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A study of thermal denaturation/renaturation in DNA using laser light scattering: A new approach

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The thermal denaturation/renaturation processes in *E. coli* and eukaryotic DNAs have been studied using the laser light scattering (LS) technique. The differential scattering intensity curve has been utilized to determine the transition temperature T_m. The effect of solution *p*H on DNA thermal denaturation has been examined. It has been shown clearly that LS is an extremely sensitive method (more than the UV absorption method) and reveals even the subtler effects such as the pre-transition fluctuations, and that the DNA denaturation is prominently affected by *p*H. The dependence of melting temperature (T_m) on composition of DNA, number of base pairs and base sequences has also been investigated. It has been observed that depending upon its base sequence, the T_m decreases in the case of renatured DNAs. The results have been compared with the UV absorption studies.

Keywords: Laser light scattering, DNA, denaturation, renaturation, *E. coli*, eukaryotic DNA

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The denaturation and renaturation studies of DNA molecule have always been of immense research interest. DNA unwinding (denaturation) is most commonly produced by the temperature, but can also be brought about by extremes of *p*H, decrease in dielectric constant of the aqueous medium by alcohol, ketones, etc., exposures to amides, urea and similar solvents[1,2] and by application of overstretching force[3,4]. Experiments[5,6] on dilute DNA solutions have provided evidence for the existence of a thermally-driven melting transition, corresponding to the sudden opening of base pairs at a critical or melting temperature T_m. The T_m, at which one half of the DNA denatures, depends on specific DNA and also on the nature of the solvent. The study on the melting of DNA plays an important role in understanding the structure and dynamics of DNA.

The co-operative thermal disruption of hydrogen bonds between complementary bases in the double helix is usually monitored by the UV absorption increment, due to unstacking of the separated bases[7]. As DNA absorbs light at 268 nm/260 nm, absorption technique has been most commonly used for investigating the melting transition in DNA. The UV absorbance of DNA is due to the nucleotide bases and arises from the π-π^* electronic transition in both purine and pyrimidine bases[8]. An increase in the absorbance reflects a change in the electronic configuration of the bases due to the decrease in double helical stacking. The absorbance increases by 30-40%, depending up on the DNA sample. Most of the investigations towards the understanding of the denaturation/renaturation processes done so far in DNA have utilized the UV absorption method[8,9].

Light scattering (LS) technique has also received interest[10,11], in exploring the phase transition processes. When a solution of DNA is heated, striking changes occur in many of the physical properties, such as scattered light flux and optical density. Therefore, like absorption, LS could also be used to explore the denaturation/renaturation processes in DNA solution. Earlier[12] study reported the results of quasi-elastic light scattering study on DNA fragments obtained from chicken erythrocytes. Using photon correlation or light beating technique aided with different models, translational diffusional constants can be determined[13-16]. In fact, LS technique can probe DNA molecule non-destructively[17], without affecting its internal structure.

In the present paper, an attempt has been made to study the thermal denaturation/renaturation in eukaryotic and *E. coli* DNAs, and the effect of solution *p*H on DNA, using static LS technique.

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Further, in order to know, if the renaturated composition regains its original state, the renatured DNAs have once again been thermally redenatured and cooled and the scattered intensity has been monitored. The variation of scattered intensity flux with temperature has been compared with that of the native DNAs.

Materials and Methods

Genomic DNA from chicken liver (eukaryotic) and *E. coli* (prokaryotic) were prepared and sheared to ~ 5 kb (i.e, of 5000 base-pairs) as described[18]. For experiments, the samples of *E. coli* and eukaryotic DNAs were made in TE buffer (10 m*M* Tris and 0.5 m*M* EDTA, *p*H ~7.4) and triply distilled water (*p*H ~7.0), respectively. The concentration of the DNA in the solution was 6 μg/ml and kept the same for all the experiments.

The experimental set-up consisted of a light scattering spectrometer with a 5 mW He-Ne laser (6328 Å), a collimating arm with provisions of focusing, an arm containing the detector assembly and the graduated scattering turn table (Fig. 1). A cooled (–10°C) photomultiplier tube (PMT) with amplifier-discriminator and photon-counter constituted the detection set-up. A scattering cell made up of quartz in a proper holder was mounted at the symmetry axis of the turn table. The details regarding the spectrometer are described elsewhere[19]. The dark count detected was less than ten. Experiments were conducted under well-stabilized conditions for both laser power and PMT.

