CHAPTER II

EXPERIMENT

2.1. Chemicals

HPLC grade solvent was used in all the experiments unless specified otherwise. Water was obtained from Merck in Thailand and acetonitrile was obtained from Merck (Thailand). The PNA and DNA were prepared by Mrs. Chotima Vilaivan. All acpcPNAs were synthesized by Fmoc-solid phase peptide synthesis protocol.

2.2. Purification and Cleavage of PNA Oligomers

The PNA was synthesized by Fmoc-solid phase peptide synthesis and cleaved from the resin by trifluoroacetic acid containing 10% triisopropylsilane as previously described [44]. HPLC experiments were carried out on a Water Delta 600TM system equipped with gradient pump and a Water 996TM photodiode array detector. The HPLC purification and analysis were performed in a reverse phase mode, eluting with a gradient system of 0.1% trifluoroacetic acid in methanol and MilliQ water. The gradient system consisted of two solution systems that are solvent A (0.1% trifluoroacetic acid in MilliQ water) and solvent B (0.1% trifluoroacetic acid in methanol). The gradient started from A: B at 90:10 for the first 5 min and then increased linearly to 10:90 over 60 min. The peaks were monitored by UV absorbance at 260 nm. The combined HPLC fractions were freeze-dried and re-dissolved in water to prepare PNA stock solutions.

2.3. Characterization of acpcPNA by ESI-MS Analysis

All acpcPNA oligomers were characterized by Electrospray ionization mass spectrometry (ESI-MS, model micrOTOF-Q II). All samples were prepared in water/acetonitrile at a ratio of 90:10 and recorded in a positive ion mode. The observed masses were the exact mass.

2.4. Experimental Conditions to Observe PNA-DNA Duplexes

In this experiment the mobile phase condition was studied by using water and acetonitrile in different ratios such as the water: acetonitrile in the ratio of 60:40, 70:30, 80:20, and 90:10 at 180 °C. The experimental procedure is to select the PNA

(T_9), which is the PNA sample to test the condition to observe the solvent mixture and select a positive mode to generate ions. The data were compared by considering the intensity of the spectrum to select the best solvent mixture condition.

2.5. Analyses of Mass Spectra of PNA-DNA Duplexes

All PNA-DNA complexes were characterized by ESI mass spectrometry. All samples were prepared in optimized mixture solvent by stock PNA and DNA solution. Preparation of the final concentration of PNA-DNA complexes = 10 μM after adjusting the volume to 100 μL with water: acetronitrile (HPLC grade). The sample mass spectra were performed on Microtof control ESI-MS (Bruker Daltonics). The data was recorded in a negative ion mode.

2.6. Studies of Noncovalent Interaction in PNA-DNA Duplexes

In the experiment we used the collision induced dissociation (CID) technique to study the noncovalent interaction of PNA-DNA complexes. After the increase in energy into a collision cell, the energy is calculated according to the equation below [31].

$$E_{CM} = E_{LAB}[m_g/(m_g+m_p)]$$

E_{CM} = centre-of-mass collision energy

E_{LAB} = ZeV (z = number of charge) = ion kinetic energy in the laboratory frame

m_g = mass of the stationary target gas

m_p = mass of projectile ion)

Factors influencing the stability of PNA-DNA complexes were studied. The experimental results obtained by ESI-MS (E_{CM} value) were compared with the results of UV-vis spectroscopy (Melting temperature; T_m).