Accessibility of Deoxyribose Protons and Cleavage by Hydroxyl Radical

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The reagent hydroxyl radical (OH^-) attacks and cleaves the DNA phosphate/deoxyribose backbone in a largely sequence-independent manner. The usefulness of this reagent emerges from its ability to recognize structural changes in DNA. For example, adenine tracts (A-tracts) have the unique property of gradually decreasing hydroxyl radical-catalyzed cleavage within the A-tract region, in the $5'$ to $3'$ direction [5]; this has been suggested to be consistent with a narrow minor groove in the A-tract region [3].

Deuterium isotope effect studies of the DNA cleavage reaction conducted by Tullius and coworkers have shown that the C4$'$ and C5$'$ protons are the primary attack sites of hydroxyl radical; other deoxyribose protons are attacked less strongly [1]. Molecular dynamics simulations have shown that the minor groove of simulated A-tract structures is narrow [5]. This narrowness restricts access to the minor groove by small molecules (such as water and hydroxyl radical) and is thereby expected to reduce the accessible surface and inhibit cleavage at attack sites. However, we do not observe a strong pattern of surface area decrease; only slight increases and decreases at C4$'$ and C5$'$, dependent on the proton position within the dodecamer (blue panels in Figure 1). This suggests that the decreased cleavage in A-tracts is not determined by the burial of any single proton and must be a cumulative effect from small decreases over several protons.

Tullius *et al.* [1] have shown that the cumulative probability of cleaving a deoxyribose with hydroxyl radical is the sum of the individual proton probabilities P_j. They have also shown that the cleavage probability of each proton is well-correlated with the proton's surface area S_{ij}. Thus, we can compute from both experimental and computed structures the accessible proton surface area and use this quantity to weigh each proton's individual cleavage probability. The average cleavage probability C_i for each deoxyribose i and the corresponding standard deviation σ_i are calculated as:

$$C_i = \left\langle \sum_{protons\ j} P_j S_{ij} \right\rangle \tag{A.1a}$$

$$\sigma_i = \left(\left\langle \sum_{protons\ j} \sigma_{P_j}^2 S_{ij}^2 \right\rangle + \sum_{protons\ j} P_j^2 \sigma_{S_{ij}}^2 \right)^{1/2} \tag{A.1b}$$

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The value for P_j, the cleavage probability for each deoxyribose proton j, is taken from Ref. [1]. The surface area of each proton j in deoxyribose i, S_{ij}, is computed by the the Lee and Richards algorithm [4] (available in CHARMM [2]). This method roughly passes a water-molecule size probe around the solute surface to determine successive planar sections of the accessible surface area.

The A-tract dodecamer cleavage probabilities deduced from our trajectories (red panels in Figure 1) show a sinusoidal pattern. The maxima and minima are spaced six base pairs apart, approximately in phase with the DNA helical repeat. The thymine strand cleavage minima occurs near the center of the A-tract; the adenine strand minima are offset by three base pairs. The overall sinusoidal profile and the three bsae pair offset are collectively similar to experimental A-tract cleavage patterns observed by Tullius and coworkers [3].

Our calculation of the expected cleavage by hydroxyl radical at each deoxyribose of the 1D89 and 1D98 A-tract dodecamers (termed S̲ and C̲, respectively in our publication [5]) agrees well with the experimental data [3], suggesting a potential for general sequence analysis. However, the effect of the base upon the proton reactivities was not systematically examined in the deuterium isotope experiments [1]; therefore, the derived reactivities are expected to retain some component of base-specific variation. The close agreement between our calculated cleavage rate and the experimental data suggests that the different behavior of A-tracts in relation to heterogeneous sequences dominates the sequence-specific variations of deoxyribose hydrogen reactivities.

