl-D-galactosamine after removal of the terminal sialic acid residues, before treatment of the sections with labeled elderberry lectin, they were incubated in a mixture of native (not conjugated with peroxidase) peanut and soy lectins for 30 min. The concentration of each of the native lectins in the incubation medium was 100 μg/ml. The presence of slatic acids in cells of the submandibular salivary gland was confirmed by staining serial sections with a 1% solution of alcian blue 8GX at pH 2.5 [9]. The intensity of staining with the different lectins was estimated in points by two independent investigators.

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TABLE 1. Results of Histochemical Staining of Sheep Submandibular Salivary Gland Cells with Elderberry, Peanut, and Soy Lectins in Different Combinations

Different versions of reactions	Mucocytes	Serocytes
Conjugate of elderberry lectin with peroxidase	2-4*	0
Methylation (37°C, 60°C) + conjugate of elderberry lectin and peroxidase	0	0-1
Methylation (37°C, 60°C) + saponification + conjugate of elderberry lectin and peroxidase	3	0
Neuraminidase + conjugate of elderberry lectin with peroxidase	3	0
Conjugate of peanut lectin and peroxidase	0	1
Neuraminidase + conjugate of peanut lectin with peroxidase	1-4	0-1
Native peanut lectin + conjugate of elderberry lectin with peroxidase	2-4	0
Neuraminidase + native peanut lectin + conjugate of elderberry lectin with peroxidase	2-3	0
Conjugate of soy lectin with peroxidase	1	0
Neuraminidase + conjugate of soy lectin with peroxidase	2-3	0
Native soy lectin + conjugate of elderberry lectin with peroxidase	3-4	0
Neuraminidase + native soy lectin + conjugate of elderberry lectin with peroxidase	2-4	0
Mixture of native peanut and soy lectins + conjugate of elderberry lectin with peroxidase	0	0-1
Neuraminidase + mixture of native peanut and soy lectins + conjugate of elderberry lectin with peroxidase	1-4	0

Legend. *) Intensity of staining, in points. 0, 1, 2, 3, 4 denote absence of reaction, very weak, weak, moderate, and strong reaction respectively.

EXPERIMENTAL RESULTS

After staining the sections of the gland with alcian blue, after preliminary mild methylation, some decrease in alcianophilia of the cytoplasm of the mucocytes was observed, in agreement with data in [7]. Rigorous methylation completely blocked the staining. At the same time, we found that as a result of saponification of the sections after preliminary methylation, the alcianophilia of cells in the terminal parts of the gland was restored, but this differs from previously published data [4, 7]. In the opinion of the authors cited, methylation leads to hydrolysis and removal of sialic acids from glycoprotein molecules synthesized by sheep submandibular gland cells. Our own data indicate rather that esterification of the terminal sialic acid residues takes place during methylation, for the methyl esters formed with their carboxyl groups are destroyed on subsequent saponification of the sections [12].

After treatment of the sections with neuraminidase the mucocytes of the terminal divisions completely lost their ability to be stained by alcian blue, evidence that they contain N-acetylated sialoglycans [11]. Meanwhile, the cytoplasm of individual serocytes preserved a weak degree of alcianophilia, due to the presence of two types of glycoproteins in them, possibly sulfated or sialidazoresistant O-acetylated sialoglycans.

The results of staining of cells of the terminal divisions of the submandibular gland, using methods of lectin histochemistry are given in Table 1. On treatment of sections with elderberry lectin all the mucocytes gave a positive reaction (Fig. 1a). However, even within the same acinus the intensity of staining varied from weak to strong, possibly due to the asynchronous course of the secretory processes.

