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KINETIC STUDIES OF THE INTERACTION BETWEEN SILVER ION AND DEOXYRIBONUCLEIC ACID

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The interactions between silver ion and DNA were studied kinetically by the stopped flow method. The experimental results show that the reaction between silver ion and GC base pair of DNA proceeds by two steps and that between silver ion and AT base pair of DNA with one step.

The conformational changes of macromolecules induced by the binding of small molecules are of interest in connection with the functions of small molecules in biological system.

Silver ion interacts preferentially with bases rather than with the phosphate group of DNA[1,2,3] and forms three types of complexes[2]. Several models[1,2,3] have been proposed for the structures of the silver ion complexes, but a definite model has not yet been presented. In this situation it is expected that a kinetic study should give important information about the structures of the complexes. In the present paper the kinetic studies of the binding of silver ion to DNA are reported.

Calf thymus DNA (type II of sigma Chemical Company) was dialyzed extensively, first against 1 mM EDTA plus 0.1 M $NaClO_4$ solution, and then against 0.1 M $NaClO_4$ solution. The DNA concentration of the stock solution was determined spectrophotometrically from the absorbance at 260 nm. Bromocresol purple (BCP) and cacodylic acid were purified exhaustively, and all other chemicals were of reagent grade and not further purified. Potentiometric titration was carried out with Hitachi-Horiba type F-5 pH meter using a silver-wire electrode connected

electrically with a standard calomel electrode. The complex formation between BCP and silver ion was assumed to be negligible judging from the invariance in both the spectra of indicator in the presence of silver ion and in the titration curve of silver ion in the presence of the indicator. Spectrophotometric measurements also suggest that BCP does not interact with DNA. The results of the potentiometric titration are shown by Scatchard plot in Fig. 1. These results are consistent qualitatively with those in the literature[1,2,3], that is, there exists one complex (complex I) at $r_b < 0.2$, where r_b is the ratio of moles of silver ion bound to moles of nucleotide bases, and at higher r_b two complexes, complex I and a second complex (complex II) exist.

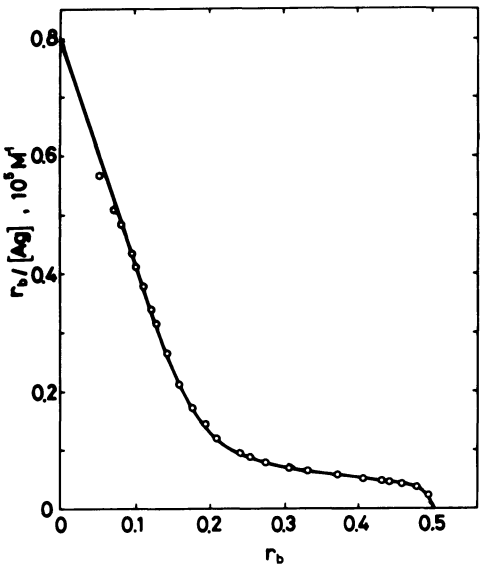


Fig. 1. Scatchard plots for the binding of silver ion to DNA in 10 mM cacodylate-0.1 M $NaClO_4$ (pH 5.6), [DNA] = 9.5×10^{-5} M.

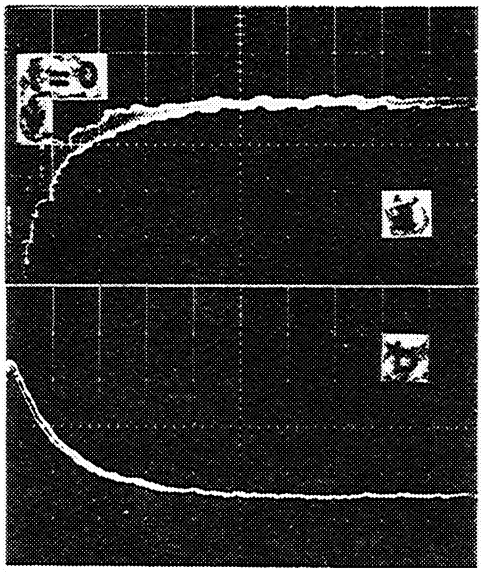


Fig. 2. Typical traces of silver ion binding to native DNA. [DNA] = 5.5×10^{-5} M, r_0 = 0.218. (a) sweep 50 ms/div, (b) sweep 2 s/div.

Kinetic studies were carried out by the stopped flow method at temperature of 20 ± 0.5°C and pH 5.6 under ionic strength of 0.1 M $NaClO_4$ with no buffer. The reaction was monitored by proton release using BCP as an indicator. Figure 2 shows the typical reaction curves. The reaction proceeds via two processes which differ in time scale by a factor of 10 or above. The fast process increases the proton concentration, while the slow process decreases the proton concentration (Fig. 2). Both processes were observed over the whole range of r_0 measured, where r_0 is the ratio of moles of silver ion added to moles of nucleotide bases. In the majority of cases, both processes were characterized by single exponential function and the

relaxation times were calculated from the semilogarithmic plot of the terminal part of the curves. Figure 3 shows the reciprocal relaxation time ($1/\tau$) of each process as a function of r_0.

