Hydrogen-Bond Breaking by O_2 and N_2. II. Melting Curves of DNA[+]

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Evidence for hydrogen bond breaking (HBB) by O_2 and N_2 was presented in a previous communication.[1] The extension of this work to a biologically significant phenomenon, the denaturation or "melting" of DNA, will now be presented. It is found that the air and oxygen significantly reduce the temperature of the DNA melting process. The possible relationship of this HBB ability of oxygen and nitrogen to phenomena observed *in vivo* will be discussed also.

The prior evidence for a HBB effect by O_2 and N_2 consisted of shifts in the melting and glass temperatures of ethanol shifts that were caused by the addition of these gases to the ethanol. These shifts were monitored by observing the phosphorescence intensity and lifetime of dissolved phenanthrene, the temperature dependencies of which are very sensitive to the condition (*i.e.*, glass, crystal, or liquid) of the solvent medium. Since the mechanism for HBB is believed[2] to be competitive complexation, implying that the "hydrogen bond breakers" can replace the intrinsic hydrogen bond acceptors of a pure H-bonded system, additional support for the thesis was adduced from infrared spectroscopic and theoretical studies.[3,4] These studies suggested the formation of weak complexes between either O_2 or N_2 and the -OH moiety.

A new effect, one similar to those observed in this "non-biological" system [1,5], is seen in an aqueous solution of calf thymus NaDNA (Sigma Chemicals). This effect is illustrated in Figure 1. Again, the temperature

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dependence of the state of the system, as observed by an increase of absorbance, is dependent on the addition or removal of oxygen and nitrogen. In each experiment the heating rate was ~1-2°C/min. The observed curves, dubbed "melting curves", are well-known.[6] The increase of absorbance corresponds to a transition from the well-ordered helix conformation to that of the less-ordered coil and is attributable to a decrease of the interaction between neighboring base-pair aromatic rings (the chromophores). The temperature of the melting transition, as well as the slope of the melting curve, is related to the composition of the DNA (i.e., to the ratio of the two base-pair combinations, A-T and G-C, which it contains), since the two different base-pair combinations contain two (A-T) and three (G-C) H-bonds, respectively. Indeed, the A-T/G-C ratio is normally held to be determinative of both the melting point and the slope of the melting curve.[7]

The melting curves are also sensitive to solvent effects (i.e., composition of solvent, ionic strength, pH, etc.). The addition of hydrogen bond breakers lowers the melting temperature: For example, urea, a strong HBB entity, lowers[8] the transition temperature of chicken DNA (in 0.0015M NaCl) by approximately 5°C/Mole. By comparison with the urea effect, the shifts observed in Figure 1 (~10-15°C for approximately $1\text{-}2\times10^{-3}$M Air or O_2[9]) are extremely large (~3 orders of magnitude larger!) Such a large difference demands rationalization.

The DNA system is considerably more complicated than the ethanol/phenanthrene system: Hydrogen bonding occurs not only in the solvent but also between solvent and solute as well as internal to the solute. Assuming that all of these H-bonds are subject to effects by the HBB species, several factors may contribute to the great magnitude of the air/oxygen effect.

1) The small size of the O_2/N_2 molecules may allow more ready access into the H-bonded DNA solute.

2) The complex with the H-bond donor, being weak, may be broken readily, in which case the HBB species may become available for attack on other H-bonds (i.e., internal DNA bonds).

3) The non-polar gases, being an order of magnitude more soluble in aromatic solvents than in water, may tend to concentrate in the hydrophobic aromatic "backbone" of the DNA helix, thereby increasing the effects on base-pair bonding.

4) Conversely, the urea, which is soluble in water but insoluble in aromatics and which forms a strong H-bond with water, may well be unavailable for significant interaction with the base-pair H-bonds.

The effect observed in Figure 1 is of undoubted biological significance. Certainly, given the importance and ubiquity of oxygen, nucleic acids and other H-bonded macromolecules in biological systems, this supposition seems reasonable. Indeed, direct HBB ability was suggested by diPaolo and Sandorfy[10] to be the crux of the anesthetic action of fluorocarbons. In turn, Nunn et al[11] and Jackson[12] showed that these same anesthetics arrested mitosis. Since O_2 and N_2 exhibit certain anesthetic effects, it is not unreasonable to suppose that they also ought to produce biological perturbations. A demonstration of such is given in Figure 1.

A correspondence between HBB by O_2 and the effect of O_2 on cellular sensitivity to radiation damage may also exist. The primary mechanism for damage is believed[13,14] to be an enhancement

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These mutated embryos of Prosophila melangaster designated "excism repair deficient mei-9 ^{L1} - embryos", differ in an allele of the mei-9 mutants. They exhibit increased mutagenic sensitivity, reduced meiotic recombinations ability and a deficiency in the repair of nucleotides. This mutation apparently causes a block in the early stage of gene repair. See also, T. Brustad, Int. J. Radiat. Biol., 22, 443(1972).