The study of carbohydrate specificity of elderberry lectin [10] showed that this lectin exhibits maximal affinity for the disaccharide structure Neu5Ac(α2,6)Gal/GalNAc. This lectin interacted much less strongly with D-galactose and N-acetyl-D-galactosamine residues. Consequently, the positive reaction of elderberry lectin with the mucocytes of the gland may be due to the presence of terminal sialic acid residues and also D-galactose or N-acetyl-D-galactosamine residues in the composition of the

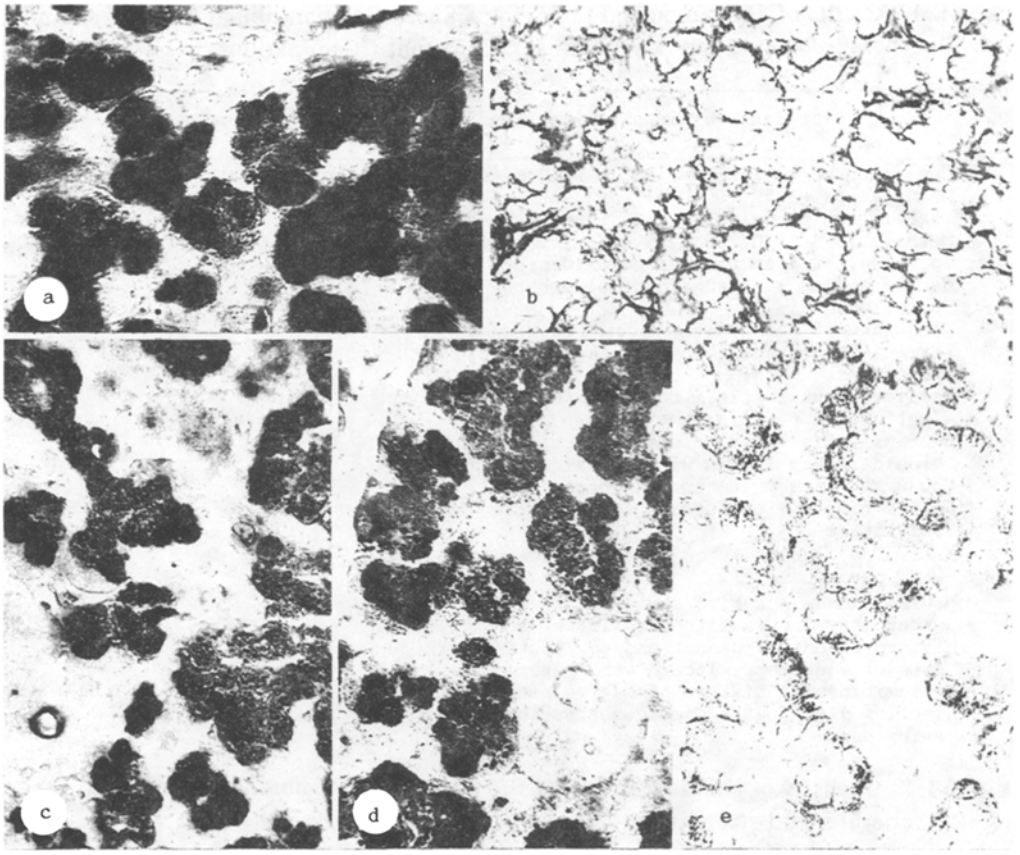

Fig. 1. Different versions of staining of sheep submandibular salivary gland during histochemical reactions using elderberry lectin, conjugated with peroxidase. 250×. a) Treatment with elderberry lectin by standard method. Intensive staining of cells of terminal divisions and lumenal surface of efferent ducts; b) areactivity of gland cells on treatment of section with elderberry lectin after preliminary rigorous methylation; c) treatment with elderberry lectin after preincubation of section with neuraminidase: removal of sialic acid does not abolish staining of acinar cells with lectin; d) incubation of section in mixture of native peanut and soy lectins followed by treatment with elderberry lectin. Some decrease in intensity of staining as a result of blocking of terminal N-acetyl-D-galactosamine residues; e) subsequent treatment of section with neuraminidase, native peanut and soy lectins, followed by elderberry lectin. Complete inhibition of reaction with elderberry lectin.

glycoprotein treated by them. Absence of D-galactosyl residues was ruled out by the total areactivity of the acinar mucocytes of the gland with peanut lectin (Fig. 2a). Terminal N-acetyl-D-galactosamine residues were discovered in the mucocytes in very small quantities by the use of soy lectin (Fig. 2c).

