

## BIOC 463A 2003-2004

### Thermal Melting of DNA Experiment

General Protocol:

Chromosomal DNA from *E. coli* (E.c.) and *Clostridium perfringens* (C.p.) is dissolved in 11 mM phosphate buffer (pH 7) and 44 mM NaCl. A baseline spectrum of the buffer alone is obtained, and then the DNA containing solution for each form of DNA. A heated water bath containing ethylene glycol based antifreeze solution is hooked up to a water-jacketed cell-holder into which you place your cuvette. An initial spectrum at 24°C is obtained. The temperature control is incrementally increased. At each temperature you allow the water bath and the DNA solution in the cuvette to come to thermal equilibrium (this must be empirically determined and will vary with the cell holder, type of cuvette, and the volume in the cuvette). After the system has come to thermal equilibrium (i.e. the temperature stays constant) you run a UV spectrum of the DNA solution. The data given below was obtained by the 2001 fall semester BIOC 463A class.

#### Chromosomal DNA

E. coli		C. perfringens	
Temp. (°C)	A <sub>260</sub>	Temp.(°C)	A <sub>260</sub>
24	0.672	24	1.012
45	0.675	50	1.016
54	0.675	55	1.042
62.5	0.674	59	1.054
68	0.678	65	1.064
76	0.695	70	1.163
78	0.712	72	1.253
80	0.732	74	1.323
82	0.767	76	1.362
84	0.830	78	1.387
86	0.884	82	1.394
88	0.908	84	1.394
90	0.914	86	1.396
95	0.918	88	1.40
99	0.919	90	1.397

**Turn in the figures and answers to the following questions at the beginning of the next lab period.**

1. Using the relationship, 66 ug/mL of double stranded DNA gives an OD<sub>260</sub> = 1, calculate the concentration of DNA in each species using the A<sub>260</sub> values obtained at 24°C.

2. Beginning with the data at 45°C for *E. coli* and 50°C for *C. perfringens*, “normalize” the data by plotting  $A_T/A_{24^\circ\text{C}}$  (where  $A_T$  represents the A<sub>260</sub> value at each temperature) vs. T for the DNA from both species on the same plot. Determine T<sub>m</sub> for the DNA of each bacterium directly from the plots. Usually “to normalize data” means to convert the data to a ratio that varies from 0 to 1. However in this case the ratio will vary from 1 to some value > 1 and the ratio is a direct measure of the magnitude of the hyperchromic effect. Briefly explain this statement.

3. Do the relative values for the  $T_m$ 's agree with those given in Table 1 of the Marmur and Doty paper?

4. Taking into consideration the buffer used to perform the experiment and using the values for the %GC content of each type of DNA from Table 1 in Marmur and Doty, calculate a theoretical  $T_m$  for each DNA based on the equation:

$$T_m = 41 X_{GC} + 16.6 \log [Na^+] + 81.5$$

Are the observed and theoretical values similar?

5. The general shape of the curve for the *C. perfringens* DNA is distinctly different from that of *E. coli*. There is a minimum of two explanations for the shape of this curve. Postulate one reason.

6. Calculate the expected  $T_m$  for *C. perfringens* DNA if the experiment were performed in 11 mM phosphate (pH 7) and 150 mM NaCl. Explain the change in  $T_m$  based on changes in the structure of the Watson-Crick model for double stranded B-form DNA.

7. The equation given above (in question 4) DOES NOT take into account the number of base pairs. An equation that includes this parameter is:

$$T_m = 81.5 + 16.6 \log [Na^+] + 0.41 (\%GC) - (625/N)$$

Where N is the length of the oligonucleotide. Using the %GC for *C. perfringens* calculate the  $T_m$  for DNA of the following size: 100 Kbp, 10 Kbp, 1 Kbp, 100 bp, 20 bp. Plot  $T_m$  vs. log number of base pairs. Using the plot you have generated and assuming the same %GC content, calculate the  $T_m$  for the lux plasmid (jHD500) that you purified in the previous experiment.

8. Considering the  $T_m$  values obtained in #7 answer the following question. With bacterial chromosomal DNA (~ 4 Mbp) hysteresis is very common, in fact it is very rare when it does not happen, no matter how slow the temperature is decreased. In shorter oligonucleotides (< 1000 bp) hysteresis is much less of a problem. How might the magnitude of the hysteretic effect for DNA be related to the  $T_m$ , which in turn is related to the number of base pairs? How might these properties be related to DNA super-structure formation in the re-annealing process?

9. Suppose you want to program a heating cycle into a PCR device that will selectively amplify an oligonucleotide of 100 bp off of intact chromosomal *E. coli* DNA. Using the data obtained in this experiment, and the equation given in #6, design a heating cycle that would accomplish the amplification of the 100 bp oligomer. (Hint: calculate the  $T_m$  of the oligo using the %GC for *E. coli* DNA).