Kinetics of the proton–deuterium exchange at position H8 of adenine and guanine in DNA

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ABSTRACT

Proton-NMR has been used to determine the activation energies and pre-exponential factors for the deuterium exchange of AH8 in poly(dA-dT)·poly(dA-dT), and for GH8 in poly(dG-dC)·poly(dG-dC). No simple relationship between the kinetic parameters and molecular conformation was found. By addition of 4.5 M NaCl a transition from the B to the Z conformation was induced for poly(dG-dC)·poly(dG-dC), and an increased exchange rate was observed. The exchange rate for poly(dA-dT)·poly(dA-dT) also increased below 64°C, and a significant decrease in activation energy on addition of 4.5 M NaCl was observed.

The exchange rates at T = 55.8°C were also measured for the AH8 and GH8 in random sequence calf thymus DNA. From the difference in exchange rates, a method of preferential labeling of either the AH8 or the GH8 in high molecular weight DNA is evaluated.

INTRODUCTION

We have recently started experiments aimed at an understanding of the dynamics and orientation of the bases in oriented solid calf thymus DNA using deuterium NMR (1). By heating DNA in a D2O solution at ~65°C the adenine H8 (AH8) and the guanine H8 (GH8) exchange with deuterium. This has previously been observed for mononucleotides (2) and polynucleotides (3). Typically, the ratio of exchange rates between the GH8 and the AH8 has been found to range from 1.1 to 11.6 at T = 50°C depending on polynucleotide sequence and conformation (3). In this study, the effects of external factors that influence this ratio of exchange rates are investigated. The optimum conditions could then be found for preferential deuteration of either the adenine or the guanine bases in high molecular weight, random sequence calf thymus DNA. Selective labeling enables the studies...
of the dynamics and orientation of these bases independently.

It was decided first to study the effects of salt concentration and temperature on the exchange rates of the GH8 and AH8 in the polynucleotides poly(dG-dC)·poly(dG-dC) and poly(dA-dT)·poly(dA-dT), respectively. The poly(dG-dC)·poly(dG-dC) sample has been shown to undergo a salt-induced transition from the B-form to the Z-form (4), accompanied by an increase in the GH8 exchange rate (3). No such transition has been observed for poly(dA-dT)·poly(dA-dT), and one might therefore expect the exchange rate to change less on addition of large amounts of NaCl. The exchange rates from these two polynucleotides will then be compared with the rates in the heterogeneous calf thymus DNA sample (measured only at \( T = 55.8^\circ C \)). Proton NMR was used to monitor the exchange since it can follow the intensity changes of each of the exchanging protons in the two bases simultaneously.

**Experimental**

**Materials**

The polynucleotides were purchased from P-L Biochemicals: poly(dA-dT)·poly(dA-dT) lot # 782-38 and poly(dG-dC)·poly(dG-dC) lot # 317910. Calf thymus DNA was purchased from Worthington (lot # W33B996) and was determined to contain \( \sim 1.1\% \) protein by a modified method of Lowry (5). Each sample was dissolved in a buffer (1.0 M NaCl, 0.04 M cacodylic acid, 0.01 M EDTA, \( \text{pH} = 7.0 \)), and sonicated with a Branson sonicator (model W-350) to reduce the molecular weight. The DNA sizes after sonication were determined by electrophoresis on a vertical polyacrylamide gel. Using PBR322 (HaeIII digest) as standard, the average lengths of poly(dA-dT)·poly(dA-dT), poly(dG-dC)·poly(dG-dC) and calf thymus DNA were found to be 100±50 base pairs (\( \sim 80\% \) of material).

After sonication, ethanol was used to precipitate the DNA. The samples were finally dissolved in a \( \text{D}_2\text{O} \) buffer (0.1 M NaCl or 4.5 M NaCl, 0.004 M cacodylic acid, 0.001 M EDTA, \( \text{pD} = 7.0 \)), and kept at \( 4^\circ C \).

