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New Concepts in Biochemistry

Alternate View on Thermal Stability of the DNA Duplex[†]

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ABSTRACT: A concept, based on the changes in internal pressure of water due to addition of salts, has been used to interpret the thermal stability of the DNA duplexes. Dependence of thermal stability of duplexes on the salt concentrations has also been described in terms of variations in the internal pressure of water observed in presence of ionic components. The thermal stabilities at high external pressures (up to 200 MPa) compared to those at atmospheric pressure are linearly related to the corresponding variations in internal pressures at several salt concentrations.

Thermal stability of a DNA duplex largely depends on some unique properties of water in an ionic environment, in addition to the basic sequences of the single complementary strands of the DNA duplex [some examples are given in Record et al. (1978), Saenger (1983), Buckin et al. (1989), Shakked et al. (1989), Zieba et al. (1991), Rentzeperis et al. (1993), and Chalikian et al. (1994)]. In fact, the very formation of the DNA duplex is governed by these parameters. In the last three decades several qualitative as well as quantitative explanations have been put forward to describe the stabilization of the DNA duplex in aqueous medium (Record et al., 1978; Manning, 1979; Troll et al., 1986; Conrad et al., 1988; Friedman & Honig, 1992). One of the views emphasizes the role of electrostriction effects in such systems. It seems to us that electrostriction effects, if expressed in terms of a measurable thermodynamic quantity, can be used to interpret several features of the thermal stability of a DNA duplex without recourse to much complex mathematics. In order to achieve this goal, we present an alternate concept described below.

Our approach aims at providing answers to three basic questions, namely, (i) why does the thermal stability, T_m of a double helix increase with the addition of salts like NaCl?, (ii) why does the duplex become thermally less stable on addition of certain other salts?, and, finally, (iii) how does the application of high external pressure affect the thermal stability of a DNA duplex? In this article, an attempt is made to provide answers to these questions and present a unified concept based upon quantitative analysis of experimental data.

Due to the high electric field of an ion, water molecules are oriented around it causing a local collapse of the bulk water structure. Since water molecules are more firmly packed around an ion than in bulk water, the net volume of the system decreases in the region. This process, called electrostriction (ES), is thus a result of ion−water interactions, and it mainly depends on the charge and the radius of an ion and the changes in local dielectric constant around the ion (Dack, 1975; Leyendekkers, 1983). This concept of electrostriction [for example, Kauzmann et al. (1962) and Conway (1981)] has been employed to offer qualitative explanations of the thermal stability of duplexes resulting from the complementary single strands. The electrostriction results in a compression of adjacent water dipoles at pressures equivalent to a few thousand bars (1 bar = 0.1 MPa). This pressure is called the thermodynamic internal pressure, P_i. In this work, the changes in the internal pressure of water due to addition of various salts are correlated with the thermal stability of DNA duplexes at atmospheric and elevated external pressures.

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Table 1: Parameters of the Equation $\Delta P_i/\mathrm{MPa} = aM + bM^2 + cM^3$ for a Few Representative Salts in Water ($P_{i,w} = 166.4$ MPa)

salts	a	b	c
NaCl	10.792	61.273	0.532
KCl	18.841	70.541	−0.096
$NaClO_4$	88.114	−1.565	0.011
KCNS	90.923	−1.734	0.011
CF_3COONa	86.464	−1.516	0.007

The term internal pressure for pure solvents was initially proposed by Hildebrand and Scott (1962) and subsequently supported by the equation of state (Ouellette & Williams, 1971). The internal pressure of a salt solution has been found to be an invaluable tool in unraveling one of the possible origins of the forces responsible for the reaction of diene with dienophile, i.e., the Diels−Alder reaction in aqueous and nonaqueous ionic media (Kumar, 1994a,b), and in understanding the thermodynamic and transport properties of aqueous ionic media (Millero, 1971; Leyendekkers, 1983). These P_i values can be reliably estimated from the thermal expansion coefficient, a, and the isothermal compressibility, β, at a given temperature T using the relationship $P_i = (aT/\beta)$. Both a and β are obtained from the experimental volumetric properties of a system.

