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Bilateral International Exchange Program (BIEP, invite) report

Send report to: Your responsible Professor in Kyoto University

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(Year/Month/Day) 2012/03/10

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Research Project

Title	**Interaction of metal ions and Nanoparticles with DNA and effect on higher order structure.**
Duration	**10th December 2011 – 20th February 2012**

Please summarize your activities and results during your stay in Kyoto University.
Also please describe how your stay has been beneficial to the graduate students in the host institute. You can add a sheet, if you need more space.
You can also write any comments and requests to the GCOE program.

During my stay at the Kyoto University Physics Dept Laboratory, I tried to understand some basic mechanism of interaction of the metal cations with the DNA polymer chain.

The change in the structure of the compact folded DNAs has attracted the interest of a major group of researchers involving biologists and biochemists. This is mainly because of the change in the higher order structure of DNAs is expected to be closely related to the mechanism of self-regulation of replication and transcription in living cells. DNA is a polyelectrolyte with a high density of negative charge. Since it has a small persistence length
(~ 50 nm), a long DNA (~ 166k Base Pair) in aqueous solution behaves like a flexible chain exhibiting an elongated coiled state. This confirmation has already been observed by Fluorescent Microscopy in fluorescent labeled DNAs. On the other hand the DNA chains exist in a very compact Globule state in both the prokaryotic and eukaryotic living cells. Thus the physiochemical studies of the large structural changes of DNA chains are becoming very important issue in modern biology.

From the studies of Bloomfield et al (1996) it is known that the condensation of the DNA chains occur due to the interaction of cations with higher (≥ 3) valencies. However from our recent studies we have observed the condensation of the DNA chains with the divalent metal cations. The purpose of our present study was to clarify the mechanism of condensation of single DNA molecule induced by different divalent cations and also their relative effects on the confirmation of the DNA molecule. We have selected Ca, Mg, and Sr as the multivalent cations. We observed the effect of these cations with varying concentration on the
confirmation of the single T4DNA through Fluorescent Microscopy. We used the YOYO as the fluorescent label for our experiments.

In our experiments we used 0.1 μM of T4DNA as the polymer chain, and 10mM Tris-HCl as the buffer. The merceptoethanol was used to stabilize the DNA under the high intense lamp source.

We observed a strong compaction of the DNA chain after the interaction with the metal divalent cations. In the absence and in the presence of minute ions the DNA assumes the elongated random coiled state, where as the concentration of the divalent cation increases the
DNA collapses and forms the globule state.

The concentration of the divalent ion and the change in the long axis length (l) is shown below in figure 1. This shows the formation of the globule state after the high concentration of the
divalent cationic interaction of Sr, Ca, and Mg.

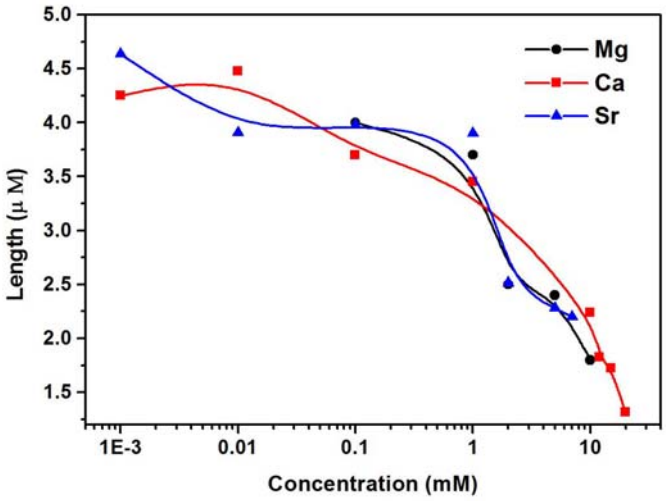

Figure 1 : Log-Log plot of the long axis length (l) and the Concentration of the divalent ions.

A more detail study of intermediate state of the concentrations of the divalent cations show a very fruitful and interesting results. The effect of Mg ions on the T4 DNA is shown in a

histogram plot of the long axis length (in nm) in Figure 2(a). Where the long axis length (in nm) of the various concentrations of the Mg ion show the formation of the globule state very prominently. In the plot, the concentration is varied from 0.001 mM to 10 mM. The 10mM concentration

shows the globule formation.