Blocking sialic acids by the methylation reaction led to total disappearance of staining of the gland cells with elderberry lectin (Fig. 1b). During subsequent saponification, restoration of staining was observed. In this case, however, reactivity of the cells was more moderate and uniform. Inhibition of binding of elderberry lectin after methylation and its restoration on subsequent saponification of the sections are evidently due to the formation, in the first place, and rupture, in the second place, of ester bonds between the carboxyl groups of the sialic acid and the methyl groups. This state of affairs is of considerable interest, for it is evidence of the possible participation of the —COOH groups of sialic acid in the mechanism of their specific binding with elderberry lectin.

Treatment of the sections with neuraminidase led to some decrease, but not total disappearance, of staining of the cells with elderberry lectin (Fig. 1c). This was probably because after desialation, elderberry lectin begins to bind with the unmasked subterminal N-acetyl-D-galactosamine and D-galactose residues. Evidence of the appearance of the latter after treatment of the section with neuraminidase was given in particular by the positive reaction with peanut and soy lectins (Fig. 2b, d). Incubation of the sections with native peanut lectin after their treatment with neuraminidase caused reduction, but not inhibition, of binding of elderberry lectin, for this procedure led to blocking only of the D-galactose residues, whereas the N-acetyl-D-galactosamine

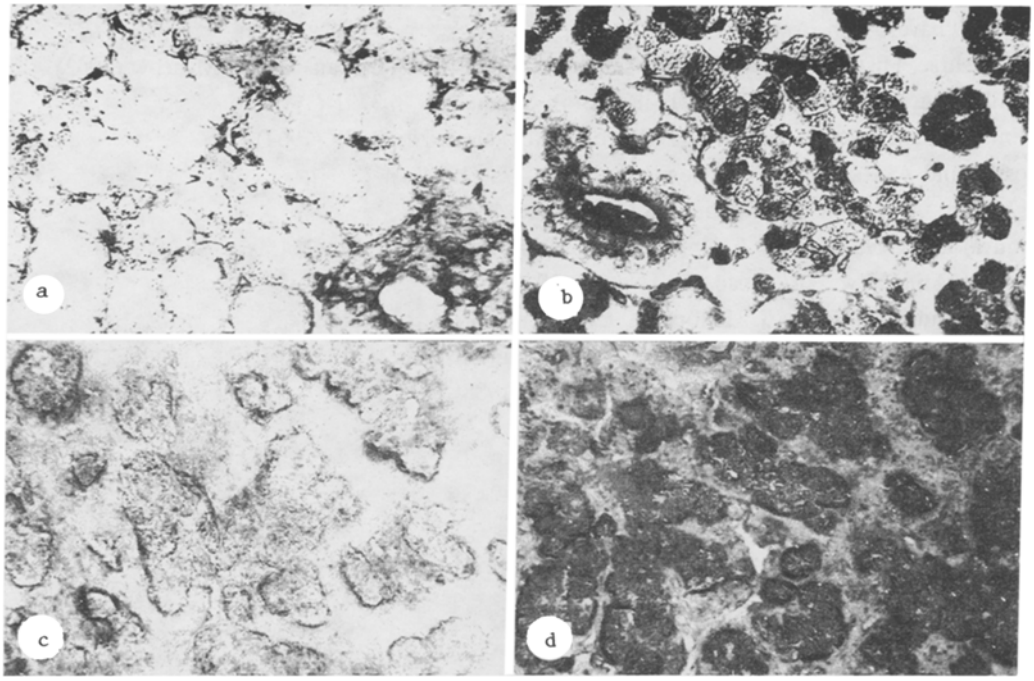

Fig. 2. Versions of staining of sheep submandibular salivary gland sections with peanut and soy lectins conjugated with peroxidase. 250×. a) Areactivity of cells of terminal divisions after treatment with peanut lectin by standard method; b) heterogeneous staining of gland cells with peanut lectin after incubation of section with neuraminidase; c) very weak reaction of secretory cells with soy lectin; d) potentiation of staining of terminal divisions after treatment of section with neuraminidase.

residues remained accessible for interaction with horseradish lectin. A similar conclusion was reached after treatment of desialated sections with native soy lectin, followed by staining with peroxidase-labeled elderberry lectin: M-acetyl-D-galactosamin e residues were blocked, but D-galactose residues were revealed by elderberry lectin.