In addition to internal pressure, the quantity useful in the present analysis of thermal stability of DNA duplex is the volume change, ΔV, accompanying the transition process. It is well-established that the DNA is heavily hydrated and consists of two discrete layers representing primary and secondary hydration shells. In fact, the bases in single strands tend to form complementary hydrogen bonding leading to the formation of hydrophobic core in the DNA duplex. The phosphate groups lie exposed to water and impart considerable charge density to single strands and to their duplex. The magnitude and sign of ΔV depend upon the base stacking, the degree of hydration, the charge density parameters of both single strands and duplex, and the electrostriction of water molecules (Falk et al., 1962; Chapman & Sturtevant, 1969; Shakked et al., 1989; Zieba et al., 1991; Chalikian et al., 1994).

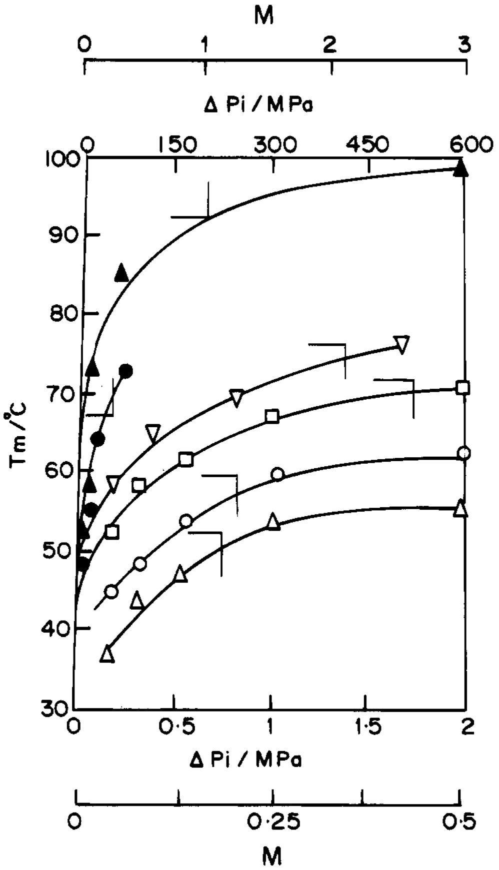

FIGURE 1: Relationship between transition temperature, T_m, and effective internal pressure, ΔP_i, for the double helix transition. ▲, poly(dA)•poly(dT); ●, poly[d(A−T)] data (Wu & Macgregor, 1993); ▽, poly(dA)•poly(dT); □, poly(rA)•poly(dT); ○, poly(rA)•poly(rU); △, poly(rA)•poly(dU) data (Rentzeperis et al., 1993). First data set up to high NaCl concentration, second set in low NaCl range; corresponding salt concentrations are also shown.

Both the P_i and ΔV parameters can now be utilized to examine the issue of thermal stability of a DNA duplex in different situations. It has been established that the thermal stability of the duplex is greatly influenced by the salt concentrations [for example, see Record et al. (1978)]. For instance, higher concentrations of NaCl induce greater stabilization of the duplex indicating higher T_m, as compared to lower T_m noted at low concentrations of salt. Our calculations of P_i using the most reliable density and compressibility data (Lo surdo et al., 1982; Hershey et al., 1984) show that P_i of water increases upon the addition of NaCl. Such a variation in P_i can be indicated by the effective internal pressure ΔP_i, as $\Delta P_i = P_i - P_{i,w}$; where $P_{i,w}$ is internal pressure of pure water. The term ΔP_i denotes the change in internal pressure of water occurring upon addition of a salt. The P_i values as a function of salt concentration are represented in the form of a polynomial expression given in Table 1 taken together with relevant parameters. The aqueous ionic environment, thus, remains under high pressure conditions, which are enhanced almost up to the solubility limit of NaCl in water. The volume changes accompanying the transition of a duplex to single strands are reported to be positive. The possible reasons for the positive ΔV values and their variations with the salt concentrations, observed in the above transition of a duplex can be understood in terms of electrostatic and stacking effects (Spiro et al., 1968; Williams & Epand, 1986; Wu & MacGregor, 1993). For example, in the case of poly(dA)•poly(dT), the ΔV values vary from 2.60 to 7.81 $cm^3\ mol^{-1}$ in the range of NaCl concentration from 0.02 to 1 M (Wu & MacGregor, 1993). As noted above, the ΔP_i at 1 M is much higher than that at 0.02 M. This high internal pressure, when coupled with positive ΔV parameter, suppresses the process of transition of a DNA duplex to single strands. Thus, in order to obtain transitions at a higher NaCl concentration, a higher temperature needs to be employed. This leads to higher T_m values with increasing NaCl concentrations. In other words, the ES or the enhanced ΔP_i resulting from the salt concentrations controls the T_m of a duplex DNA. Such observations are shown in Figure 1, where the relationship for the transitions of six duplexes of poly(dA)•poly(dT) or the polymers containing A•U base pairs chosen for illustration is demonstrated between T_m and ΔP_i at several NaCl concentrations, ranging from low to high, at atmospheric pressure (Wu & MacGregor, 1993; Rentzeperis et al., 1993). The plots of T_m versus ΔP_i for the melting of natural duplexes from *Escherichia coli* and *Diplococcus pneumoniae* with high G•C content in KCl solutions are presented in Figure 2. The shape of the curves is a typical temperature−pressure plot encountered in transition reaction kinetics. In the case of higher valent salts like $MgCl_2$, higher values of ΔP_i are obtained as