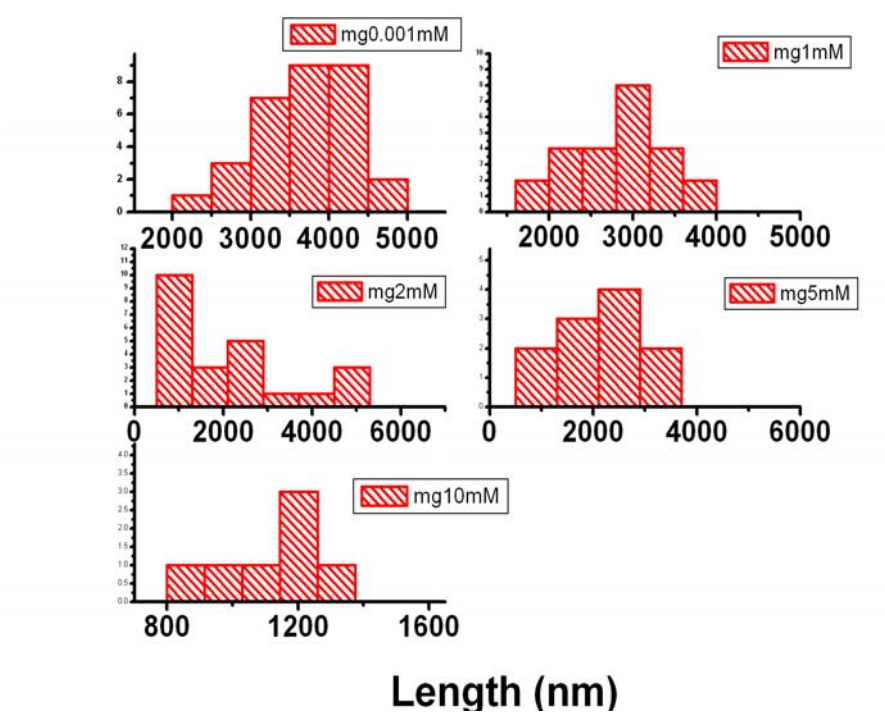

Figure 2(a): Long axis length histogram plot of the T4DNA molecule after the Mg ion interaction

Similarly for Ca and Sr cations also, the long axis length histograms are shown below. These data show a very unusual but strong evidence of the formation of globule state of the long DNA molecule under the effect of the divalent cations.