All samples showed normal melting behavior when heated up to 95\(^\circ C \). The lowest melting temperature was found to be 66.5\(^\circ C \).
For poly(dA-dT)-poly(dA-dT) in 0.1 M NaCl, while all other samples had melting transitions above 76°C. It was therefore considered safe to use a highest temperature of 55.8°C for the exchange studies without risking denaturation.

For the NMR measurements, the samples were put into 5 mm microcells (Wilmad 529-E) with a volume of 100 μl. The DNA concentration was ~0.9 mM in base pairs for all samples except for the calf thymus DNA sample which had a concentration of ~13 mM in base pairs.

The exchange of the AH8 and GH8 with deuterium was controlled by immersing the NMR tubes in waterbaths for fixed periods of time at the following temperatures (±0.1°C): 34.5°C, 41.3°C, 48.4°C, and 55.8°C. After removal from the waterbaths, the samples were maintained at a temperature of 4°C except during the NMR measurements (1/2 h at 22°C). A total of 18 samples were used in this study: the two polynucleotides poly(dA-dT)-poly(dA-dT) and poly(dG-dC)-poly(dG-dC) in 0.1 M NaCl and 4.5 M NaCl, incubated at the four different temperatures, and calf thymus DNA in 0.1 M NaCl and 4.5 M NaCl heated at 55.8°C. The samples which had the slowest exchange rates were monitored for a total time of ~973 hours.

NMR measurements

A JEOL GX 400 NMR instrument operating at 400 MHz was used to monitor the exchange by measuring the intensity (peak height) of the GH8 and AH8 resonances. Variations in instrumental sensitivity between measurements were corrected for by scaling individual spectra so that the intensity of a non-exchangeable proton was kept constant. In poly(G-C)-poly(G-C) the CH6 was used for this purpose, while the overlapping resonances of AH2 and TH6 were used in poly(A-T)-poly(A-T). The sum intensities of the GH1', CH1', AH1' and TH1' (at 5.8 ppm) were used similarly in the measurements of calf thymus DNA.

RESULTS

Polynucleotides

For all polynucleotide samples, first-order kinetics were assumed according to the relationship

\[ I(t) = C_0 e^{-kt} \]  

[1a]
Fig. 1. Example of a set of NMR spectra as a function of heating time for poly(dG-dC)-poly(dG-dC) in 0.1 M NaCl at T = 55.8°C. Spectra were measured at times t = 0 h, 19.7 h, 40.4 h, 61.4 h, 82.0 h, and 175.3 h. The intensity of the GH8 resonance decreases as the time of the exchange increases.

where \( C_0 \) is the amplitude, and \( k \) is the rate constant. The rate constant, \( k \) (and \( C_0 \)), was calculated from a weighted, nonlinear least squares fit of errors by comparing \( I(t) \) with the experimentally determined intensity \( I_{\text{exp}}(t_n) \):

\[
I_{\text{exp}}(t_n) = I_{\text{obs}}(t_n) - I_{\text{obs}}(\infty)
\]

[1b]

In this equation, \( I_{\text{obs}}(t_n) \) is the intensity (peak height) of the GH8 or the AH8 at time \( t_n \), and \( I_{\text{obs}}(\infty) \) is the measured intensity when all protons have been exchanged with deuterons. Figure 1
Fig. 2. Example of fits of measured intensities (points) to single exponential decays (lines) (Eq. [1]) for poly(dG-dC) poly(dG-dC). T = 55.8°C (●, ——), T = 48.4°C (○, ———), T = 41.3°C (■, ———) and T = 34.5°C (□, ———).

shows a typical set of spectra as function of heating time for poly(dG-dC) poly(dG-dC) in 0.1 M NaCl at T = 55.8°C, and examples of fit of Eq. [1] to the intensities are shown in Fig. 2 for all four different temperatures. The calculated exchange rates are tabulated in Table 1 for both polynucleotides at the four different temperatures and both salt concentrations. These data have been used to calculate the activation energy $E_a$ and the frequency factor $A$ using a weighted, linear least squares fit of $\ln k$ to the Arrhenius equation given by

$$\ln k = \ln A - \frac{E_a}{RT}$$  \[2\]

where $R$ is the gas constant, and $T$ is the absolute temperature. Fig. 3 shows the fit of the exchange rates in Table 1 to the
Table 1. Calculated exchange rates \( (k) \), activation energies \( (E_a) \) and pre-exponential factors \( (A) \).