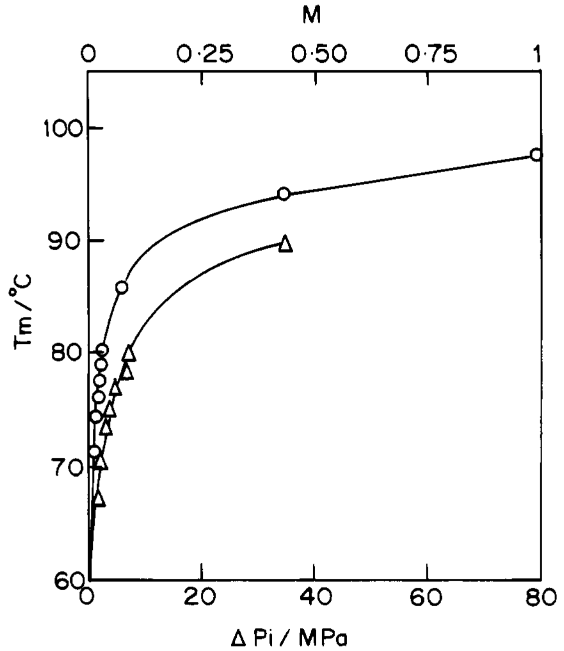

FIGURE 2: Demonstration of relationship between T_m and ΔP_i for the denaturation of *E. coli* (O) and *D. pneumoniae* (△) with high G•C contents in KCl; corresponding concentrations are given (Marmur & Doty 1962).

compared to those for NaCl at corresponding concentrations. In general, values of ΔP_i for $MgCl_2$ are almost three times as high as those for NaCl up to 4 M, as calculated from literature data (Lo surdo et al., 1982; Hershey et al., 1984). Thus, almost the same stability for a given duplex can be obtained using a much lesser concentration of $MgCl_2$ as compared to a given concentration of NaCl (Thomas, 1954; Dove & Davidson, 1962; Kotin, 1963; Krakauer, 1974; Record et al., 1978). The ES effect enhances when one moves from uni- to multivalent ionic salts (Millero, 1971), for which the thermodynamic support comes from the activity coefficient data (Lyon & Kotin, 1964).