The scattering angle was fixed at 90° and the scattered intensity was monitored at each 1°C rise in temperature with the rate of increase approx. 1°C/1.5 min. On reaching the temperature to 95°C, where the scattered intensity flux was found to be well saturated, the cell was allowed to cool and the scattered intensity was again monitored for each 1°C decrease in temperature up to the normal room temperature. The rate of cooling was approx. 1°C/4 min. The observations were recorded in exactly the similar manner for both the samples.

Results and Discussion

The variation of scattered intensity with temperature recorded at fixed scattering angle of 90° for native *E. coli* and eukaryotic DNAs is shown in

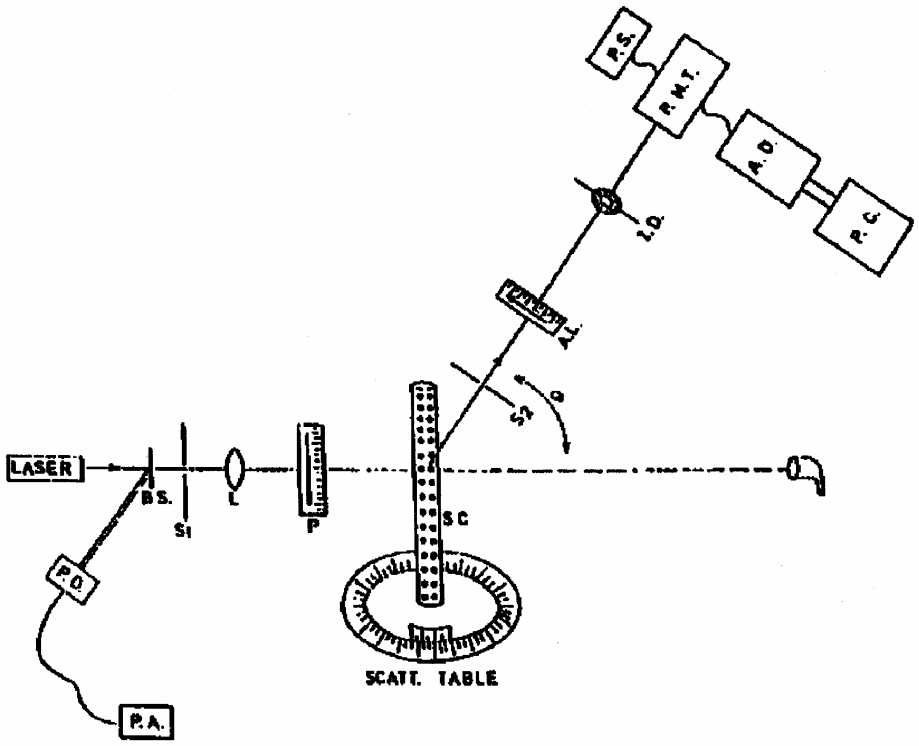

Fig. 1—Homemade laser light scattering spectrometer. [B.S., Beam splitter; P.D., photo diode; P.A., pico ammeter; S_1, S_2, slits; L, lens; P, polarizer; S.C., scattering cell; A.L., analyzer; I.D., iris diaphragm; P.M.T., photo multiplier tube; A.D., amplifier discriminator; P.C., photon counter; P.S., power supply]