<table>
<thead>
<tr>
<th>Sample (Exchanging proton)</th>
<th>[NaCl] (M)</th>
<th>( T^{-1}(K^{-1}) \times 10^3 )</th>
<th>( k(h^{-1}) \times 10^3 )</th>
<th>( E_a(kJ/mol) )</th>
<th>( \ln(A/h^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(dA-dT)•poly(dA-dT)</td>
<td>0.1</td>
<td>3.25 0.12±0.02</td>
<td>0.41±0.06</td>
<td>128.3±4.1</td>
<td>41.2±1.6</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>3.25 0.19±0.06</td>
<td>0.68±0.10</td>
<td>106.9±4.2</td>
<td>33.5±1.6</td>
</tr>
<tr>
<td>Poly(dG-dC)•poly(dG-dC)</td>
<td>0.1</td>
<td>3.25 1.14±0.08</td>
<td>3.04±1.17</td>
<td>109.8±2.5</td>
<td>36.2±0.9</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>3.25 3.31±0.28</td>
<td>7.05±0.65</td>
<td>106.8±4.4</td>
<td>36.0±1.65</td>
</tr>
<tr>
<td>Calf thymus DNA</td>
<td>0.1</td>
<td>3.04 6.0±0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>3.04 8.6±1.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Calf thymus DNA</td>
<td>0.1</td>
<td>3.04 13.3±1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>3.04 18.0±3.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Arrhenius equation, and the parameters \( E_a \) and \( \ln A \) thus obtained are given in Table 1 for the two polynucleotides at 0.1 M and 4.5 M NaCl.

Calf thymus DNA

The exchange rates of the AH8 and GH8 in the calf thymus DNA-samples are more difficult to obtain (see Fig. 4). Since the two resonances overlap each other and also overlap with resonances from nonexchangeable protons (adenine H2, cytosine H6 and thymine H6), a simple first-order kinetic fit is not adequate. Typically, the AH8 and GH8 resonances are found at a chemical shift \( \delta = 8.18±0.11 \) ppm and \( \delta = 7.76±0.14 \) ppm, respectively, for a variety of polynucleotides (6-11). The variation in chemical
Fig. 3. Arrhenius fits (lines) (Eq. [2]) of the calculated exchange rates (points): Poly(dG-dC)•poly(dG-dC) in 0.1 M NaCl (□, - - -); poly(dG-dC)•poly(dG-dC) in 4.5 M NaCl (■, —); poly(dA-dT)•poly(dA-dT) in 0.1 M NaCl (○, - - -); and poly(dA-dT)•poly(dA-dT) in 4.5 M NaCl (●, —). The calculated exchange rates for calf thymus DNA are also shown at T = 55.8°C: GH8 in 4.5 M NaCl (●), GH8 in 0.1 M NaCl (○), AH8 in 4.5 M NaCl (▲) and AH8 in 0.1 M NaCl (▲).

The shift for respective base depends on the surrounding bases. The fact that the chemical shift for the AH8 and GH8 resonances differ, can be used to determine their individual rates if the observed intensities are treated as a function of the chemical shift δ. For this purpose, the following relationship was used to fit the data:

\[ I(t, \delta) = C_0(\delta) e^{-k(\delta)t} + D(\delta) \]  

For \( \delta > 8.0 \) ppm, the amplitude \( C_0 \) and the rate constant \( k \) will mainly depend on the AH8 exchange, while for \( \delta < 7.7 \) ppm, \( C_0 \) and \( k \) are determined by the GH8 exchange. The term \( D(\delta) \) corresponds...
Fig. 4. NMR spectra of calf thymus DNA at $T = 55.8^\circ C$ as function of heating time; $t = 0$ h, 19.2 h, 39.8 h, 61.1 h, 80.5 h, 146.7 h, 222.7 h and 384.6 h. The intensity at 8 ppm is decreasing for increasing time.

to the contribution to the observed intensity from the non-exchangeable protons. In principle, the exponential term in Eq. [3] could be replaced by a biexponential function, corresponding to the decays of the $AH_8$ and the $GH_8$ intensities which are assumed to have different rate constants. Such an approach however, is not feasible because of the small number of measured intensities as a function of time.