On the other hand, the presence of certain other salts causes a decrease in T_m with increasing ionic concentration. For example, the T_m values of a duplex in presence of $NaClO_4$, KCNS and CF_3COONa at several ionic concentrations are reported to be decreasing with increasing concentration (Hamaguchi & Geiduschek, 1962). This can also be explained using the concept of negative changes in ΔP_i upon addition of salts. For calculating ΔP_i induced by the presence of these salts, we used experimental data (Lobo & Quaresma 1985) and the scaled particle theory for molecules of different sizes (Lebowitz et al., 1965; Kumar, 1993), which yielded reliable a and β values. The ΔP_i values for a few salts discussed herein are given in Table 1. The plots of T_m versus ΔP_i caused by the above salts are given in Figure 3. These plots offer interesting correlation in two respects: (i) the ΔP_i values are negative and (ii) the curves are smooth up to high concentrations of salts. An examination of Figure 3 reveals that the proposed correlation is applicable even to a situation where the T_m decreases by 60 °C. The ΔP_i values for CF_3COONa demonstrate the salt's capability as the most powerful denaturant out of the three studied. In such cases, the negative ΔP_i values are the results of the antielectrostriction effect caused by salts as indicated above. In other words, the difference between molar volume of a liquid salt and its partial molar volume tends to become negative. One may think of this situation as a result of the salting-in effect. Salts like those plotted in Figure 3 have very large anions that make the charge density smaller than that of water dipole, thus decreasing the interactions as compared to those among water molecules. This argument is supported by several theoretical calculations [for example, see Friedman (1981)]. Since the internal pressure is calculated from α and β, it is interesting to analyze their behavior for both the T_m-enhancing and the T_m-lowering salts. We note that the salts, which enhance the thermal stability with increasing concentration, are accompanied by a noticeable increase in thermal expansion and a decrease in the isothermal compressibility with the increase in salt concentrations. On the other hand, for the T_m-lowering salts, an opposite trend is observed, i.e., an increase in isothermal compressibility overpowers the thermal expansion when one moves from dilute to concentrated salt. For the salts shown in Figure 3, a situation of internal pressure drop over pure water is created due to ion–solvent interactions, which, when coupled with positive ΔV values, tends to destabilize the duplex, with the consequent lowering of T_m. This situation continues to dominate with the increasing salt concentration. Our calculations on such systems show that the internal pressure drop is also a function of anionic size. Figure 4 shows the changes in T_m with respect to ΔP_i, i.e., $\partial T_m/\partial(\Delta P_i)$ as a function of concentrations for the T_m-enhancing and -lowering salts.

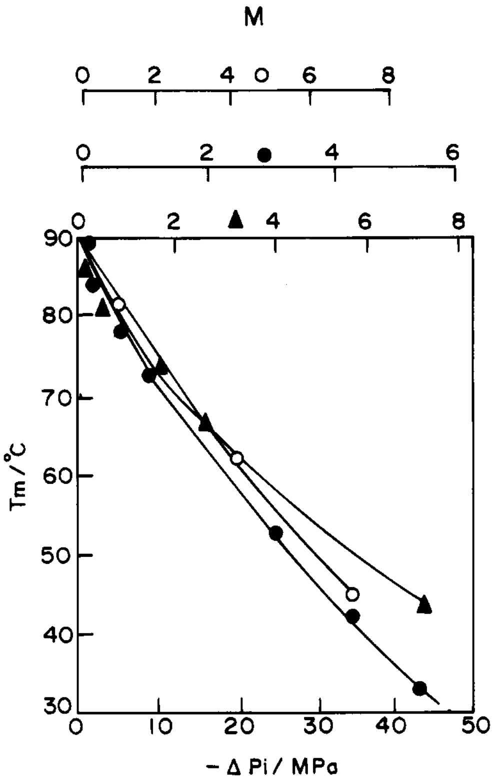

FIGURE 3: Plot of T_m against ΔP_i for denaturation of DNA duplex in presence of salts. △, $NaClO_4$; O, KCNS; ●, CF_3COONa. Data (Hamaguchi & Geiduschek, 1962).

The difference in the T_m values for different duplexes at a given NaCl concentration can be analyzed in a similar fashion. Since the ΔV values for the duplex depend upon several parameters described earlier, the effect of a given NaCl concentration or thermodynamic internal pressure can easily explain the differences in T_m values for the different duplexes. For example, the ΔV values for the transitions of poly(dA)•poly(dT) and poly[d(A-T)] at 0.2 M NaCl con-