Incubation of desialated sections of the gland in a mixture of native peanut and soy lectins followed by their treatment with a conjugate of elderberry lectin with peroxidase led to the virtually total disappearance of staining of the mucocytes (Fig. 1e). In this case, the subterminal D-galactose and N-acetyl-D-galactosamine residues, bound with native peanut and soy lectins respectively, became inaccessible for interaction with elderberry lectin. Successive incubation of intact (not treated with neuraminidase) sections of the gland with native peanut arid soy lectins, followed by elderberry lectin, caused a very slight decrease in the intensity of staining (Fig. 1d). This can be explained by the fact that the terminal residues in the composition of sheep salivary gland glycoconjugates have mainly the NeuNAc-GalNAc-sequence [4, 6], and only a small proportion have terminal N-acetyl-D-galactosamine residues (Fig. 2c).

The results of this investigation thus provide a basis for the possibility of using elderberry lectin for the selective histochemical detection of sialic acids. With the aim of blocking binding of elderberry lectin with terminal D-galactose and N-acetyl-D-galactosamine residues, they must be blocked by preincubation of sections with native peanut and soy lectins, or with other lectins possessing affinity for these particular monosaccharides. The further study of the possibility of using elderberry lectin in histochemical research is promising. In particular, it is essential to determine the effect of the character of the glycoside bond between the terminal and subterminal monosaccharide residues in the glycopolymer molecule on lectin-receptor interaction, to rule out the possibility of creation of stereochemical hindrances for binding of sialic acid residues with elderberry lectin by molecules of native peanut and soy lectins, and also, to compare the histochemical specificity of the latter and of other sialo-specific lectins.

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KINETICS OF REPAIR OF SUBLETHAL RADIATION INJURIES IN EARLY HEMATOPOIETIC PRECURSORS

T. V. Todriya and O. I. Gan

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Hematopoietic precursor cells, namely splenic colony-forming units (CFUs), possess high ability to repair sublethal radiation injuries (SLRI), as has been demonstrated on Elkind's model of repair for precursors forming colonies in the spleen of a lethally irradiated recipient on the 7th-10th day. The degree of repair of SLRI during fractional irradiation depends on several parameters of radiosensitivity, proliferative activity, proliferation rate, and position of the cells in the cell cycle. For mature CFUs (CFUs at 7-10 days) maximal repair is recorded with a 5-h interval between the first and second fractions of irradiation [11], but by contrast, with the same conditions of fractionation, less mature hematopoietic precursors (CFUs at 11 days) exhibit reduced ability to repair SLRI [1, 2]. It is not yet clear, however, whether reduced ability for early postradiation repair of sublethal injuries is a property possessed by early hematopoietic precursors (CFUs at 11 days), or whether the 5-h interval used between fractions does not permit the completeness of repair of SLRI to be estimated. The present investigation was conducted to shed light on these problems.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL)F_1 mice. Repair of radiation injuries was studied on Elkind's model of repair [4], adapted for hematopoietic tissue [11]. Recipient mice were irradiated in a dose of 6 Gy on an IPK γ-ray apparatus (^{137}Cs), with a dose rate of 0.185 Gy/min, after which the irradiated animals (10 mice in each group) were given an injection of $5 \cdot 10^6$ bone marrow cells per mouse. Some of the experimental mice were irradiated 1 h after transplantation of the cells with a single dose of 6 Gy (unfractionated irradiation group — UFI). Other recipient mice were irradiated fractionally, with intervals of 1, 2, 3, 4, 5, and 6 h between two equal doses, each of 3 Gy, of irradiation — the fractional irradiation group (FI). Thus the recipients in all the experiments received a total dose of irradiation of 12 Gy, and the injected cells received 6 Gy (a single dose or in fractions with an interval of 1-6 h). Colonies were counted in the spleens on the 8th day (control) and 11th day

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