THE IMPORTANCE OF LONDON DISPERSION FORCES IN THE MAINTENANCE OF THE DEOXYRIBONUCLEIC ACID HELIX[1]

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Van der Waals interactions, per se, are generally thought to play a relatively minor role in the stabilization of native conformations of biologically important macromolecules. In the case of deoxyribonucleic acid (DNA), nevertheless, de Voe and Tinoco (1962) have suggested that this general class of interactions, and, in particular, London dispersion forces, are among the principal factors which maintain the bases in the stacked conformation in the double stranded helix.

This conclusion, however, has been more or less ignored in discussions of the denaturing action of a wide variety of organic molecules on the Watson-Crick structure of DNA. It has been proposed that this conformation is stabilized in aqueous solution primarily by "hydrophobic interactions" (Herskovits, 1962, 1963) as these have been defined by Kauzmann (Kauzmann, 1959). The effectiveness of organic compounds as denaturants has been attributed primarily to their replacement of or interference with the tendency of water to form ice like hydration layers around apolar groups in solution. In the presence of such perturbants, therefore, the entropic reasons for base stacking are abolished and the polynucleotide strands assume a more random disposition.

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According to this picture, the reaction

$$\text{helix} + \text{HOH} \rightleftharpoons \text{coil}\cdot\text{HOH} \qquad (1)$$

should have a negative standard enthalpy and a very large negative standard entropy (Kauzmann, 1959; Nemethy and Scheraga, 1962). Unfortunately for the hydrophobic hypothesis, studies on DNA (Bunville, et. al., 1965) as well as on simpler models (Rawitscher, et. al., 1963; Van Holde, et. al., 1965) have shown that reaction (1) goes with a positive enthalpy (ca. 8 kcal.) and a large positive entropy (20 to 30 e.u.) at 25° C. These results would seem to rule out the fact that hydrophobic interactions as described play a significant role in the stabilization of the DNA helix.

If London dispersion forces do indeed make an important contribution to the "stacking energy" of the DNA helix, there is a crude experimental test which one may apply: The London dispersion energy of interaction, E_L, between two molecules or groups, a and b, is related to their respective molar refractions, R_a and R_b, by the approximate equation (2) below (Waugh, 1954)

$$E_L \cong -\left[\frac{3}{2}\right]\left[\frac{3}{4\pi N}\right]\left[\frac{I_a I_b}{I_a + I_b}\right]\left[\frac{R_a R_b}{r_{ab}^6}\right] \qquad (2)$$

where I_a and I_b are the ionization energies of a and b, respectively, r_{ab} is the center to center distance between a and b, and N is Avogadro's number. (In principle, R is the molar refraction at infinite wavelength, but, in practice, the value at the sodium D line is sufficiently close and may be substituted.) Most of the neutral organic molecules which denature DNA (Herskovits, 1962, 1963; Levine, et. al., 1963) possess ionization potentials and average molecular radii which do not vary greatly from each other. To a first approximation, therefore, the molar refraction should be a reasonably good index of a given molecule's ability to provide a high density of dispersion centers and to act as an effective competitor for the DNA bases. As long as the entropy changes in the denaturation process are

the same for all compounds, the order of the effectiveness of a given denaturant should more or less parallel the order of its molar refraction. In this preliminary communication a report is given of such a correlation found for the existing data in the literature.

The molar refractions at the sodium D line (R_D) have been calculated for a variety of solutes and solvents which lower the T_m of DNA (Herskovits, 1962, 1963; Levine, et. al., 1963). If a given compound is liquid at room temperature, $R_D^{20\text{-}25^\circ}$ was obtained from data given in "The Handbook of Chemistry and Physics" (Weast, et. al., Ed., 1964) and in "Solvents Guide" (Marsden and Mann, Ed., 1963) using the equation

$$R_D = \left[\frac{M}{\rho^t}\right]\left[\frac{(n_D^t)^2 - 1}{(n_D^t)^2 + 2}\right] \qquad (3)$$

where M is the molecular weight, ρ^t is the density of pure liquid at temperature t (20 to 25° C) and n_D^t is the index of refraction at the sodium D line at temperature t. For those solutes which are solids at 25° C, R_D was derived by summing the bond refractions using the system of Vogel (Batsanov, 1961). The values of R_D were then compared with the efficacy of these denaturants as measured by the molar concentration required to effect 50% denaturation of the total population of DNA molecules in mixed solutions containing water. As Table I and Figure 1 show for salmon testes DNA and for T-4 bacteriophage DNA, respectively, a definite correlation is observed between these two parameters.

