Bull. Environ. Contam. Toxicol. (1989) 43:534–540

Bulletin of Environmental Contamination and Toxicology

Interaction of 7,10-Dimethylbenz[c]acridine with Deoxyribonucleic Acid

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Many aza-heterocyclic hydrocarbons (aza-arenes) including 7,10-dimethylbenz[c]acridine (BAc) are potentially hazardous environmental contaminants because of their carcinogenic and mutagenic character (Lacassagne et al. 1956) . As trace components, aza-arenes have been detected in tobacco smoke (Grimmer et al. 1987), automobile exhausts (Sawicki et al. 1965) , aquatic sediments (Wakeham 1979) and urban atomospheres (Nielsen et al. 1986). Especially, BAc has strong carcinogenicity. The recent synthesis of a large number of potential metabolites of benz[c]acridine has permitted an analysis of the mutagenic activity of these compounds in bacterial and mammalian cells (Wood et al. 1983) and mouse skin (Levin et al. 1983). These results provided initial evidence for bay-region activation of benz[c]acridine to ultimate mutagenic and carcinogenic metabolites. Apart from some structure-activity relationships on BAc (Kamata et al. 1986), alittle work has been done with BAc in vitro. Okano has shown the interaction of BAc and deoxyribonucleic acid (DNA) by using ultraviolet measurement. Consequently, the interaction showed that the interaction of BAc and DNA has been evidenced by a remarkable hypochromism in the ultraviolet region. The double-helical structure of DNA has appeared to play an essential role in the interaction. DNA-BAc complex has exhibited greater ultraviolet hypochromism than BAc alone (Okano et al. 1975). Moreover, Okano has discussed the formation of free radical from BAc in the presence of proteins. There was a corresponding correlation among the electron spin resonance (ESR) signal intensity of the free radical formed system of BAc and protein, charge of the K-region or ring nitrogen of BAc (Okano et al. 1978). The present study is aimed at an advance on the mechanisms of the interaction of BAc with DNA in vitro by using mainly the measurement of the fluorescence and viscosity.

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MATERIALS AND METHODS

7,10-Dimethylbenz[c]acridine was synthesized according to literature (Buu -Hoi et al. 1944), and purified as described in previous paper (Motohashi et al. 1983). Calf thymus DNA (sodium salt) was purchased from Sigma Chemical Co.(St, Louis, Mo., USA). All chemicals were of analytical grade and water was doubly distilled. The standard solution of BAc was prepared in ethanol (4 μg/ml). DNA (1.26×10^{3} μg/ml) was dissolved in buffer solution of 7×10^{-3}M sodium chloride (NaCl) + 5×10^{-4}M sodium citrate (pH 7.0), and diluted as occasion demands at the buffer solution. For the preparation of denaturated DNA, native DNA solution was heated in boiling water bath for 10 min and cooled rapidly in ice. The DNA solution was mixed with an equal volume of ethanol to prepare the BAc solution.

The fluorescence spectra were measured with a Hitachi Model 650 - 40 fluorescence spectrophotometer, equipped with grating systems for both excitation and fluorescence, using a cell of 1 cm optical path. Samples were scanned at 20 nm/min. The fluorescence spectrum of BAc in ethanol -buffer (1:1) solution excited at 290 nm showed peaks at 412 and 427 nm. The viscosity of 0.5 ml sample was measured at 30° C with a coneplate viscometer (Tokyo Keiki Instrument, Tokyo, Japan), equipped with a rotor producing a shear stress calculated to 0.233 dyn/cm^{2}.

