Temperature-Dependent Staining Reaction of Chromatin by Alcian Blue

J. C. Stockert and A. Juarranz

Departamento de Citología e Histología, Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, Madrid-34, Spain

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After treatment with Alcian blue at high temperatures, nuclei from chicken blood smears showed an intense staining rection, which proved to be dependent on the DNA content of chromatin. The possibility that interactions between the phthalocyanine chromophore and unpaired bases accounts for this temperature-dependent staining of chromatin is briefly discussed.

Today, the copper phthalocyanine dye, Alcian blue 8 GS has become widely used for the histochemical demonstration of acid mucopolysaccharides [1–3]. This basic dye possesses unusual staining properties by showing a very low affinity for nucleic acids [4], and only occasionally, a staining reaction in chromatin [1, 5, 6].

However, strong hydrophobic, face-to-face interactions would be expected to occur between the planar phthalocyanine chromophore (Fig. 1) and the bases of polynucleotides [4]. On account of the temperature-dependent intercalative binding of a porphyrine derivative to DNA [7], we have examined several staining conditions which could reveal a similar reactivity of chromatin toward Alcian Blue.

Smears of chicken blood were fixed in methanol for 2 min and then air dried; in some cases they were post-fixed in 5% formaldehyde for 15 min. Alcian blue 8 GS (Serva) was used at a concentration of 0.1% in 0.5% acetic acid or in 20% formamide, and applied for 5 min at room (20 °C) or at higher temperatures as seen in Fig. 2. In the latter case, staining solutions were allowed to cool slowly. Preparations were washed in distilled water, air dried, and examined under oil immersion (×100). Cytophotometric measurements were carried out at 610 nm by using the previously described procedure [8]. Extraction methods, applied on methanol-fixed smears, were the following: 50% acetic acid for 5 min; 5 N HCl for 30 min at room temperature; 5% perchloric acid (PCA) at 4 °C for 18 h; 5% trichloroacetic acid (TCA) at boiling temperature for 20 min; DNase, 0.5 mg/ml in 1 mM $MgCl_2$ at 37 °C for 2 h, followed by washing in cold TCA for 5 min. After treatments, slides were rinsed in distilled water, air dried and stained. Solutions of daunomycin (DNM, 10^{-5} M), ethidium bromide (EB, 10^{-6} M), and acridine orange (AO, 10^{-6} M) in distilled water were applied on smears for 5 min, after heating at 100 °C in water or in Alcian blue solutions and slow cooling.

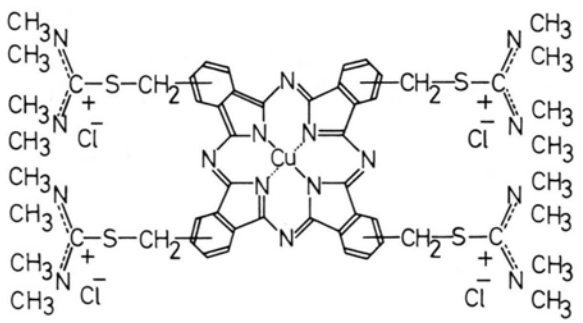

Fig. 1. Chemical structure of Alcian blue 8 GS [3, 12].

Reprint requests to Prof. Dr. Juan C. Stockert.
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Table I summarizes the results of several extraction methods and competition experiments. Untreated or acid hydrolyzed chromatin does not stain with Alcian blue when used at 20 °C, either for 5 min or 30 h (No. 1). However, an intensive blue staining of erythrocyte chromatin, which depends on the DNA component, occurs by heating prepara-

Table I. Effect of several extraction procedures and experimental conditions on the Alcian blue (AB) staining of chromatin. The dye was used either at 20 °C or at 100 °C. + and – indicate positive or negative staining reactions, respectively.

Experimental design, No.	Chromatin	
	Staining	Fluorescence
1. AB 20	–	
Acetic, AB 20	–	
5 N HCl, AB 20	–	
PCA, AB 20	–	
2. AB 100	+ (blue)	
DNase, AB 100	–	
TCA, AB 100	–	
3. DNM	–	+ (red)
EB	–	+ (red)
AO	–	+ (green)
AB 100, DNM	+ (blue)	–
AB 100, EB	+ (blue)	–
AB 100, AO	+ (blue)	–

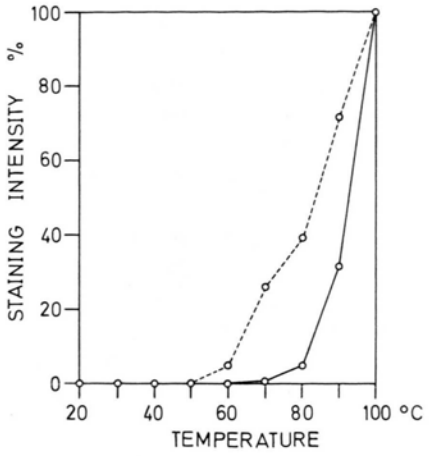

Fig. 2. Intensity of Alcian blue staining of erythrocyte chromatin in function of the staining temperature (solid line, Alcian blue in 0.5% acetic acid; dashed line, Alcian blue in 20% formamide). Each point represents the mean value of 4 measured nuclei.

tions at 90–100 °C in presence of Alcian blue (No. 2). The characteristic chromatin fluorescence by daunomycin, ethidium bromide and acridine orange [9, 11] is also obliterated when fluorochromes are applied after staining with Alcian blue at high temperature (No. 3).

The intensity of chromatin staining by the dye at increasing temperatures was analyzed in chicken blood smears after formaldehyde postfixation. Fig. 2 shows the result of two of these measurements, which appear very similar to the melting kinetics of chromatin as evaluated by hyperchromicity at 260 nm [10].

According to these observations, Alcian blue selectively stains the DNA component of chromatin under conditions which are known to cause DNA denaturation in fixed cells [11]. Dye-competition experiments show that the previous Alcian blue staining abolishes the specific fluorescence of chromatin induced by intercalating fluorochromes, which suggests competition for the same binding sites. In native DNA, the interaction between base pairs and the phthalocyanine ring is prevented because of the four bulky side chains of Alcian blue [4]. The "quinolinic phthalocyanine", Cuprolinic blue, lacks side chains and intercalative modes of binding to nucleic acids have been suggested [4]. A binding mechanism based on hydrophobic and Van der Waals interactions of the Alcian blue chromophore with unpaired bases from denatured DNA could account for the temperature-dependent staining reaction of chromatin. Further investigations to analyze the kinetics of thermal denaturation of DNA and the staining mechanism of Alcian blue are in course.

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