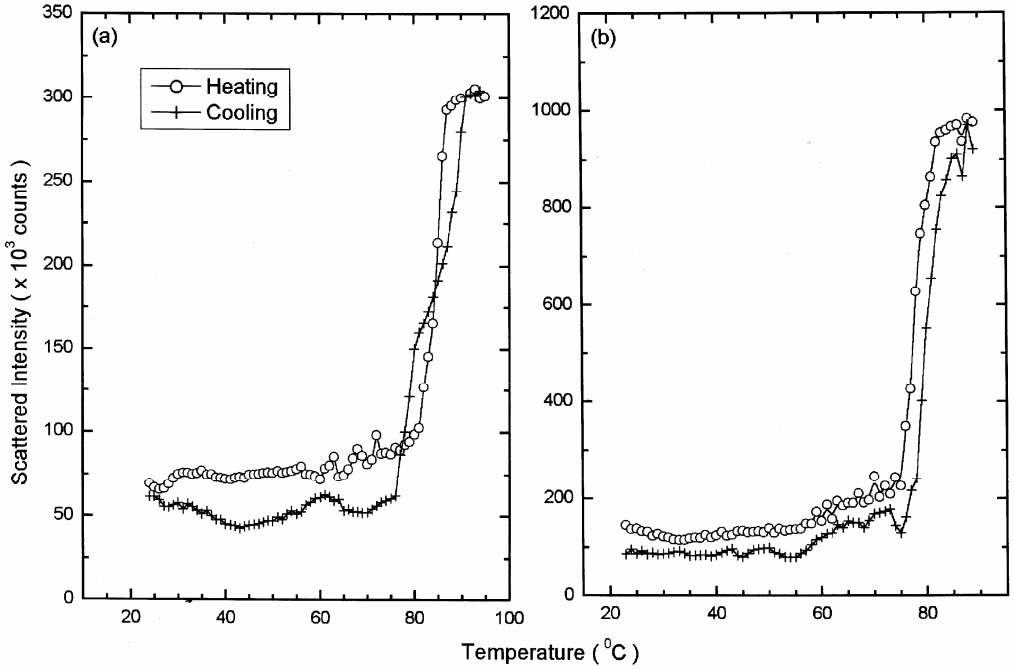

Fig. 2—Variation of scattered intensity with temperature for native (a) *E. coli* DNA; and (b) eukaryotic DNA

Fig. 2a and b respectively. The heating up to approx. 60°C does not affect the composition of the native *E. coli* DNA (Fig. 2a) as the intensity remains almost constant. In between 60 to 70°C, the oscillations set in the scattered intensity with gradual rise. At about 70°C, a sudden rise in the scattered intensity is observed which becomes almost steep around 80°C. Heating after 82°C again does not affect the scattered intensity and it gets saturated thereafter. The rise in the scattered intensity flux is approx. 500-600% and occurs with a transition width of approx. 3-4°C. Almost the similar nature of the variation of scattered intensity has been observed in native eukaryotic DNA (Fig. 2b). The scattered intensity remains constant up to approx. 55°C rise in temperature, whereafter the oscillations set in the scattered intensity with a slight gradual increase. At about 73°C, the intensity starts increasing steeply and gets saturated around 81-82°C, having the transition width of about 5-6°C.

As the temperature is raised, two possible hydrogen bonded base pairs — adenine-thymine (A-T) and guanine-cytosine (G-C) between complementary strands begin to open, inducing the unwinding of the strands. Opening of the base pairs occurs in a highly co-operative manner, causing the strands to completely separate from each other. After the complete strands separation, almost a linear increase in scattered intensity is observed on further increase in the temperature. Comparing the Figs 2a and b, it is clear that T_m is higher for *E. coli* DNA than eukaryotic DNA. This is primarily due to the variation in base pair composition, as G-C content in former (51%) is higher than the latter (42%)[20]. The T_m is precisely correlated with the average base pairs composition[5,21,22] of the DNA. This suggests that DNAs having higher G-C base pairs content are more stable. As G-C base pairs contain three hydrogen bonds, they require more heat energy to dissociate than A-T base pairs and as a consequence, DNA with a greater G-C content would be comparatively more stable.

It is also clear from Figs 2a and b that on cooling the heated samples slowly, DNA nearly regains its original helical form. This is quite evident from the renaturation curve. The original intensity is nearly regained, showing clearly that the renaturation of the molecule is almost complete in both *E. coli* and eukaryotic DNAs having only a very minor difference amongst them[6,23].

Pre-transition fluctuations

In both the cases (Figs 2a & b), some fluctuations have been observed in the scattered intensity before the start of the denaturation process. It is interpreted as the 'fluctuational opening', which is caused due to the large amplitude motions that set in the DNA,

before the denaturation temperature. In fluctuational opening[24], the base pairs open for a very short time and then close again. Thus, it could be considered as intrinsic precursor to the process of denaturation.

