DICHROMATIC FLUORESCENCE OF DIFFERENTIALLY CONDENSED CHROMOSOMES STAINED WITH ACRIDINE ORANGE

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Metaphase chromosomes of the Chinese hamster, differentially condensed by the action of: 1) 5-bromodeoxyuridine, 2) colcemid, 3) "cold shock," were stained with acridine orange in concentrations of between 1.5×10^{-7} and 3×10^{-5} g/ml at pH values of between 4.1 and 8.5. The stretched segments of the chromosomes gave a reddish-orange fluorescence, whereas the condensed segments gave a green fluorescence. Distribution of the color along the length of chromosome depended chiefly on the acridine orange concentration and on the exposure in ultraviolet light and was unchanged by variations in the pH of the dye and the molarity of the buffer in which it was made up. The phenomenon is evidently unconnected with variations in denaturation of DNA but is most probably due to structural-chemical differences between euchromatin and heterochromatin.

KEY WORDS: fluorescence; acridine orange; chromosomes.

Acridine orange (AO) is often used to assess the degree of denaturation of chromosomal DNA after various experimental treatments of cytological preparations [4, 5, 8]. It has recently been shown [6] that chromosomes differentially condensed by the action of 5-bromodeoxyuridine (5-BDU) give green fluorescence after staining with AO in the condensed segments and red fluorescence in the stretched segments. It is postulated that this effect may be due to incorporation of a brominated precursor of thymine into the DNA, modifying its conformation. Fluorescence of chromosomes in which differential condensation was induced by 5-BDU, colcemid, and "cold shock " was investigated.

EXPERIMENTAL METHOD

The investigation was carried out mainly on a culture of Chinese hamster fibroblast-like cells for which the karyotype and technique of culture were described previously [3]. Some experiments were carried out on a culture of human peripheral blood. Mitotic condensation was delayed by the use of: a) 5-BDU in a dose of 200 μg/ml 5-6 h before fixation of the cells, b) prolonged culture in medium with colcemid [1], and c) "cold shock" – keeping the growing culture for 24 h at 3°C followed by incubation for 1 h at 20°C and further culture at 37°C for another 6 h. The chromosomal preparations were obtained by the standard method, using methanol–acetic acid (3 : 1) fixative. The AO reagent (Serva, West Germany) was made up in citrate-phosphate buffer with a concentration of dye of between 1.5×10^{-7} and 3.0×10^{-5} g/ml. The molarity of the buffer varied from 0.02 to 2.0 M and its pH from 4.1 to 8.5. The preparations were stained for 10-15 min and mounted directly in the solution of the dye.

EXPERIMENTAL RESULTS

In all cases regardless of the method of obtaining differential condensation of the chromosomes the distribution of color along their length was uniform: the stretched segments fluoresced in the longer-wave

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TABLE 1. Color of Fluorescence of Condensed and Stretched Segments of Chromosomes after Treatment with Different Concentrations of AO and with Different Irradiation Times

AO concentration (in g/ml)	Irradiation time	Color of fluorescence	
		of condensed segment of chromosome	stretched segment of chromosome
$1.5 \cdot 10^{-7}$–$1 \cdot 10^{-6}$	First 1-2 min	Green	Green
	Next 5-10 min	Green	Orange-green
$5 \cdot 10^{-6}$–$1 \cdot 10^{-5}$	First 3-5 min	Yellow-orange	Yellow-orange
	Next 20-30 min	Green	Orange-red
$3 \cdot 10^{-5}$	First 3-5 min	Orange-red	Orange-red
	Next 30-40 min	Green	Orange-red

region of the spectrum. Variations in the pattern of fluorescence were connected with the AO concentration and the time of irradiation (Table 1). The molarity of the buffer and its pH had no significant effect, although with increasing alkalinity of the solution the color contrast was increased slightly.

The results show that the distinctive coloring of the differentially condensed chromosomes cannot be explained either by incorporation of 5-BDU into DNA or by differences in the degree of DNA denaturation. The possibility cannot be ruled out that the observed color differences are due to factors nonspecific with respect to DNA, as shown by the absence of any fundamental changes in color resulting from variation in the pH of the dye. In particular, condensed and stretched regions of chromosomes can be considered to adsorb the dye differently, for the stretched regions consist of heterochromatin [1, 3], characterized by its own special type of replication, mitotic condensation and, evidently, three-dimensional organization. Meanwhile, the mechanism of color transformation under the influence of an exciting color, when in low concentrations of AO mainly the stretched segments change color (toward the long-wave region) and with an increase in its concentration the color change takes place chiefly in the condensed segments (toward short waves), has not yet been completely explained. The fact that fluorescence was estimated in a medium of dye does not change the situation, for during control standard staining, as recommended by Zelenin [2] and Rigler [7], the same effects could be observed although less clearly. Within the limits of the AO concentrations used in the layer of fluorochrome under the coverslip hardly any additional background was created, but an equilibrium state was thereby maintained between the bound dye and the surrounding medium, uncontrollable effects of washing and differentiation were eliminated, and the light-resistance of the dye was increased. The conditions as used also enabled a rough estimate to be made (from the relative brightness of fluorescence and the thickness of the layer of dye and of the chromosome) of the AO concentration in the object, which was hundreds or thousands of times higher than in the dye solution. On these grounds the appearance of associations of molecules of the fluorochrome in the chromosomal substrates and a shift of fluorescence toward the long-wave region can evidently be expected even when the concentrations of the dye in solution are so low that usually only a monomolecular type of binding can be implied [2, 7].

The results described above, showing how fluorescence depends on the properties of the chromatin and the conditions of analysis, thus enable a very cautious approach to be made to the interpretation of the color picture in cases when AO is used to assess the degree of denaturation of DNA in cytological preparations. A further study of this phenomenon may also prove useful to the understanding of the nature of chromatin organization in the structure of the mitotic chromosome.

LITERATURE CITED

1. A. F. Zakharov and N. A. Egolina, Genetika, 5, No. 6, 90 (1969).
2. A. V. Zelenin, Luminescence Cytochemistry of Nucleic Acids [in Russian], Moscow (1967).
3. L. P. Tupitsyna, Byull. Éksperim. Biol. i Med., No. 10, 101 (1974).
4. M. Bobrov and K. Madan, Cytogenetics, 12, 145 (1973).
5. A. de la Chapelle, J. Schroder, and R. K. Selander, Chromosoma, 40, 347 (1973).
6. M. B. Dutrillaux, M. C. Laurent, M. J. Couturier, et al., C. R. Acad. Sci., 276, 3197 (1973).
7. R. Rigler, Acta Physiol. Scand., 67, Suppl. 257, 1 (1966).
8. J. C. Stockert and J. A. Lisanti, Chromosoma, 37, 117 (1972).