Observations on the Chromatin Staining by Aluminium-Hematoxylin

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The staining reaction of chromatin by aluminium-hematoxylin was investigated. Intense staining, which proved dependent on nucleic acid content, was achieved by using either the preformed lake, mordanting followed by hematoxylin, hematoxylin alone or the lake at high ionic strength. Extraction procedures removed aluminium ions from chromatin but the lake was retained. Chromatin fluorescence induced by ethidium bromide and acridine orange was abolished after staining with the lake. Intercalating-like forces are suggested for aluminium-hematoxylin binding to chromatin.

It is known that, when used with an aluminium mordant, hematoxylin stains the chromatin very sharply and in a selective way [1 – 4]. For a long time, the basophilia of chromatin has been attributed to a saltlike binding between the nucleic acid phosphate groups and basic dyes or metal-dye complexes [3 – 6], but several other forces (hydrogen, van der Waals and hydrophobic bonds) also occur in dye-nucleic acid interaction [7, 8], as well as in chromatin staining [9 – 11]. To analyze more precisely the staining mechanism of chromatin by aluminium-hematoxylin, the effect of extraction procedures and several staining conditions were investigated.

Human blood smears were fixed in methanol for 2 minutes and then air dried. Aluminium-hematoxylin (Al-H) was prepared by mixing a 0.2% aqueous solution of spontaneously oxidized hematoxylin and a 5% solution of ammonium aluminium sulfate in the proportion 1 : 1. NaCl was also added to samples of Al-H at levels of 2.0 and 4.0 M. Ethidium bromide (EB) and acridine orange (AO) were used as 0.05 mg/ml solutions in 1 M NaCl. Mordanting with ammonium aluminium sulfate (Al) was performed with a 2.5% solution for 15 minutes. Staining was carried out with Al-H, Al-H-NaCl, 0.1% oxidized hematoxylin (H), EB and AO for 15 minutes. After washing in 0.2% Na_2CO_3 solution for 15 minutes, slides were air dried and observed in a Zeiss Photomicroscope by using either bright field illumination or fluorescence. Extraction procedures (applied before or after staining, as seen in Table I) were the following: 5% trichloroacetic acid (TCA) at 90 °C for 20 minutes; DNase I, 1 mg/ml in 1 mM $MgCl_2$ at 37 °C for 60 minutes; 0.2 M EDTA or 2 M NaCl for 30 minutes.

Table I summarizes the results of the different staining reactions. Chromatin staining (Nr 1) is achieved by using the preformed lake (A1-H) or mordanting followed by the dye. An unexpected chromatin staining also occurs by hematoxylin alone or alkaline solutions of hematoxylin. It is interesting to note that a paradoxical increase of electron opacity in chromatin was observed with hematoxylin alone [11].

Removal of nucleic acids (Nr. 2) severely affects the affinity of chromatin for Al-H. Treatments with 0.2 M EDTA or 2 M NaCl (Nr. 3) cause extraction of aluminium ions, but the lake is not removed. Furthermore, chromatin stains intensively by using Al-H in 2 or 4 M NaCl. The characteristic fluorescence of chromatin by EB or AO (Nr. 4) is slightly diminished when preceded by aluminium ions, but it is abolished after Al-H staining.

According to these results, hematoxylin lakes stain the nucleic acid components of chromatin selective-

Table I. Effect of several extraction procedures and experimental conditions on the aluminium-hematoxylin staining reaction. + + + and – indicate intense and no staining, respectively.

Experimental design No.	Chromatin			
	Staining		Fluorescence	
1. (Al-H)	violet	+ + +		
Al, H	violet red	+ +		
H	blue	+ +		
2. TCA, (Al-H)	pink	±		
DNase, (Al-H)		–		
3. Al, EDTA, H		–		
Al, NaCl, H		–		
(Al-H), EDTA	violet blue	+ + +		
(Al-H), NaCl	violet	+ + +		
(Al-H-NaCl)	violet	+ + +		
4. EB		–	red	+ + +
AO		–	green	+ + +
Al, EB		–	red	+ +
Al, AO		–	green	+ +
(Al-H), EB	violet	+ + +		–
(Al-H), AO	violet	+ + +		–

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ly, in agreement with observations by other authors [2, 3]. However, an electrostatic binding of lakes to the phosphate groups appears clearly insufficient to account for all the staining characteristics of chromatin. Extraction procedures, which remove metal ions [12], do not have effect on the lake-substrate complex. Likewise, high ionic strength does not diminish the intensity of chromatin staining, which agress with other reports [11]. Dye-competition experiments also show that a previous Al-H staining abolishes the specific chromatin fluorescence induced by intercalating fluorochromes, which suggests competition for the same binding sites.

Taking these results into account, together with structural considerations on the dye, it seems logical to assume that, in addition to the aluminium-phosphate interaction, the aluminium-hematoxylin lake also complexes with nucleic acids by means of other forces, like those which characterize the intercalative mode of binding. Further investigations to analyze the mechanism of this interaction more precisely are under way.

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