The variation of scattered intensity with temperature on heating as well as cooling the renatured *E. coli* DNA is shown in Fig. 3a. The transition temperature is observed to be 77°C with a transition width of approx. ~ 20°C. It is also obvious that the pre-transition fluctuations are quite reduced. It can very well be inferred by comparing the curve of Fig. 3a with Fig. 2a that the denaturation of renatured *E. coli* has changed in its character. The transition temperature T_m has decreased with increase in the transition width. Of course, the factor by which the scattered intensity increases remains almost the same.

The differential scattering intensity (melting) curve for native and renatured *E. coli* DNAs is shown in Fig. 4a, which highlight the difference (in composition) between the native and renatured *E. coli* DNA. Similarly, the corresponding curves for the eukaryotic DNA are given in Figs 3b and 4b, respectively. The transition temperature T_m, as noted from the Fig. 4b for eukaryotic DNA is 55°C, with a transition width of about 20-25°C. The decrease in T_m of renatured DNAs suggests that there has been some

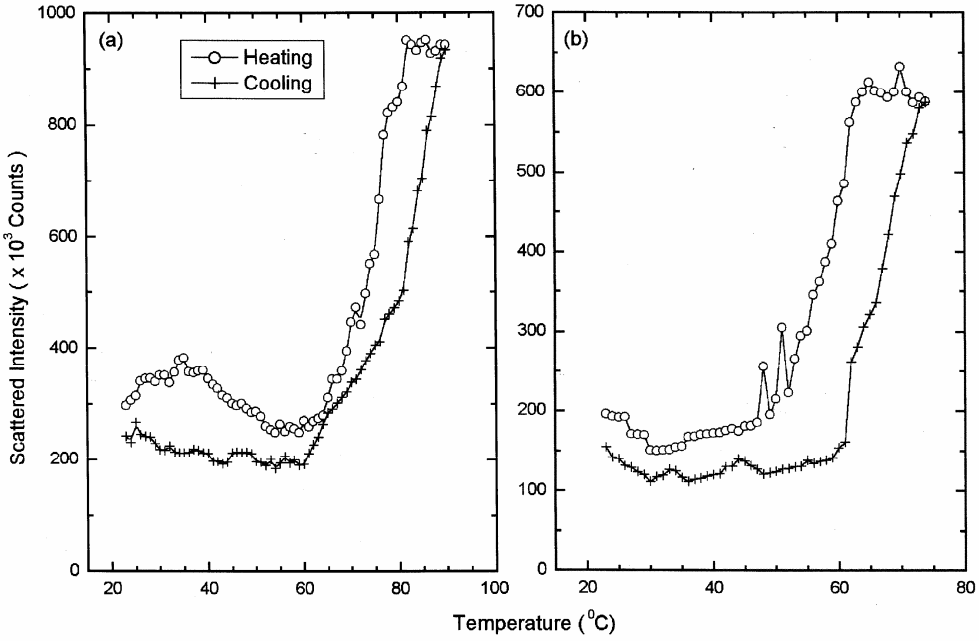

Fig. 3—Variation of scattered intensity with temperature on heating the renatured (a) *E. coli* DNA; and (b) eukaryotic DNA

