Metachromatic staining and electron dense reaction of glycosaminoglycans by means of Cuprolinic Blue

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Summary

The cationic phthalocyanin-like dye Cuprolinic Blue, unlike phthalocyanin dyes such as Alcian Blue or Astra Blue, can definitely exhibit a clear metachromatic reaction with appropriate substrates. The application of Cuprolinic Blue to epoxy-embedded semithin sections revealed that mast cell cytoplasmic granules, goblet cell mucin and cartilage matrix stained in violet shades (metachromatic), whereas nuclear chromatin presented a bright blue coloration (orthochromatic). The metachromatic structures showed a high degree of contrast when ultrathin sections treated with Cuprolinic Blue were examined by electron microscopy. Cytophotometric measurements of stained components from the large intestine showed different absorption maxima: at 580 nm for mucin and at 640 nm for nuclei. The spectroscopical analysis revealed a clear-cut metachromatic shift when the dye was in the presence of chondroitin-4-sulphate. The addition of aluminium metal to Cuprolinic Blue solutions resulted in a striking spectral change; under such conditions the dye showed absorption maximum at 530 nm.

Introduction

A wide variety of basic dyes from the thiazine and acridine groups have been shown to exhibit unusually useful properties of metachromatic staining (Michaelis & Granick, 1949; Bergeron & Singer, 1958; Bradley & Wolf, 1959; Semmel & Huppert, 1964; Feder & Wolf, 1965; Comings, 1975; Armas-Portela et al., 1983). Aqueous solutions of cationic phthalocyanin dyes (e.g. Alcian Blue and Astra Blue) were found to show a high degree of chromophore aggregation, and the well-accepted metachromatic spectrum (Scott, 1970). Although a characteristic orthochromatic shift can be observed both for basic and acidic phthalocyanin dyes under monomerizing conditions (e.g. organic solvents, detergents) (Scott, 1970; Juarranz et al., 1985), the ortho- and metachromatic staining reactions of these dyes are difficult to distinguish by light microscopy. This is because the corresponding wavelengths are hardly recognized as being different by the naked eye.

Cuprolinic Blue, 'quinolinic phthalocyanin', (Scott, 1972) is a cationic, intensely blue coloured copper-containing phthalocyanin-like dye (relative molecular mass, Mw, 1,0845) (Fig. 1), which is regarded as an analogue of the well-known Alcian Blue and Astra Blue (Scott, 1960a). This dye has been employed as a selective stain for the histochemical demonstration of RNA (Scott, 1972, 1973; Lampert & Scott, 1974; Corsellis et al., 1975; Mendelson et al., 1983; Tas et al., 1983) and lately of glycosaminoglycans (Scott, 1980b; Scott et al., 1981; Van Kuppevelt et al., 1984a,b). During the course of cytochemical studies on phthalocyanin dyes and their substrates (Juarranz et al., 1985, 1986) we have noted that in epoxy-embedded tissues, glycosaminoglycans were stained by Cuprolinic Blue in a shade very different from that of nuclei. The aim of the present study is to investigate the microscopical and spectroscopical properties of the ortho- and metachromatic reaction of Cuprolinic Blue as well as the selective contrast induced by this dye in electron microscopy.
Materials and methods

Normal animals of different species were employed in the present study. Tissue specimens of trachea from chicken, large intestine, uterus, stomach and testes from mice, bone marrow from rats and salivary glands from Drosophila hydei were fixed in 3% glutaraldehyde in Sörensen's buffer at pH 6.8 for 24 h at 4 °C and then rinsed in the same buffer for 2-3 h. To avoid the well-known interference of osmium deposits with the stainability of semithin sections as well as the identification of electron dense reaction products in the sections, postfixation with osmium tetroxide was not performed. After dehydration in graded ethanol or acetone series, the specimens were embedded in Epon 812 or Durcupan ACM (Glauert, 1975). Semithin and ultrathin sections were prepared on an Ultracut (Reichert-Jung) or a Jura-7 (Jeol) ultramicrotome. Semithin sections (1 to 2 μm) were transferred to a drop of water on glass slides and then dried on a hot plate. Ultrathin sections (silver interference colour) were mounted on gold grids without formvar.

Semithin and ultrathin sections were stained at 60 °C for 18 h by means of a freshly prepared 1 mg/ml solution of Cuprolinic Blue (BDH Chemicals) in distilled water at pH 5.9, to facilitate the penetration of the dye into the plastic. Some semithin sections were also stained with Cuprolinic Blue in the presence of 1 M MgCl₂ or with a Cuprolinic Blue solution to which a small sheet (0.015 mm thick) of aluminium metal (100-120 mg per 10 ml of the staining solution) was added 18 h prior to staining. The metal was retained in the Cuprolinic Blue solution during staining. Slides were rinsed in distilled water for 5 to 10 s, dried in air and mounted in Eukitt or examined directly in immersion oil.

Microscopic observations were made by means of a Zeiss photomicroscope III and a Jeol 100-S electron microscope operating at an accelerating voltage of 60 kV. Cytophotometric measurements were performed with the Zeiss III photomicroscope, equipped with an 03 photometer head, a photomultiplier (HTVR 446), and a Hewlett-Packard 97-S microcomputer. A continuous interference monochromator filter (Schott) was situated under the condenser, in order to select any desired wavelength. Absorption spectra of Cuprolinic Blue solutions, either in the presence of aluminium metal, chondroitin-4-sulphate sodium salt (Serva), or without any added substances, were obtained employing a double beam Perkin-Elmer 551-S UV/VIS spectrophotometer, equipped with a Hitachi 561 recorder. Measurements were performed employing 1 cm wide quartz cells.

Results

Light microscopic examination of semithin tissue sections stained by Cuprolinic Blue showed a bright blue colour of chromatin, nucleoli and basophilic cytoplasm (orthochromatic staining reaction), whereas the goblet cell mucin, microvilli of intestinal epithelial cells, mast cell granules and cartilage matrix were coloured differently in violet shades (Fig. 2). Such a metachromatic shift was comparatively minor in other structures examined. Thus, the acrosome granules, basal laminae and collagen fibres stained in a light violet or bluish pink shade. The gastric cell zymogen granules and salivary gland cell granules in Drosophila failed to show any differential staining reaction.

When ultrathin sections from the above-mentioned tissues were examined by electron microscopy, all the vividly metachromatic cell components identified by light microscopy exhibited a definite opacity. Fig. 3(a–c) illustrates such contrasting reactions with Cuprolinic Blue: a high electron opacity of mast cell granules is distinctly