Fluorescence Reaction of Chromatin by Curcumin

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Treatment of cell smears, paraffin, and Epon tissue sections with aqueous solutions of curcumin results in a green fluorescence reaction in chromatin under violet-blue excitation which is abolished after extraction procedures with DNase and TCA. The selective fluorescence characteristics of curcumin support the possibility of employing this dye as a new fluorochrome.

The natural dye curcumin (diferuloyl methane; turmeric yellow; C.I. 75,300, Fig. 1) extracted from the roots of several *Curcuma* species, is widely used as a flavouring, colouring, and antioxidant food additive (E-100) [1]. Curcumin shows interesting anti-inflammatory [2], anti-mutagenic [3], anti-tumor-promoting [4], and anti-hepatotoxic properties [5]. In some organisms [6, 7], clastogenic effects caused by this drug, were found. This was not the case in mammalian cells [8, 9].

Curcumin is a pH indicator and forms complexes with di- and trivalent metal ions, but its histological use seems to have been overlooked [10]. The dye also shows fluorescence properties which could be of interest in microscopical techniques. It is the aim of this work to describe the selective chromatin fluorescence after curcumin staining.

Smears of chicken blood, mouse spleen, and *Trypanosoma cruzi* epimastigotes growing in culture

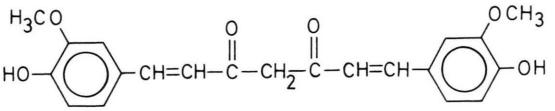

Fig. 1. Chemical structure of curcumin.

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were fixed in methanol for 2 min and air-dried. Polytene chromosomes of *Drosophila melanogaster* larvae were obtained by fixing salivary glands in methanol: acetic acid (3:1), followed by squashing in 50% acetic acid. Paraffin sections of mouse kidney and testis were prepared after having been treated with formaldehyde. Samples of mouse kidney, liver, uterus, spleen, and cerebellum cortex were fixed in 3% glutaraldehyde for 6 h, dehydrated in ethanol, and embedded in Epon.

Extraction procedures for nucleic acids were performed on cell smears by using DNase I (Serva, 0.5 mg/ml in 10^{-3} M $MgCl_2$) at 37 °C for 3 h, and 5% trichloroacetic acid (TCA) at boiling temperature for 20 min. Unstained smears, paraffin, and Epon sections were also checked for autofluorescence.

A 2 mg/ml stock solution of curcumin (Merck, dye content: 97%) was prepared in absolute ethanol and then diluted to the appropriate concentration with distilled water. Cell smears were treated with 10 µg/ml curcumin for 5 min; paraffin and Epon sections were stained with 0.05 mg/ml curcumin for 5 min (paraffin), or 30 min (Epon). The preparations were washed in distilled water, mounted either in distilled water, 70% sucrose, glycerol, 0.5% $SnCl_2$ in glycerol, immersion oil, Damar resin, or Eukitt and then observed in a Zeiss photomicroscope III, equipped with the epifluorescence condenser III RS. A Perkin-Elmer 650-10S fluorescence spectrophotometer was used to study the emission features of curcumin solutions (5 µg/ml either in distilled water, 0.01 N HCl, or 0.01 N NaOH) under 440 nm excitation.

When observed under violet-blue (436 nm) exciting light, the chromatin of nuclei and of chromosomes showed a green fluorescence. The kinetoplast of *T. cruzi*, bands of polytene chromosomes, and masses of dense chromatin of interphase nuclei were the highest fluorescent structures in cell smears and tissue sections (Fig. 2). The basophilic cytoplasm and nucleoli presented a lower emission, or none at all. While using UV (365 nm), violet (405 nm), and blue (450–490 nm) excitation, the chromatin fluorescence was lower too. No fluorescence was noticed in the chromatin of cell smears following DNase, or TCA treatments.