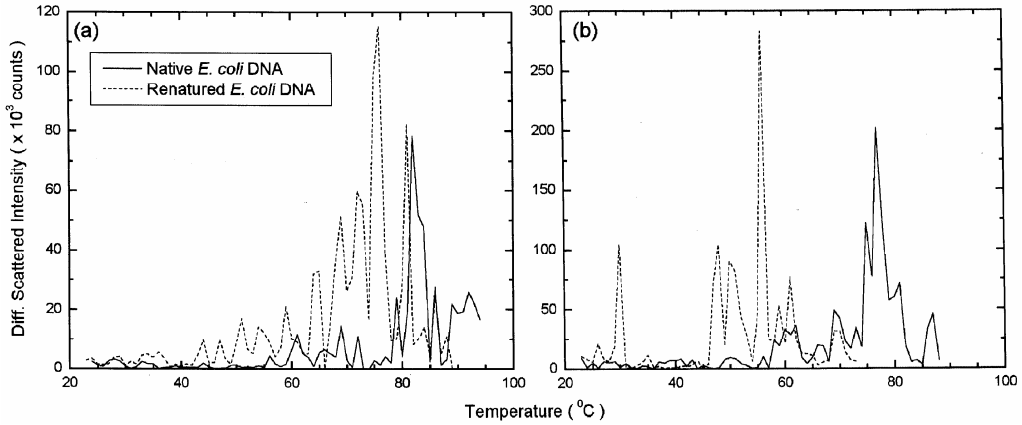

Fig. 4—Differential melting curves for native and renatured (a) *E. coli*; and (b) eukaryotic DNA

mispairing or mismatch between base pairs that has occurred during renaturation. For example, if one strand of DNA has adenine on a site, the other strand would have guanine or cytosine, instead of thymine on the same site. Any mispairing between bases leads to the decrease in stability of a DNA duplex[25]. In a duplex of native DNA comprising two strands that are perfectly paired, every base pair must get disrupted before the strands can separate. The presence of any mismatched pairs of bases that are not held together by hydrogen bonds, therefore, reduces the energy required for separation. In differential scattered intensity *versus* temperature graph shown in Figs 4b and 5b, it is obvious that the melting of the DNAs, as seen in the respective figures is a multi-step process i.e., opening of the strands does not take place in one stretch. This type of feature has also been reported by earlier workers[24,26,27].

It is clear from Figs 3 & 4 that decrease in T_m of renatured DNA is higher in eukaryotic DNA than *E. coli*, indicating that during renaturation, mispairing is greater to some extent in eukaryotic DNA. The eukaryotic DNA consists of more repetitive sequences[20] of base pairs in comparison to *E. coli*, that are related but not identical results in the formation of imperfectly paired duplex molecules during renaturation, causing decrease in stability. Earlier[28], it has also been reported that the repetitive components consist of families of related sequences of base pairs. The members of each family comprise a set of nucleotide sequences that have sufficient similarity with each other to renature, but are not identical.

Effect of *p*H on denaturation/renaturation

The *p*H of the solvent as mentioned above plays a major role in the process of denaturation/renaturation of DNA. The effect of *p*H on the conformational stability of DNA has been investigated by various physico-chemical methods[29-31]. The viscosity and UV absorption undergo a sudden change as the *p*H decreased from 4 to 3[32]. In the present work, this aspect, in particular of the solution *p*H has been examined. The variation of scattered intensity with temperature for eukaryotic DNA at different *p*H values is shown in Figs 5a, b and c, respectively. Fig. 5a shows the denaturation and renaturation curves for native eukaryotic DNA at *p*H 5.4. In the present case, the DNA melts (T_m) or denatures at about 85°C, which is somewhat higher as compared to the T_m for the DNA in a normal *p*H 7.2 (see Fig. 2b) solvent. Also, the melting transition at this *p*H is very sharp. It is seen from renaturation curve that on cooling the denatured helix nearly regains its original helical form. It further suggests that at *p*H 5.4, the DNA helix is more stable. The slight increase in T_m around *p*H 5.4 may be explained by partial protonation of adenine and thymine, which may cause charge-dependent stabilization of DNA helix[34].

At *p*H 4.4, the scattered intensity does not increase sharply at a particular temperature (Fig. 5b). Instead,