References

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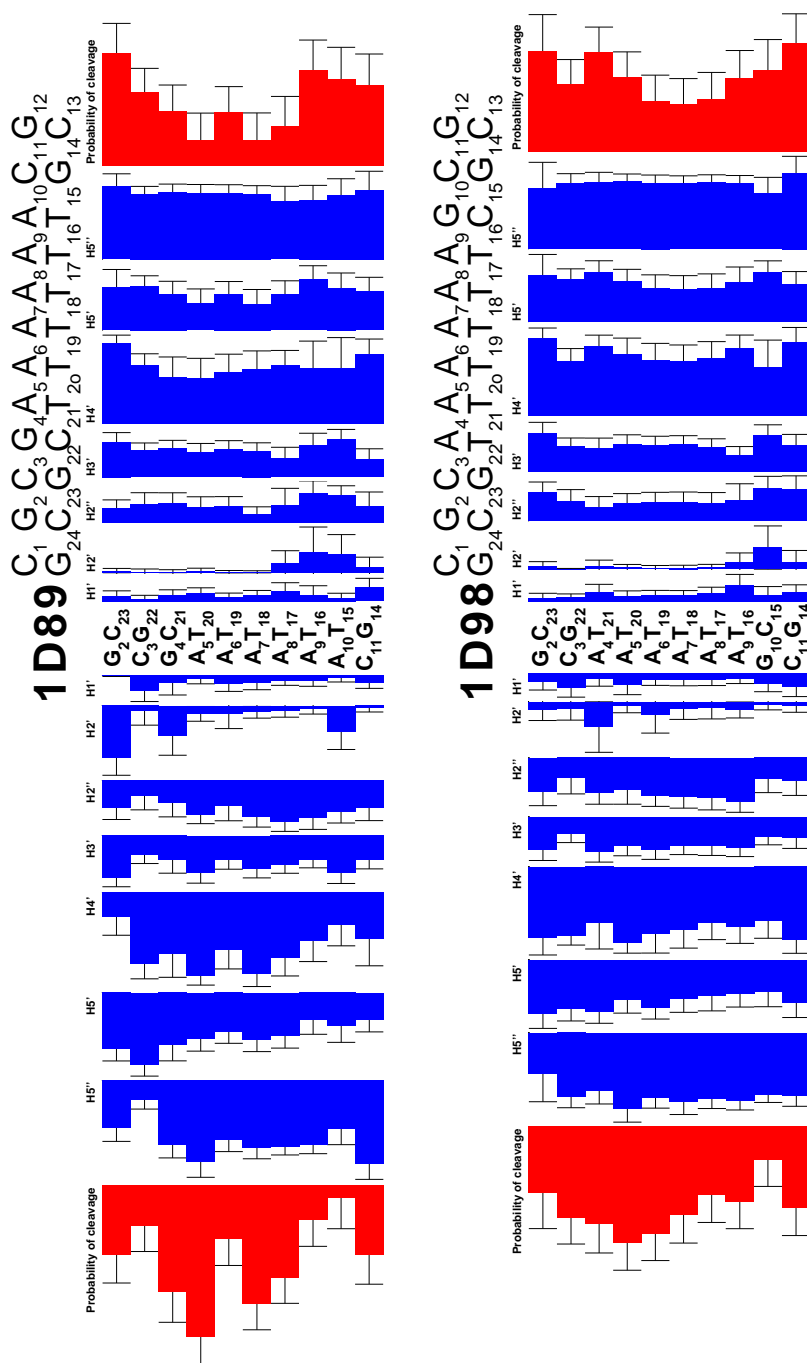
Figure 1: Deoxyribose proton surface accessibility and calculated cleavage probability for the two A-tract dodecamers (1D89 and 1D98). The sequence and numbering scheme for each dodecamer is indicated. The surface area of each proton (blue bars) was calculated at a frequency of 1 structure per picosecond using a probe with a radius of 1.4 Å [4]; one standard deviation of the surface area is indicated. Similarly displayed is the cleavage probability (red) for each deoxyribose (described in the text). The cleavage probabilities C_i are unnormalized as is generally done [3]; the proton cleavage probability P_j has not been normalized to compare with Ref. [1].