The fading of curcumin fluorescence was rather high; preparations, mounted in the strong reducing medium glycerol-$SnCl_2$ [12], showed the lowest fading rate. When observed under bright-field illumination, the chromatin appeared unstained. No auto-

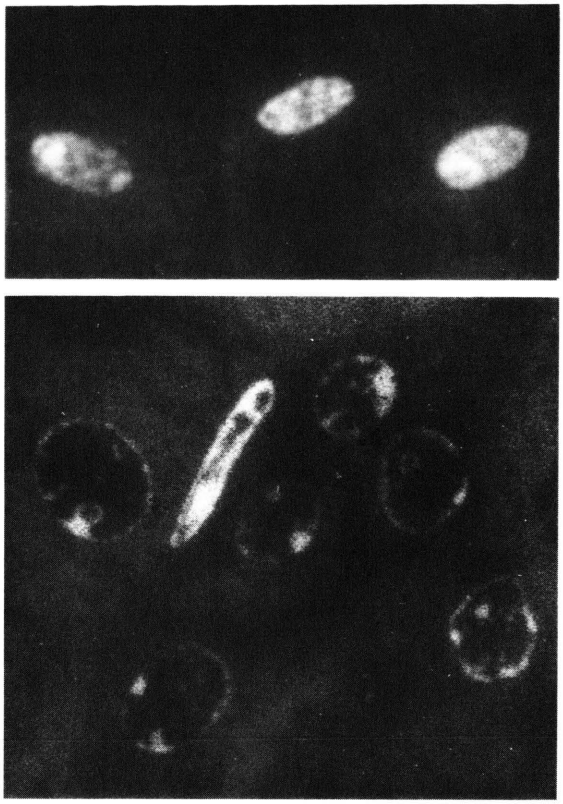

Fig. 2. Top: Chicken blood smear showing the fluorescence of erythrocyte nuclei after curcumin staining. ×2000. Bottom: Semithin Epon section of mouse kidney stained in curcumin. Note the fluorescence of chromatin in nuclei from proximal tubules and in a fibroblast nucleus. ×2000.

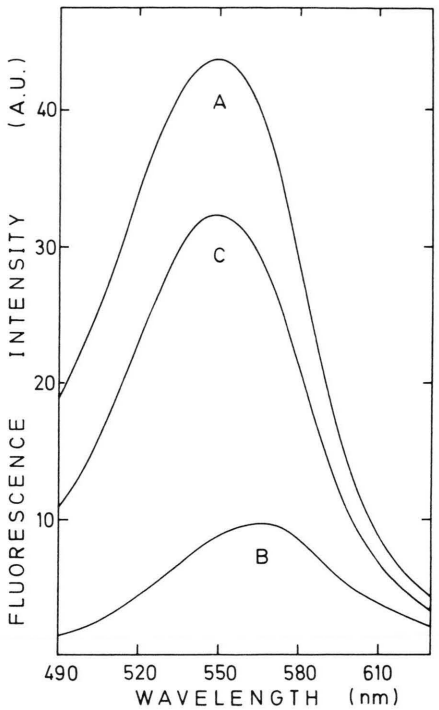

Fig. 3. Fluorescence emission spectra of 5 μg/ml curcumin solutions in distilled water (C), 0.01 N HCl (A), and 0.01 N NaOH (B). Exciting light: 440 nm; A.U.: arbitrary units. The spectra were corrected for the Raman scatter of the solvents.

fluorescence was found in nuclei and chromosomes of cell smears, and only a pale greenish emission was noticed in paraffin, or very thick Epon sections under violet-blue excitation [11].

A preliminary spectral study of the emission of curcumin was made to substantiate the microscopical fluorescence reaction (Fig. 3). Under excitation at 440 nm, emission peaks of 550 and 565 nm were found for the free dye in acid and alkaline media, respectively.

The nature of chromatin fluorescence caused by curcumin is intriguing. Unpublished results show that the emission of the dye greatly enhances when the viscosity of the solution increases. This phenomenon could account for the fluorescence reaction which occurs when curcumin becomes bound to chromatin. Extraction procedures indicate that DNA is the responsible component for chromatin fluorescence. Taking into account the chemical structure of curcumin, electrostatic or intercalative binding to DNA seems unlikely. While comparing the similarity of the crescent shape characteristics of the minor groove binders [13], it is tempting to speculate that curcumin could also insert along this groove in B-DNA; this binding mechanism would increase the molecular rigidity of the dye with the corresponding fluorescence enhancement.

In view of the wide use of fluorescence techniques in cytochemistry and cell biology [14–16], the investigation of new fluorochromes may be of considerable interest. Further studies on the fluorescence and binding mechanism of curcumin are under way.

Acknowledgements

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