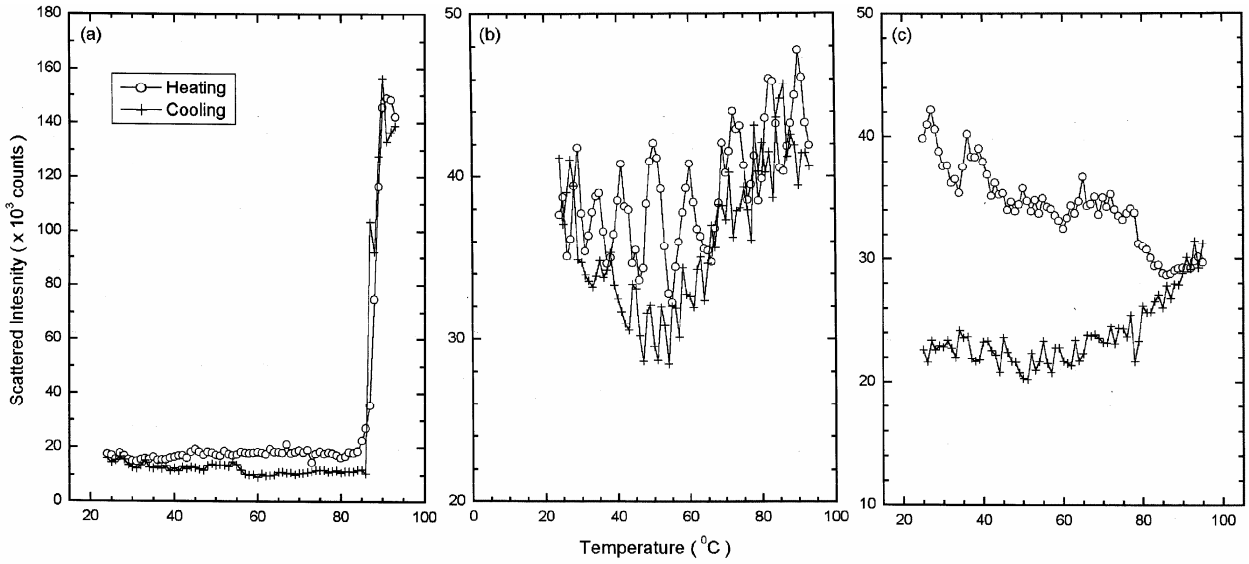

Fig. 5—Variation of scattered intensity with temperature for native eukaryotic DNA (a) at solution *p*H 5.4; (b) at solution *p*H 4.4; and (c) at solution *p*H 3.2

it shows oscillations all along with gradual increase from 60°C onward. It appears that though increase in temperature denatures the DNA at this *p*H also, but it occurs in a quite small segment-wise. That is the reason that it spreads over whole of the temperature width. This suggests that at this *p*H, DNA that changes its structure on cooling, after its denaturation, comes back to its natural state almost in the same manner. The reason may be understood in the following way: It is a known fact that DNA denatures in a strong acidic and alkaline medium. At *p*H 4.4, the double helical structure of DNA is, therefore, likely to get distorted, resulting in the change of the parameters (such as the number of particles, shape and size, etc.) that affect the scattered light intensity by it. It appears, therefore, obvious that the pattern of the scattered intensity showing the denaturation is quite different, as compared to at the normal *p*H (7.2).

It is interesting to note that in case of the solution having *p*H 3.2, the scattered intensity oscillates (Fig. 5c) and also decreases slightly, with the rise in temperature. It appears that at lower *p*H (3.2), the DNAs were already in the denatured state and, therefore, there was no significant change in its conformation on heating/cooling. It seems logical also, as after the denaturation almost all the DNAs being in coil form (single strand), possibility of change in the number of scattering centers with the temperature is negligibly small. Our results at *p*H 3.2 are consistent with the earlier reported results[30,31,34] in this respect.

Conclusions

The static laser LS technique has been shown to be another sensitive method of studying the thermal denaturation/renaturation processes in DNAs, besides the UV absorption method. The melting temperatures (T_m) 82 and 78°C determined for *E. coli* and eukaryotic DNAs, respectively were in agreement with the earlier investigations[8]. It was clearly demonstrated that T_m depends on the composition of DNAs. On heating the renatured DNAs, T_m was found to decrease, depending upon their base sequences, suggesting that some mispairing occurs during the renaturation. The results also showed that for DNAs comprising more repetitive sequences of base pairs, the probability of mismatch during renaturation increases. The thermal denaturation at different *p*H (in acidic region) shows that DNA could be denatured by varying the *p*H of the solution. The present study revealed that the denaturation/renaturation processes in DNA are more sensitive to the LS, as compared to UV absorption. In addition, LS may provide new dimensions to the understanding of the mechanisms of these processes.

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