Aldehyde Bisulfite-Toluidine Blue (ABT) Staining as a Topo-Optical Reaction for Demonstration of Linear Order of Vicinal OH Groups in Biological Structures

Gy. Romhányi, Gy. Deák, and J. Fischer
Institute of Pathological Anatomy, University Medical School, Pécs, Hungary
Received March 10, 1975

Summary. The aldehyde-bisulfite-toluidine blue reaction followed by poststaining stabilization with potassium ferricyanide (ABT) is described as a topo-optical, oriented staining reaction of the vicinal OH groups of complex carbohydrates in biological structures such as polysaccharides, glycoproteins and glycolipids.

The birefringence as induced by the oriented dye binding as a result of ABT is indicative of linear order of the vicinal OH groups and, in turn, provides information on the ultrastructural pattern of carbohydrate moieties in biological substances, which pattern is often not demonstrable by other ultrastructural methods.

The possibilities of this new approach to the ultrastructural analysis of complex carbohydrates with ABT in a great number of biological substances is demonstrated and its practical value in histopathology discussed.

Introduction

Carbohydrate moieties are common building blocks of a great variety of biological structures such as polysaccharides, glycoproteins and glycolipids. There is electron microscopic as well as polarization optical evidence to indicate a micellar texture of some of these macromolecular elements in biological structures (Frey-Wyssling, 1954; Drochmans, 1962; Missmahl and Kühler, 1961; Musy et al., 1972; Módis, 1974; Luzardo-Baptista, 1972; Thiery, 1967; De Marsh and Kautz, 1957). However, no evidence is available for the order at the molecular level of the vicinal OH groups of carbohydrate components in these structural elements.

There are histochemical methods known to demonstrate vicinal OH groups at the light and electron microscopic levels. The electron microscopic methods, based on the reduction of silver (Movat, 1961; Rambourg, 1967; Thiery, 1967; Csuka and Sugár, 1971), alkaline bismuth (Ainsworth et al., 1972) by the aldehyde groups formed from glycol groups by periodic acid; or on the condensation of the aldehyde groups by fluorophenyl-hydrazine (Bradbury and Steward, 1967), can readily be used to localize vicinal OH groups at the electron microscopic level. However, they offer no information about the molecular order of the reacting OH groups and in turn about the macromolecular components to which they are attached.

The PAS reaction, developed for the selective demonstration of vicinal OH groups by McManus (1946), Lillie (1947) and Hotchkiss (1948) has gained great significance in the histochemistry and histopathology of carbohydrate components. However, it does not produce anisotropy effects in the reactive structures and therefore it cannot be used to study the molecular order of the vicinal OH groups by polarization microscopy. This aim could only be achieved by selective and oriented dye binding (topooptical staining reaction) of the vicinal OH groups.
However, OH groups lack affinity for dye binding and cannot be demonstrated as such by a selective oriented topo-optical staining reaction only after their transformation into groups which are capable of dye binding. In a previous paper (Romhányi et al., 1973) we reported on the selective inversive topo-optical reaction of sulfate collagen and basement membranes with toluidine blue at pH 1.0 based on the selective dye binding of the sulfated OH groups of collagen. This was achieved by pretreatment with periodic acid, which oxidized the vicinal OH groups of the carbohydrate components to aldehydes and prevented them from participating in the sulfation reaction. In this way practically only the OH groups of collagen (of hydroxyproline and hydroxylysine) remained available for sulfation (Romhányi et al., 1973).

In this paper we will report on our polarization optical results obtained by means of the topo-optical staining reaction of vicinal OH groups based on the aldehyde-bisulfite addition reaction followed by toluidine blue staining at pH 1.0 and stabilization with potassium ferri cyanide (ABT). During this reaction the vicinal OH groups are transformed into dialdehydes by periodic acids and then transformed into negatively charged groups by the bisulfite addition reaction. In this way they are rendered capable of binding toluidine blue at pH 1.0, which results in strong basophilia and birefringence indicating linear order of the OH groups in the reacting macromolecular structures.

Malinin (1970) was the first to use the aldehyde-bisulfite addition reaction for the demonstration of glycogen by means of its apparent selective metachromatic staining with toluidine blue at acidic pH values. However, he carried out only light microscopic observations without stabilization of the most labile metachromatic staining reaction and performed no polarization optical investigations.

Even earlier Shackleford (1963) had reacted periodate-oxidized 1,2 glycol groups of mucous substances with p-hydrazine-benzene-sulfonic acid. The sulfonated mucous substances showed strong toluidine blue basophilia at pH 1.0. By using poststaining stabilization with potassium ferrocyanide we have succeeded in developing the ABT method into a standard topo-optical staining reaction of the vicinal OH groups, which is characterized by strong, toluidine blue (pH 1.0) induced basophilia and birefringence in structures in which OH groups are present in a linear order, and by basophilia with isotropy in structures in which the reactive glycol groups are present in random distribution. We have already used this method in earlier investigations in which we called it a PA-BSA (periodic acid bisulfite aldehyde) reaction; we now propose the shorter term, ABT.

The stabilization of the ABT reaction with potassium ferrocyanide was based on our previous experience with the stabilization of the labile metachromatic staining reaction (Romhányi, 1963). It proved essential also for maintaining on the reactive structures the oriented dye binding as caused by the ABT reaction. The omission of poststaining stabilization resulted in a rapid change of the metachromatic staining of the ABT reaction into orthochromasia with isotropy, which no longer provided information about the structural order of the OH groups. Metachromatic hues were a common characteristic of the ABT reaction as first reported for glycogen by Malinin (1970). This aspect of this staining reaction and its correlation to the stereochemical distribution of the negatively charged groups on the carbohydrate rings will be the subject of a separate paper. We have already