The results of a least squares fit of the measured intensities with Eq. [3] are shown in Figures 5A and 5B. In these figures, the parameters $C_0(\delta)$, $D(\delta)$ and $k(\delta)$ are shown as functions of the chemical shift for the low salt form of calf thymus DNA. The exchange rate $k = (6.0\pm0.4)\cdot10^{-3}\cdot h^{-1}$ of the $AH_8$ is obtained in the range $7.9$ ppm $\leq \delta \leq 8.2$ ppm, and a rate $k = (13.3\pm1.0)\cdot10^{-3}\cdot h^{-1}$ is estimated for the $GH_8$ in the range $7.5$ ppm $\leq \delta \leq 7.7$ ppm (Fig. 5B).
Fig. 5. Calculated parameters from fits (Eq. [4]) of the NMR spectra of calf thymus DNA in 0.1 M NaCl. (A) Amplitudes of nonexchangeable protons (O), and the sum of the exchangeable GH8 and AH8 (■). The amplitudes are measured relative to the peak at 5.8 ppm (see "Experimental"). (B) Calculated exchange rates. The lines (———) and (-----) have been drawn merely as an aid for the eye.

The rates from the high-salt sample have larger uncertainties, and were estimated to \( k = (8.6 \pm 1.3) \times 10^{-3} \, \text{h}^{-1} \) for the AH8 and \( k = (18.0 \pm 3.6) \times 10^{-3} \, \text{h}^{-1} \) for the GH8.

**DISCUSSION**

**Polynucleotides**

In this study, we have investigated the effects of temperature, salt concentration and molecular conformation on the exchange rates of the AH8 in poly(dA-dT)•poly(dA-dT) and the GH8 in poly(dG-dC)•poly(dG-dC). The pH was not varied, since a range of pH = 4-11 does not seem to affect the exchange rates in DNA (12).
Fig. 6. NMR spectra of poly(dG-dC)-poly(dG-dC) in 4.5 M NaCl, T = 48.4°C. Times for the measured spectra were: t = 0 h, 34.2 h, 63.7 h, 81.8 h, 100.5 h and "infinity" (t > 140 h at T = 60°C). Observe that the decrease in intensity of the GH8 peak height as heating time is increased is accompanied by an increase in peak height of the GH1'.

The only obvious change in the molecular conformation, judged from the NMR spectra was observed for the poly(dG-dC)-poly(dG-dC) sample that changed from a B-form to a Z-form as the salt concentration was increased from 0.1 M NaCl to 4.5 M NaCl. In the Z-form, a strong dipolar coupling between the GH8 and the sugar H1' of the guanine residue (GH1') has been observed (13). In our case, this coupling resulted in a linebroadening of the GH1' resonance and a corresponding reduction of the GH1' peak height. As can be seen in Fig. 6, the peak height returns to its maximum value as the GH8 is exchanged with deuterium. (Note: these spectra have poorer signal-to-noise ratios than for the corresponding high-salt poly(dA-dT)-poly(dA-dT). This is probably caused by signal loss owing to low solubility and aggregation of the Z-conformation.)
The linebroadening can be explained as follows. When all the GH8 remains (i.e. \( t = 0 \)), the linewidth of the GH1' resonance is given by

\[
\nu_{1/2}(t=0) = \nu_{1/2}^{GH8} + \nu_{1/2}(r)
\]  

where \( \nu_{1/2}^{GH1} \) is the linewidth measured at half height of the GH1' resonance, \( \nu_{1/2}^{GH8} \) is the contribution to the GH1' linewidth from dipolar relaxation with the GH8, and \( \nu_{1/2}(r) \) contains other contributions to the linewidth.