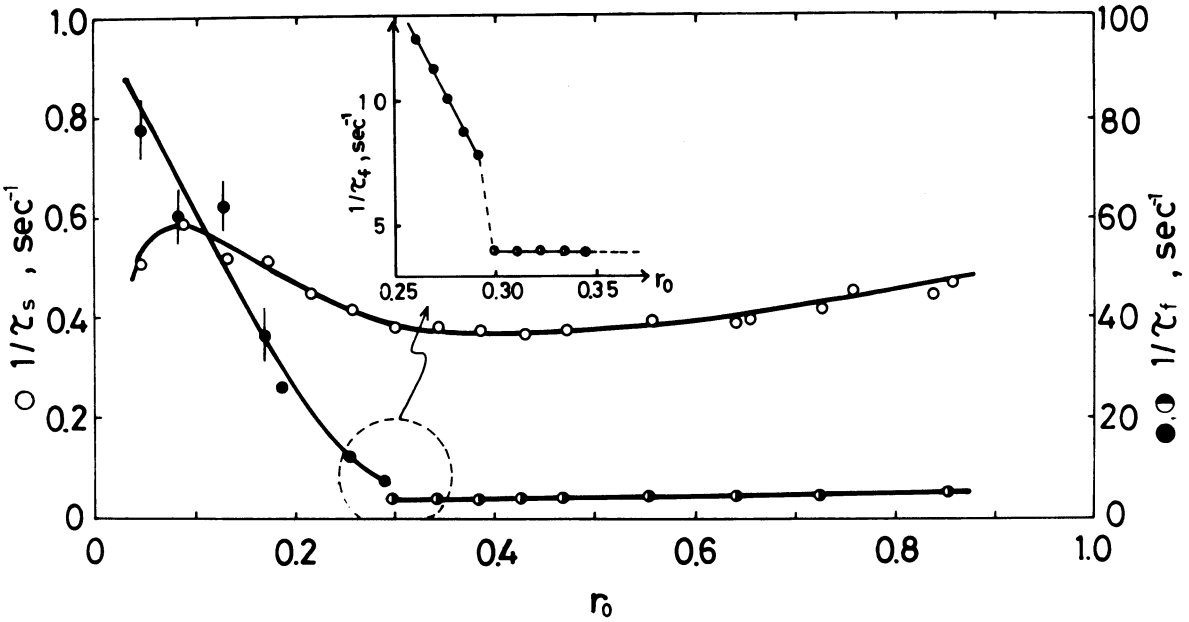


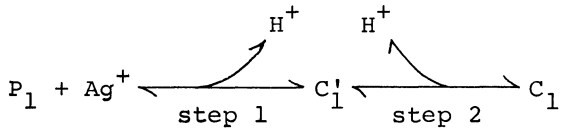
Fig. 3. The reciprocal relaxation times of silver ion binding to native DNA in 0.1 M $NaClO_4$ (pH 5.6). [DNA] = 5.5×10^{-5}, [BCP] = 1×10^{-5} M.
(●, ◑) fast process, (○) slow process.

For the fast process, as can be seen in the insert of Fig. 3, there exists a distinct discontinuity in the relaxation time at $r_0 = 0.3$. This continuity is much too large to be simply attributed to experimental error. Also, an abrupt increase in the relaxation amplitude could be recognized at r_0 above 0.3 where the type II binding emerges. Furthermore, by our preliminary stopped flow experiments with UV detection, two distinguishable rapid relaxations, which seem to arise from either type I and type II bindings, could be observed at r_0 above 0.3. All these results indicate that the fast process observed by pH indicator should be divided into two parts; one at r_0 below 0.3 is attributed to the type I binding, and the other at r_0 above 0.3 is associated with the type II binding or a coupled process of type II to type I binding.

On the other hand, observed over the entire range of r_0 measured, the slow process did not show any significant change either in the signal amplitude or the relaxation time. Consequently, it is likely that this slow process is due to the type I binding only, rather than to the type II binding.

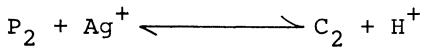
Taking account of these results, the following mechanisms can be proposed as qualitatively satisfactory for the complex formation between silver ion and DNA.

The formation of complex I proceeds via two steps as follows;



(1)

where P_1 is the free site for binding I, and C_1' and C_1 are two types of complex I. In step 1 the proton is released and in step 2 the proton binds again. Meanwhile the formation of complex II proceeds apparantly by a one step reaction so far as proton is concerned,



(2)

where P_2 and C_2 are the free site and complex for the type II binding, respectively.

According to the static studies reported for the binding of silver ion to DNA[1,2,3], the type I binding, which results from the interaction between silver ion and the GC base pair of DNA, has been regarded as being proton independent. However, the present kinetic experiments using pH indicator elucidated that the reaction consists of two steps; one is the proton release and the other is the proton uptake, and in the overall reaction, proton concentration is unchanged. The mechanism proposed above for the type II binding is consistent with that from the static experiments[1,2,3]. In order to discuss the above mechanisms quantitatively, more detailed experiments for the effects of pH, ionic strength and conformation of DNA upon the reaction rate are now in progress.

References

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