In Table I, the solvents found by Herskovits (1962, 1963) to denature DNA are listed in order of their increasing effectiveness. (The molar concentration of solvent required to achieve 50% denaturation was calculated from the author's reported values of the mole fractions together with the assumption that the apparent density of the organic component in solution is approximately equal to the density of the pure organic liquid.) With the

TABLE I: DENATURATION OF SALMON TESTES DNA BY ORGANIC SOLVENTS AND SOLUTES.

Compound	Denaturation Midpoint (moles/l)[a]	R_D (cc)
H_2O	-	3.7
Methanol	22.1[b]	8.2
Formamide	18.8[b]	10.6
Ethylene Glycol	16.5[b]	14.5
Dimethylsulfoxide	8.4[b]	20.1
N,N dimethylformamide	7.3[b]	20.0
Tetramethyl urea	5.1[b]	33.2
Urea	9.5[c]	13.7
Dimethyl urea	7.5[c]	23.3
Tetramethyl urea	4.8[c]	33.2

[a] The molar concentration required to effect 50% of the maximum DNA absorbance increase at 259 mμ at 25° C.

[b] Calculated from the mole fraction values given in Table II of Herskovits, 1962. Solutions contained 1 to 5 x 10^{-2} M electrolyte.

[c] Calculated from the mole fraction values given in Figure 1 of Herskovits, 1963. Solutions contained 1 x 10^{-3} M electrolyte.

exception of ethylene glycol, this order closely follows the order of increasing R_D. For comparison the value of R_D of water has been included. It is significantly lower than any of the organic solvents.

This correlation is even more striking when the data of Levine, et. al., (1963) are examined from this viewpoint (Figure 1). These authors have reported the denaturing efficiency of a wide variety of compounds in terms of the molar concentration of solute at which 50% of T-4 phage DNA is denatured in aqueous solutions at 72° C, and an ionic strength of 0.043 M. In Figure 1, these concentrations have been plotted against their molar refractions. Again the glycol family (points 5, 11, and 22) as well as inositol (point 48) depart from the general trend, but otherwise the correlation is good. The scatter about the line is very likely due to the