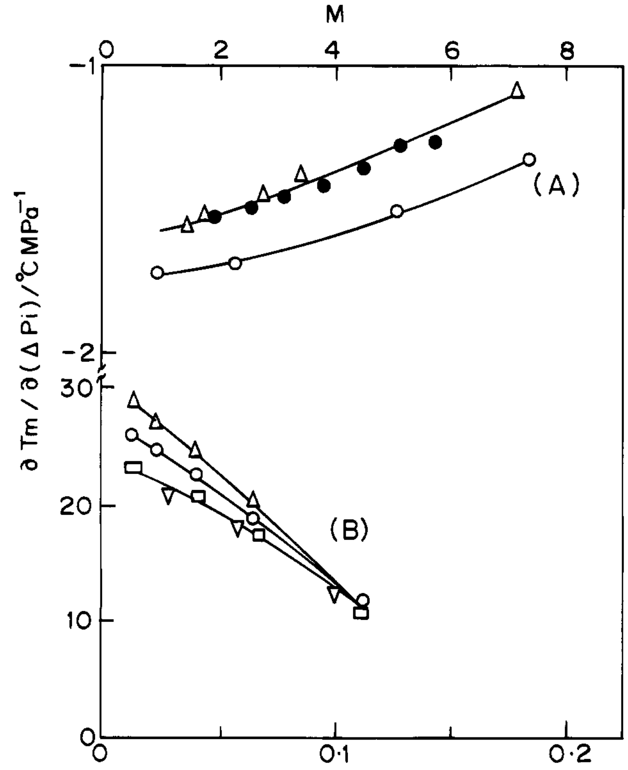

FIGURE 4: Variation of ($\partial T_m/\partial \Delta P_i$) with respect to salt concentration, M. Curve A for lowering T_m with salts; symbols are identical to those of Figure 3. Curve B for enhancing effect on T_m; hollow symbols are identical to those in Figure 1.

centration are 4.59 and 2.14 cm^3 mol^{-1}, respectively (Wu & MacGregor, 1993). The internal pressure of the medium being the same (~189 MPa) at a fixed NaCl concentration (0.2 M), the transition for poly(dA)•poly(dT) would, therefore, require a higher temperature than that for poly[d(A-T)], considering the basic thermodynamic relationship between pressure and volume with respect to kinetics of the process. Thus, T_m for poly(dA)•poly(dT) is higher (73.1 °C) than that for poly[d(A-T)] (64.3 °C). Thus, the value of ΔV, an indication of hydration and other structural properties of any duplex, as described earlier, controls the variation in thermal stability at a definite internal pressure at a given NaCl concentration is available.

Though the base stacking resulting from dispersion forces and hydrophobic effects is an important factor in stabilizing the duplex, electrostatic effects also play a significant role in the stability of a DNA duplex. Interactions involved between PO_4^- ion of the DNA strands and Na^+ ions are the major source of electrostatic effects. The polyion condensation theory of these interactions between PO_4^- (polyion) and Na^+ (counterion) was developed by Manning (1969) [for review, see Manning (1979)]. Later Record et al. (1978) used the polyion condensation theory of Manning and incorporated the concept of thermodynamic binding in it for describing the effect of ions on polyelectrolytes. First, in presence of a salt, the counterion condenses on the polyion in order to reduce the axial charge density of a strand. This is followed by the second step, in which the remaining unneutralized polyions are shielded (screening effect) by an atmosphere of mobile counterions. It is not yet clear how much difference exists in counterion condensation in single strands and duplex (Zieba et al., 1991). In the present approach, internal pressure induced by the salt is a bulk solution property and acts as a driving force for the formation of a duplex from its complementary single strands based upon the volume changes accompanying duplex formation. It appears that both the condensation and screening effects are indirectly accounted for in the internal pressure term of a salt solution.