At long times, all the GH8 have exchanged with deuterium, and the linewidth of the GH1' resonance is given by

\[
\nu_{1/2}(t=\infty) = \nu_{1/2}(r)
\]  

From this, the linewidth contribution from the GH8 can be calculated:

\[
\nu_{1/2}^{GH8} = \nu_{1/2}(t=0) - \nu_{1/2}(t=\infty)
\]

The linewidths \( \nu_{1/2}^{GH1'}(t=0) \) and \( \nu_{1/2}^{GH1'}(t=\infty) \) were estimated to 124±8 Hz and 86±4 Hz, respectively, so \( \nu_{1/2}^{GH8} = 38±9 \) Hz, corresponding to a relaxation rate \( R_2 = 119±28 \) s\(^{-1}\) for the GH8-GH1' dipolar relaxation rate. The value of this rate can be used to obtain dynamical information about the motions in Z-DNA (14). It is however out of the scope of this paper to present such an analysis.

Since the B to Z transformation causes a change in the microenvironment of the GH8, it has been proposed that steric effects are responsible for the differences in their exchange rates (3). This is expected to primarily be reflected in the frequency factor \( A \). From the data in Table 1, the more than two-fold increase in the rate constant (at \( T = 50^\circ C \)) for the exchange in Z-DNA compared to B-DNA might as well be caused by a decrease in the activation energy.

It is surprising that the poly(dA-dT)•poly(dA-dT) sample shows an even larger decrease in the activation energy accompanied by a decrease in the pre-exponential factor on addition of 4.5 M NaCl. The simultaneous change of both these
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parameters has the result that the low-salt sample would have a higher exchange rate than the high-salt sample above \( T = 64.3^\circ C \) (extrapolated crossing point), while the high-salt sample is the faster at lower temperatures (Fig. 3). It is unlikely that the decrease in the activation energy for the high-salt samples is due only to a conformational change of the DNA, because the poly(dA-dT)·poly(dA-dT) sample does not show any major conformational change on addition of NaCl (4,15,16). The AH8 and the GH8 have been proposed to exchange with an ionic intermediate in the rate determining step (12). In a highly dipolar medium or in a solvent of high ionic strength, the so-called "salt effect" might then change the rate of exchange (17). However, this effect is expected to affect the rates in both the poly(dG-dC)·poly(dG-dC) and the poly(dA-dT)·poly(dA-dT) by about the same amount. The difference of 20 kJ/mol in activation energy on addition of high salt for the poly(dA-dT)·poly(dA-dT) sample, compared to 3 kJ/mol for the poly(dG-dC)·poly(dG-dC) sample must be explained in some other way.

Since DNA is a highly charged polyelectrolyte, added salt is not only important for the structure of the DNA, but also for the DNA-D\(_2\)O interaction in the hydration shell. Additions of large amounts of NaCl is expected to reduce the water activity and to disrupt any "ordered" water structure around the DNA. Density gradient centrifugation studies (18) and theoretical calculations (19) have shown that A-T base pairs typically bind one or two water molecules more than a G-C pair. The more pronounced water hydration in the low salt poly(dA-dT)·poly(dA-dT) sample might explain the increase in activation energy needed to break up the extra stabilization of the one or two extra water molecules per base pair (20-21).

One can also attempt to rationalize the data of Table 1 in terms of the Eyring equation (for example, see ref. 22) given by

\[
k = \frac{k_B T}{h} e^{\frac{\Delta S^*}{R}} e^{\frac{-\Delta H^*}{RT}}
\]

