KINETIC STUDIES ON ACRIDINE ORANGE-DNA INTERACTION BY FLUORESCENCE STOPPED-FLOW METHOD

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Received August 5, 1970

SUMMARY: The fluorescence stopped-flow technique was applied to the kinetic investigation of acridine orange(AO)-DNA interaction. Increased sensitivity of detection has enabled one to study with much lower concentration range of dye (as low as 4×10^{-8} M) than so far employed in the rapid reaction technique by light absorption measurement. The process of dimerization of AO on the outside of DNA and that of intercalation into base pairs have been separately observed.

It appears generally accepted that a cationic dye interacts with DNA through two modes of binding, intercalation between DNA bases and binding to the outside of DNA, depending on the ratio of DNA phosphate to dye (P/D ratio) (1). From the concentration dependence of relaxation times in the temperature-jump studies, Li and Crothers proposed a mechanism of intercalation via outside binding for proflavine-DNA interaction (2). In our studies of AO-DNA interaction (3), changes in absorption spectra during various time ranges of reaction were studied with the stopped-flow method, to discriminate the two modes of binding, utilizing the strong "dimer"-forming tendency of the dye when bound outside of DNA. (The term "dimer" includes higher aggregates of dye.) However, no separate observation of the two individual binding processes has ever been successful.

Since the fluorescence intensity of AO around 540 nm is greately increased upon intercalation (4,5), while quenched when "dimerized" on the outside of DNA (6), the use of fluorescence of AO would provide a powerful means for distinguishing the individual processes in both static and kinetic investigations. Additional advantage of fluorescence over light absorption resides in its high sensitivity of detection, which afforded us to reach much lower concentration ranges, down to 4×10^{-8} M of AO.

In this paper, a kinetic study of AO-DNA interaction by the fluorescence stopped-flow method will be reported. The process of intercalation and that of dimerization of outside bound dye were clearly distinguished, and the kinetics of the two processes were studied separately.

EXPERIMENTAL

AO and salmon sperm DNA used were prepared in the same way as those in the previous study (3). The reaction was performed at 25°C in 1 mM phosphate buffer of pH 6.9, the ionic strength being 0.004. The concentrations of AO and DNA were changed about five hundred fold.

The stopped-flow apparatus (Yanagimoto SPU-1) used in the previous study was modified for the measurement of fluorescence. The excitation beam at 492 nm was obtained with a monochrometer, a 50 W tungsten lamp being used as light source. A quartz observation cell of 2 mm square in cross section was used with 5 mm vertical slit. The fluorescence emission was observed from the right angle to the excitation beam, through an interference filter of 552 nm, or through a cut-off filter (Nikon Y52) transmitting above 520 nm when higher sensitivity is needed. The dead-time of the apparatus was about 1 msec under the operating

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