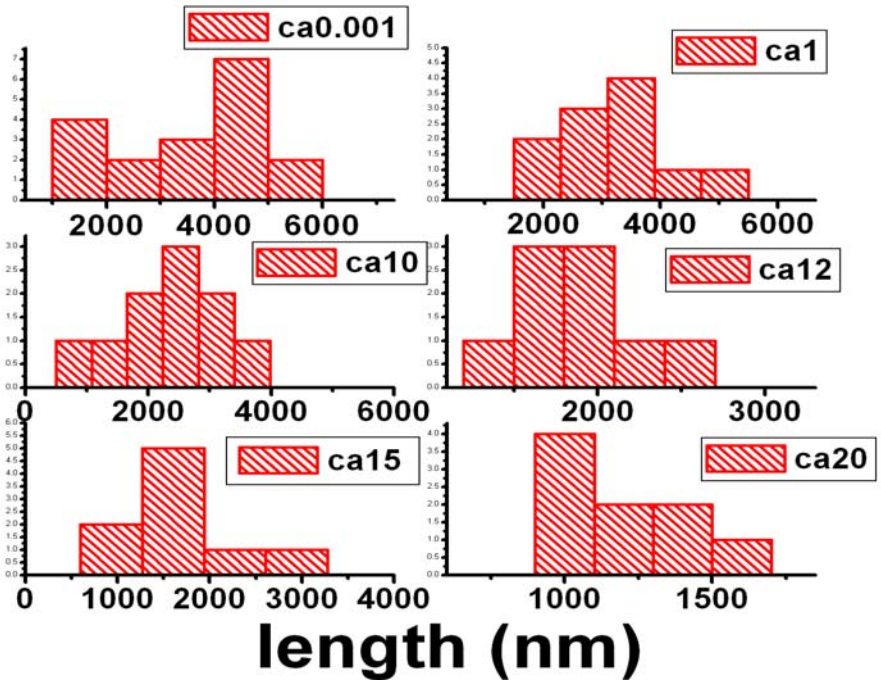

Figure 2(b): Long axis length histogram plot of the T4DNA molecule after the Ca ion Interaction. The inset value shows the concentration of the Ca ion in mM.

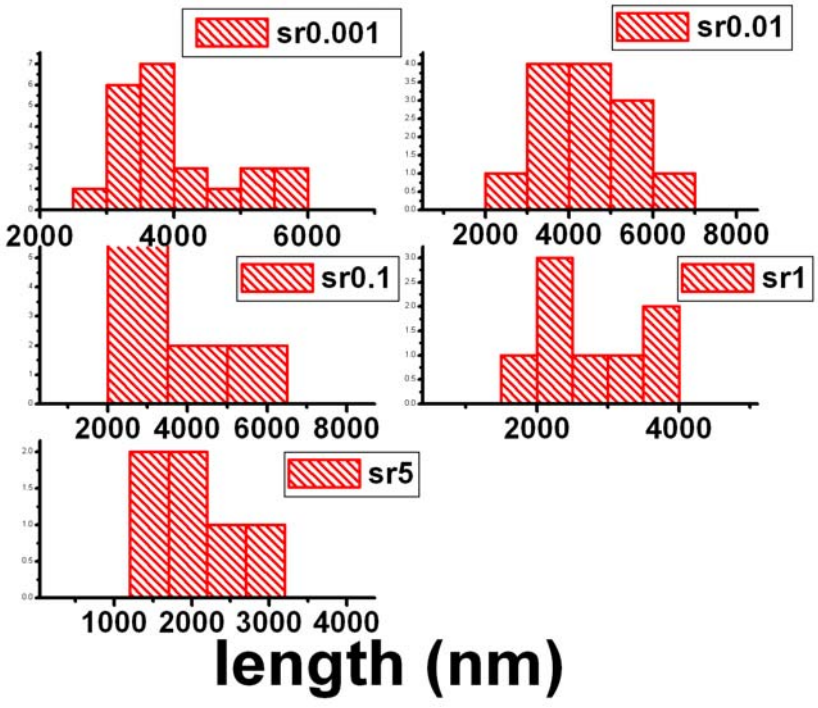

Figure 2(c): Long axis length histogram plot of the T4DNA molecule after the Sr ion Interaction. The inset value shows the concentration of the Sr ion in mM.

So, in conclusion, we have observed the dramatic effect of the divalent cation of Ca, Mg, and Sr
on the confirmation of the T4DNA which were not seen earlier so effectively. In this study the single molecular observation through the Fluorescent Microscopy plays a very crucial

role,
as it gives the real picture of the interaction of the single molecule with the present of the divalent cation. We have also seen the relative difference of the interaction of the cations (Mg, ca, and Sr). As for the Ca, we observe the globule formation in ≥ 20mM (figure 2(b)), whereas for Mg it
happens at ≥ 10 mM (figure 2(a)) concentration, and for the Sr cation it is just ≥ 5 mM (figure 2(c)). Thus it is clear that Sr interacts more strongly with the DNA than the other two cations.

I am extremely thankful to Prof. K. Yoshikawa and Prof. Ichikawa for their support and my stay at the Laboratory of Dept Of Physics, Kyoto University. I am also thankful to the GCOE program for sponsoring me for my stay during the time 10th Dec' 2011 – 20th Feb' 2012.

During my stay in the laboratory I interacted with the graduate students of the laboratory And shared my experience and work theme. It helps us to work in a team spirit in the laboratory. I spend a very enjoyable time in the laboratory. I got some new ideas and have initiated to form a strong collaboration with the laboratory for my future work.