RESULTS AND DISCUSSION

The fluorescence spectra of DNA-BAc complex were measured as Figure 1. The fluorescence waveforms in visible regions did not show changes. On the other hand, 1.556×10^{-5}M BAc was added at each three DNA concentrations, respectively. Consequently, DNA-BAc complex showed a decrease in fluorescence intensity compared to BAc only. The decrease in fluorescence intensity may be closely correlated to a phenomenon termed static concentration quenchings. The quenchings are ascribed to self-association or aggregation of the fluorecence BAc. On the other hand, in case of the increase in fluorescence intensity, the increase is probably correlated with rigidity of a molecule and atomic compactness in a molecule (Sadler 1960). Molecular rigidity and atomic compactness may be achieved by the interaction of BAc molecules inside the DNA helix. The changes of fluorescence intensity of DNA-BAc complex shown in Figure 1 is well consistent with the above idea, provided that the major reaction pathway at this reactive site is an exterior binding site, DNA-BAc complex by BAc and DNA -phosphate site (Kamata et al. unpublished data).

Figure 2 showed the influence of pH on the fluorescence intensity of the DNA-BAc complex. The changes have not been observed pH values 6 to 8. DNA-BAc complex was a little unstable at the low pH value ranges. Furthermore, Figure 3 shows the influence of changes in the salt (NaCl) concentration on the fluorescence intensity of DNA-BAc complex containing

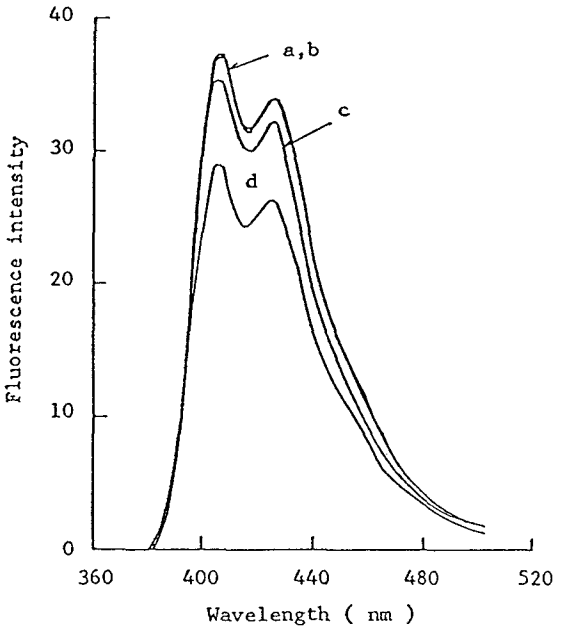

Figure 1. Fluorescence spectra of DNA-BAc complex. The excitation wavelength was 290 nm. The concentration of BAc was 1.556×10^{-5}M, and those of DNA were varied as follows: (a) BAc alone, no DNA, (b) 1.26 μg/ml, (c) 1.26×10 μg/ml, (d) 1.26×10^{2} μg/ml.

5×10^{-4}M sodium citrate. DNA-BAc complex in sodium citrate solution did not show the influence of the addition of 10^{-6} to 10^{-2}M NaCl. On the other hand, in water alone, the fluorescence intensity of DNA-BAc complex tends to increase with the addition of 10^{-6} to 10^{-2}M NaCl. From the data in Figures 2 and 3, it suggests that DNA-BAc complex was not influenced by pH and changes in salt concentration.

The changes of the fluorescence spectra of BAc from denatured DNA-BAc complex are shown in Figure 4. Denatured DNA-BAc complex showed a lower fluorescence intensity than the fluorescence intensity of the native DNA -BAc complex. At higher temperatures, a lower fluorescence intensity was observed indicating thermal denaturation. From Figure 1, curve a in Figure 4 contains mainly the external binding of DNA-BAc complex. By the way, both curve b and c of denatured DNA-BAc complex decreased the fluorescence intensity, which means curve a contains a little intercalating binding of DNA-BAc complex. The decrease in intensity due to thermal treatment probably corresponds to a transition of DNA structure, single strands of DNA only forming the complex of external binding type with BAc. From the data in Figure 4, it suggests that the intercalation mechanism was involved in the intercalation of BAc molecules inside the