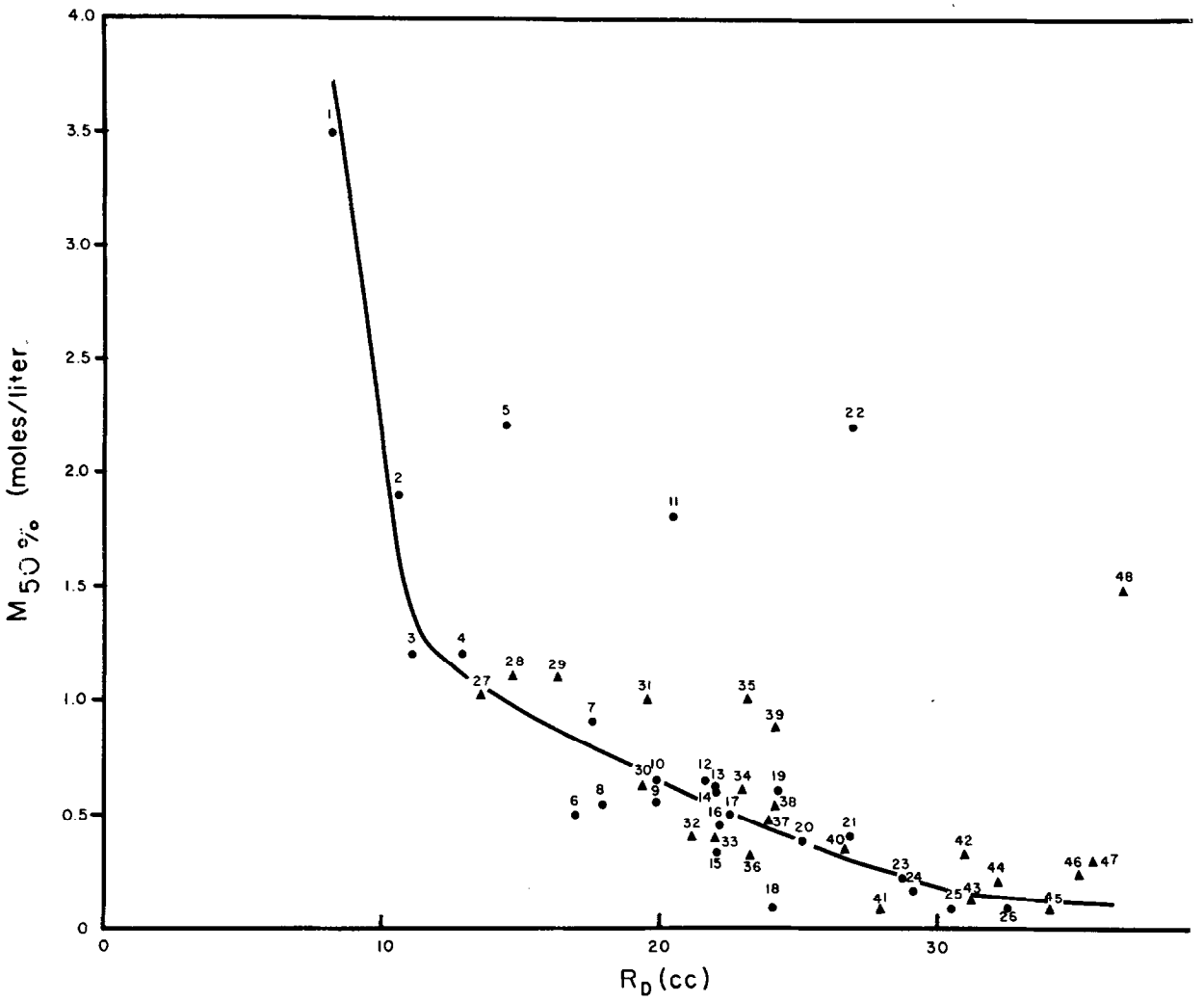

Figure 1. The Efficiency of DNA Denaturants as a Function of Their Molar Refraction: The value of the molar concentration, $M_{50\%}$, of a solute required to effect 50% denaturation of T-4 Bacteriophage DNA in aqueous solution at 73° C and an ionic strength of 0.043 (data of Levine, et. al., 1963) is plotted on the ordinate against the solute's molar refraction at the sodium D line, R_D, on the abscissa. (●) represents values of R_D calculated from equation (2) in text; (▲) represents values obtained by summing bond refractions (Batsanov, 1961). The solutes are coded as follows: (1) methanol, (2) formamide, (3) acetonitrile, (4) ethanol, (5) ethylene glycol, (6) allyl alcohol, (7) isopropyl alcohol, (8) n-propyl alcohol, (9) γ-butyrolactone, (10) N,N dimethylformamide, (11) glycerol, (12) 1,4 dioxane, (13) sec-butyl alcohol, (14) tert-butyl alcohol, (15) n-butyl alcohol, (16) isobutyl alcohol, (17) urethan, (18) pyridine, (19) N,N dimethylacetamide, (20) N-methyl urethan, (21) tert-amyl alcohol, (22) dithioglycol, (23) cyclohexyl alcohol, (24) hexanamide, (25) aniline, (26) benzyl alcohol, (27) urea, (28) acetamide, (29) glycolamide, (30) propionamide, (31) N-methylformamide, (32) 3-aminotriazole, (33) thiourea, (34) ethylurea, (35) 1,3 dimethylurea, (36) thioacetamide, (37) butyramide, (38) ethyleneurea, (39) N-ethylacetamide, (40) δ-valerolactam, (41) phenol, (42) ethylene thiourea, (43) purine, (44) tert-butyl urea, (45) p-methoxy phenol, (46) N-propyl urethan, (47) allyl thiourea, (48) inositol.

omission of the ionization energy and the distance functions. Branched molecules with larger radii will have lower energies of interaction, whereas relatively planar or aromatic molecules, whose distance of closest approach will be considerably smaller, will have higher energies. This is evidenced experimentally by the fact that the former lie, in general, above the average line (which means that they are less effective as denaturants) whereas the latter fall more or less below (and are more efficient). There may, of course, be additional minor contributions to the scatter from other factors such as charge transfer or electrostatic interactions.

The deviation of the glycol family and inositol is probably due to two factors. There is a minor contribution due to the larger molecular radii of these compounds. In addition, the "head group" effect is marked. For the alcohols, the predominant London dispersion interactions are undoubtedly between the apolar groups and the DNA bases. The polar OH groups (and even, to a certain extent, the SH groups) are no more effective than an equivalent volume of water. Thus, the effective molar refraction of these compounds is something close to $(R_{total} - nR_{OH \text{ or } SH})$ where n is the number of OH or SH groups in the molecule. In addition, the extensive hydration shell around these polar groups may prevent the close approach of the apolar portions, thus leading to a lower energy of interaction.

In contrast to the data presented in Table I and in Figure 1, no such correlation was found between $M_{50\%}$ and other physical properties (when available) such as permanent dipole moment or surface tension. The effectiveness of a given denaturant thus seems to be due primarily to its ability to furnish a high local concentration of dispersion centers (high polarizability). Water is presumably an ineffectual competitor because of its extremely low polarizability. It is unique in this regard as no other neutral solvent possesses such a small molar refraction. These results lend strong support to the conclusion that London dispersion interactions between the bases make a sizable contribution to the "stacking energy" of

the DNA helix.

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