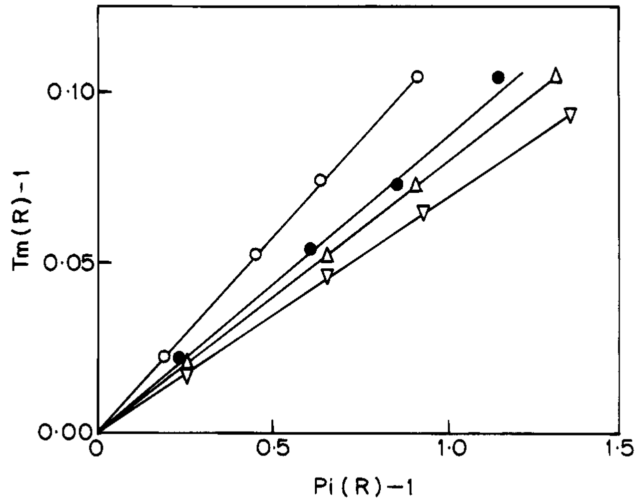

FIGURE 5: Relative transition temperature at various pressures, $T_m(R) - 1$ as a function of relative internal pressures, $P_i(R) - 1$ at various pressures in several NaCl concentrations. ○, 1 M; ●, 0.2 M; △, 500 mM; ▽, 200 mM duplex: poly(dA)•poly(dT), range of external pressure covered 200 MPa, at atmospheric pressure (0.1 MPa) $T_m(R) - 1 = P_i(R) - 1 = 0$. Points in plots are chosen to shown extent of linearity, though linearity is observed throughout. Experimental data are from Wu and Macgregor (1993), identical observations for other duplexes also.

The pressure dependence of the transition of a duplex to single strands (Wu & MacGregor, 1993) is now considered. Application of external pressure has observed to result in an increase in T_m for several duplex−single strand transitions. The calculations in the present investigation of P_i for NaCl using extensive data (Hilbert, 1979; Pitzer & Rozers, 1982) at each applied external pressure value demonstrate that P_i increases with increase of external pressure. Thus, the total internal pressure of the system at a concentration of NaCl is higher at an elevated external pressure than at atmospheric pressure. This increase in the total internal pressure, coupled with the positive volume of transition, inhibits the transition of duplex to single strands. In order to achieve the transition, therefore, a higher T_m value at an external pressure higher than the atmospheric pressure is needed. We demonstrate this by defining two quantities: $T_m(R) - 1$ [where $T_m(R)$ is the ratio of T_m at any external pressure to that at atmospheric pressure] and $P_i(R) - 1$ (with an analogous definition as used for T_m). Some valuable information emerges out of plots between $T_m(R) - 1$ and $P_i(R) - 1$ at several NaCl concentrations. For example, in the case of poly(dA)•poly(dT), for which extensive data are available, the plots $T_m(R) - 1$ versus $P_i(R) - 1$ are linear (Figure 5) at each NaCl concentration in the range from 200 mM to 1 M. The extent of linearity is seen up to 200 MPa with slopes of significant magnitude, suggesting the strong influence of internal pressure on thermal stability. Values of these slopes vary from 0.067 at 200 mM to 0.118 at 1 M NaCl, and these are in relatively satisfactory agreement with the temperature−pressure slopes reported by Wu and MacGregor (1993). Analogous plots were obtained for other duplexes also. This demonstrates that the stabilization of DNA duplexes due to an application of external pressure at a given NaCl concentration can be characterized and correlated using the concept of internal pressure.

As seen above, the ΔV values for the transition of a DNA duplex to single strands are positive. In contrast to this transition, the ΔV values for the formation of duplex from complementary strands, in general, are negative. A higher internal pressure coupled with negative ΔV values facilitates the duplex formation from two complementary strands. In some cases, however, very small positive ΔV are noted at extremely low Na^+ concentrations. The positive ΔV values at such concentrations are presumably the result of the combined effect of specific base-pairing and the low degree of electrostriction. For example, Rentzeperis et al. (1993) noted positive ΔV at 16 mM Na^+ for poly(rA)·poly(rU), which became negative at higher Na^+ concentration (116 mM). They described this variation in terms of changes in the degree of hydrations as a function of concentrations of salt.

In conclusion, in the present study the concept of change in internal pressure induced by the addition of salts is employed to explain the thermal stability of DNA duplex both at atmospheric and high external pressures. Viewed in the context of the other theories like those dealing with differential uptake of ions, polyion condensation, salt bridge, etc. (for review, see Record et al. (1978) and Manning (1979)] and their relevance to the topic of the present study, internal pressure, being a direct measure of electrostriction effect, in conjunction with volume profile of a process, can offer us a powerful tool to correlate thermal stability of the DNA duplexes, including that at high pressures. While looking at the physical etiology in sea water, which is a multicomponent ionic system, it is felt that an application of the concept might offer some reliable answers to the question how the DNA duplex could have been stabilized or destabilized. Although a limited number of data sets from the recent studies have been employed to build up and verify the proposed concept for the thermal stability of the DNA duplexes, it is believed that the approach might be useful in future for correlating large arrays of data on the thermal stability of such helices.

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