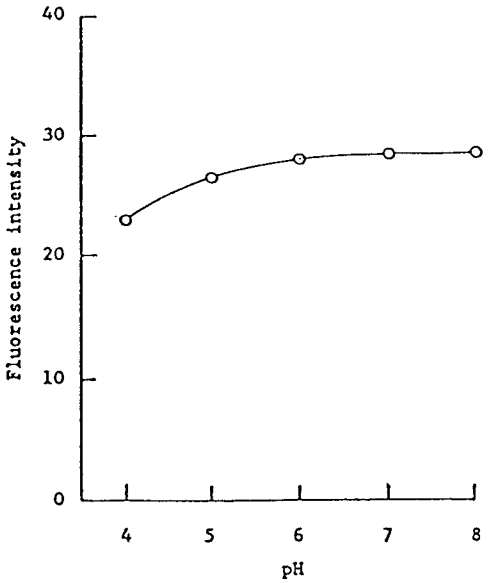

Figure 2. Effect of pH on the fluorescence intensity of DNA-BAc complex. The concentration of DNA was 1.26×10^{2} μg/ml and BAc was 1.556×10^{-5}M.

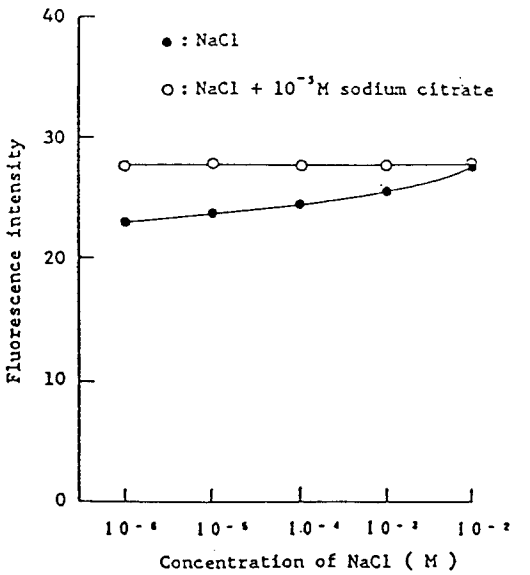

Figure 3. Effect of added NaCl concentration on the fluorescence intensity of DNA-BAc complex. The concentration of DNA was 1.26×10^{2} μg/ml and BAc was 1.556×10^{-5}M.

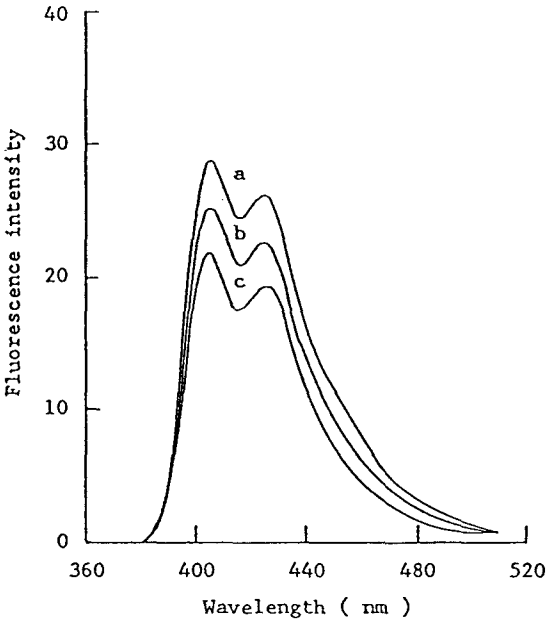

Figure 4. Effect of DNA thermal denaturation on the fluorescence intensity of DNA-BAc complex. Temperature of DNA thermal denaturation was varied as follows: (a) native DNA and 40° C, (b) 60° C, (c) 80° C and 95° C.

DNA helix. Consequently, there were two different binding sites in which BAc binded with DNA, one being an exterior binding site, and the other an intercalative site.

Figure 5 showed the viscosity changes of DNA-BAc complexes. In here, the horizontal line expresses the shear rate and the vertical line is viscosity (η). The η (cp: 10^{-3}dyn · s/cm^2) of DNA-BAc complex in regions of small velocity gradient showed large values. On the other hand, η tends to change to smaller according to the larger shear rate. This fact shows that DNA-BAc complex was destroyed by the increasing of the velocity gradient values. The heat denatured DNA alone did not show the increasing of η. Furthermore, the heat denatured DNA did not show η difference when BAc was added in. From the above η' s measurements, DNA-BAc complex has comparatively large η, therefore, it is a very stable complex.