Here \( k_B \) is the Boltzmann factor, \( h \) is Planck's constant, \( R \) is the gas constant, \( T \) is the absolute temperature, \( \Delta S^* \) is the entropy change for the activated complex and \( \Delta H^* \) is the enthalpy...
change for activation. The results from a weighted, nonlinear least squares fit of equation [5] are given in Table 2, where the activation energy $E_a$ and the change in free energy of activation, $\Delta G^\neq$ are given by

$$E_a = \Delta H^\neq + RT \quad [6]$$

$$\Delta G^\neq = \Delta H^\neq - \Delta S^\neq T \quad [7]$$

As expected, the activation energy values are identical to those found from the Arrhenius fit (Table 1). The entropy change $\Delta S^\neq$ is largest for the low-salt form of poly(dA·dT)·poly(dA·dT) which also has the largest enthalpy change. This is consistent with a model where a "protective" water shell has to be disrupted for the base catalyzed (12) activation complex to be formed. It is therefore possible that the differences in activation energy ($E_a$) and entropy change ($\Delta S^\neq$) between the poly(dA·dT)·poly(dA·dT) and poly(dG·dC)·poly(dG·dC) samples at low salt are not caused only by differences in their molecular structure, but also in the "structure" of the D$_2$O near the DNA.

From the measurement on mononucleotides Benevides et al. (2) found the activation energies to be 99.2±1.7 kJ/mol and 99.6±2.5 kJ/mol for the AH$_8$ in 5'-dAMP and GH$_8$ in 5'-dCMP, respectively. The pre-exponential factors were estimated to $0.11 \cdot 10^{15}$·h$^{-1}$ and $0.21 \cdot 10^{15}$·h$^{-1}$ for the AH$_8$ and GH$_8$, respectively. These values are smaller than for the polynucleotides studied here, and could

<table>
<thead>
<tr>
<th>Sample</th>
<th><a href="M">NaCl</a></th>
<th>$\Delta H^\neq$(kJ/mol)</th>
<th>$\Delta S^\neq$(J/K·mol)</th>
<th>$\Delta G^\neq$(kJ/mol)</th>
<th>$E_a$(kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(dA·dT)·poly(dA·dT)</td>
<td>0.1</td>
<td>126.0±5.0</td>
<td>89.7±15.5</td>
<td>97.0±10.0</td>
<td>128.7±5.0</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>106.1±8.4</td>
<td>30.8±26.1</td>
<td>96.2±16.8</td>
<td>108.8±6.4</td>
</tr>
<tr>
<td>Poly(dG·dC)·poly(dG·dC)</td>
<td>0.1</td>
<td>107.1±4.7</td>
<td>46.8±14.9</td>
<td>92.0±9.5</td>
<td>109.8±4.7</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>104.3±11.5</td>
<td>45.7±36.1</td>
<td>89.5±23.2</td>
<td>107.0±11.5</td>
</tr>
</tbody>
</table>
be caused by a difference in the stability of the C-H bond, steric factors caused by nearby bases, and possibly by hydration differences in the polynucleotides compared with the mononucleotides.

The similarity in exchange rates at a given temperature for different polynucleotides has been used as a method to point out conformational similarities between them (3). From the reported results above, it is evident that independently of the cause of the differences in $E_a$ and $A$ (Table 1), a meaningful comparison can only be made by measurements at several temperatures.

**Calf thymus DNA**

The exchange rates for calf thymus DNA at $T = 55.8^\circ C$ is also enhanced by addition of NaCl, even if the effect is less pronounced than in the alternating polynucleotides. At this temperature, the exchange rates of both the GH8 and AH8 are increased by 30-40% on addition of high salt.

In the introduction, it was suggested that a difference in exchange rates of the AH8 and GH8 might be used as a method of preferential labeling of the bases by deuteration. At present, this method is the simplest and least expensive technique to obtain large amounts (hundreds of milligrams) of labeled high molecular weight ($>10^7$) DNA. The high molecular weight is necessary for the wetspinning method used to orient the DNA (1,23).

Since there are as yet no data on the temperature dependence of the exchange rates in calf thymus DNA, the selectivity can only be calculated from the two rates at $T = 55.8^\circ C$ and 0.1 M NaCl. The exchange rate $k_G$ for the GH8 is ~71% lower than the rate of the GH8 in low-salt poly(dG-dC)•poly(dG-dC). On the other hand, the AH8 rate, $k_A$, is ~184% faster in calf thymus DNA than in poly(dA-dT)•poly(dA-dT). This unfortunately results in a relatively small ratio of the exchange rates $k_G/k_A$ ~2.22 in calf thymus DNA as compared with $k_G/k_A$ ~17.4 for the polynucleotides at $T = 34.5^\circ C$ and high salt.