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of free radical generation upon irradiation of oxygenated or aerated system. Recently, however, it has been suggested[15,16] that two mechanisms are involved in the damage process. This supposition was supported by the considerably greater sensitivity of repair-deficient fruit fly embryos[17] than normal fruit fly embryos[16] to irradiation under low-oxygen atmospheres. The normal embryos were equally sensitive, however, under high-oxygen atmospheres, implying that O_2 had interfered with some repair mechanism commonly present in normal embryos but lacking in mutated embryos. No mechanism for repair-inhibition was suggested but, again, the HBB ability of oxygen may contribute to this effect. The involvement of HBB in repair-inhibition might be tested by performing the embryo experiments under low-oxygen atmospheres composed or argon and also nitrogen and determining the difference, if any, of the two.

The HBB ability of oxygen and nitrogen and the complexation of these species to H-bond donors can be deemed "biologically significant" even if unrelated to effects in either meiosis or radiation sensitivity. The recent discovery of a hydrogen bond (or, more likely, a weakly bound complex) between oxygen and oxymyoglobin[18] and the shifts, as reported here, in the melting curves of DNA caused by O_2 and N_2 are sufficient, in and of themselves, to validate that contention.

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This paper was not submitted for publication due to the ineproducibility of the results. The shift cauaed by O_2 and N_2, if any appears to be smaller than the experimental error ($r \pm 2K$). The shift observed in these curves may arise from several factors - different heating rates and/or different molecular weights of DNA (chain lengths) caused by different times of sonification.

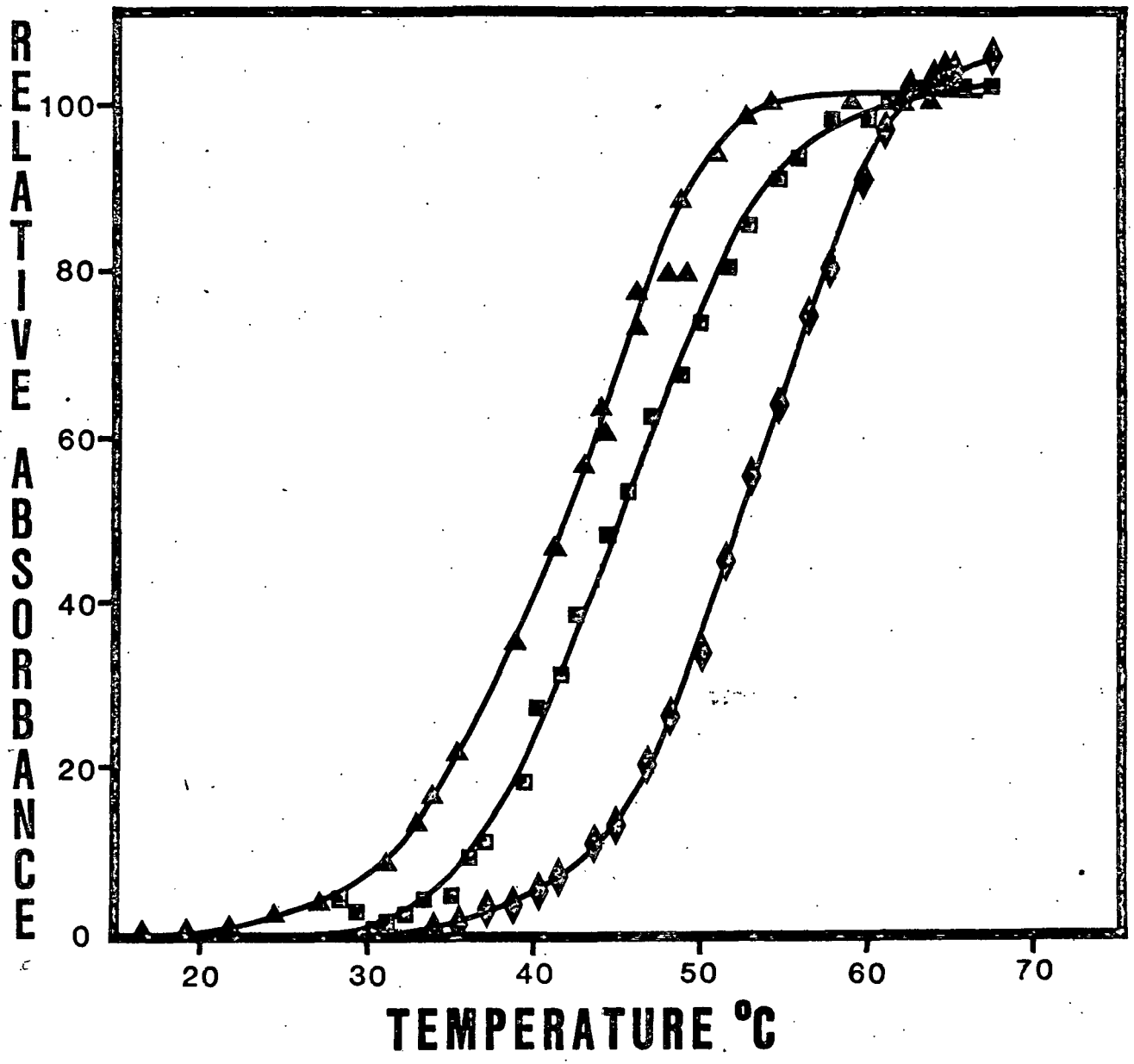

Figure 1 - Warming Curves - Absorption of Calf Thymus Na DNA as a function of temperature. Triangles - O_2 saturated; Squares - Air saturated; Diamonds - Argon saturated.

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