Figure 6 showed the histerisis loop of the shear stress for shear rates on DNA-BAc complex. The horizontal line in the shear stress (dyn/cm^2) and the vertical line is shear rate (s^{-1}). This loop shows that DNA-BAc complex belongs to a thixotropy, a kind of non-Newtonian types. Namely, this complex has a non-Newtonian, thixotropy properties.

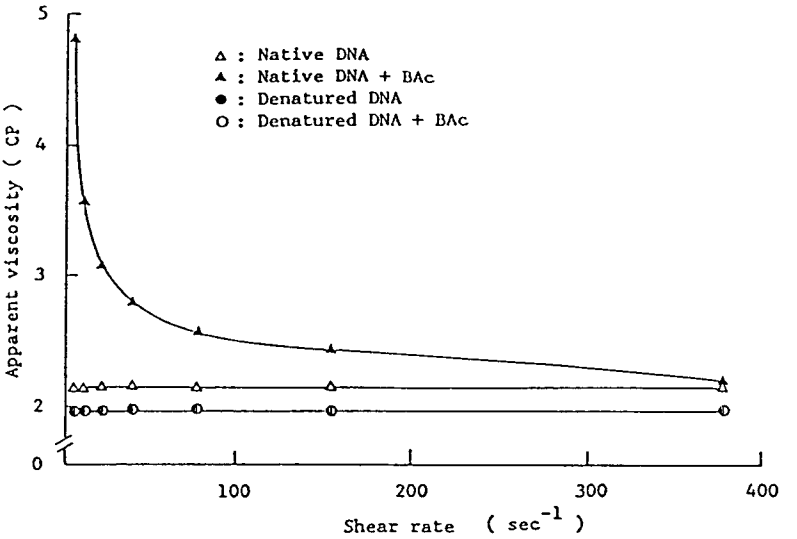

Figure 5. Relationship between apparent viscosity and various shear rates in DNA-BAc complex. The concentration of DNA was 1.26×10^{2} μg/ml and BAc was 1.556×10^{-5}M.

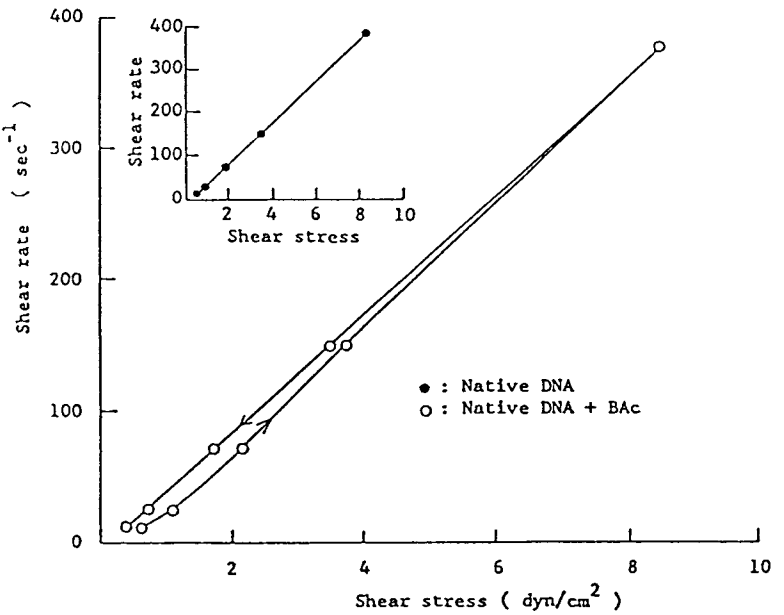

Figure 6. Histerisis loop of shear stress for shear rate in DNA-BAc complex. The concentration of DNA was 1.26×10^{2} μg/ml and BAc was 1.556×10^{-5}M.

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Received January 10, 1989; accepted April 11, 1989.