The fraction of AD8 that is present in the DNA as a function of exchange time $t$ is given by

$$AD8 = 1 - e^{-k_A t}$$

[8]
where $k_A$ is the rate constant for the AH8 exchange. Similarly, the time $t_G$ that is the time necessary to exchange a fraction of the GH8 with deuterium, is given by

$$t_G = -\frac{1}{k_G} \ln (1 - GD8)$$  \[9\]

Here GD8 is the fraction of GH8 that has been exchanged.

If Eqs. [8] and [9] are combined with $t = t_G$, the ratio of number of GD8 to AD8 may be obtained as a function of the fraction of exchanged GD8 according to

$$\frac{[GD8]}{[AD8]}_{H+D} = \frac{GD8}{1 - (1-GD8) k_A/k_G}$$  \[10\]

If all the protons are first exchanged with deuterium, and then back-exchanged in $H_2O$, the ratio of the AD8 to GD8 can similarly be found according to

$$\frac{[AD8]}{[GD8]}_{D+H} = \frac{(1-k_G/k_A)}{[AD8]}$$  \[11\]

Here AD8 is the fraction remaining in the DNA, and not yet exchanged back to AH8.

In Eq. [11] it has been assumed that the exchange rate is the same for deuterium as it is for protons. At least for nucleosides (nucleotides), this seems to be true. Tomasz et al. (12) found an exchange rate $k \approx 4.0 \cdot 10^{-3} \cdot h^{-1}$ for $G^3H8$ in guanosine and a rate $k \approx 2.4 \cdot 10^{-3} \cdot h^{-1}$ for $A^3H8$ in adenosine. They studied the exchange rates of tritium for protons at $T = 37^\circ C$, while Benevides et al. (2) found similar rates in nucleotides by proton exchange for deuterium. Therefore, the mass of the nucleus does not seem to play an important role.

In Fig. 7, the two ratios described in Eqs. [10] and [11] above are shown as functions of the fraction of incorporated GD8 and AD8, respectively (for $k_G/k_A = 2.2$). It should be noted that the two ratios differ, and that it is possible to obtain a higher relative amount of labeled adenine than guanine. As expected, for a higher deuteration level, the selectivity is
Fig. 7. Expected ratios of incorporated AD8 and GD8 in calf thymus DNA for a ratio of exchange rates: $k(GH8)/k(AH8) = 2.2$. Calculated ratio of $(GD8/AD8)_{H\rightarrow D}$ by heating in $D_2O$ (Eq. [10]) as function of fraction incorporated GD8 (-----). Calculated ratio of the $(AD8/GD8)_{D\rightarrow H}$ by subsequent heating in $H_2O$ (Eq. [11]) as function of remaining fraction AD8 (-----).

worse. For example, at a level of 30% of exchanged protons, the ratios are found to be $(GD8/AD8)_{H\rightarrow D} = 2.0$ and $(AD8/GD8)_{D\rightarrow H} = 4.2$. This results in that the GD8 rich sample contains at least 8 times more labelled guanine bases than the AD8 rich sample (which instead contains 8 times more labeled adenine bases). Even better selectivity could be obtained if, for example, the AH8 in calf thymus DNA has a higher activation energy than the GH8 (as was found for poly(dA-dT)$\cdot$poly(dA-dT) compared with poly(dG-dC)$\cdot$poly(dG-dC) in low salt). For example, a ratio of $k_G/k_A = 5$ would, at a level of 30% of exchanged protons, give the following ratios: $(GD8/AD8)_{H\rightarrow D} = 4.4$ and $(AD8/GD8)_{D\rightarrow H} = 12.3$. The optimum ratio obtainable can only be found by further temperature studies on the calf thymus DNA.
This method of preferential labeling in native DNA opens up the possibility to study the dynamics (24) and orientation of the two bases separately by solid state NMR (1).

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