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SECOND SUMMER COLLEGE IN BIOPHYSICS

30 July - 7 September 1984

Dynamics of Conformational Fluctuations in DNA from Hydrogen Exchange Rate Measurements.

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LECTURE 1.

INTRODUCTION TO EXCHANGE : CHEMICAL ASPECTS

A. GENERAL BACKGROUND.

1. DNA IS A FLEXIBLE MOLECULE. Some indications :

   a NMR relaxation of ^{1}H ^{13}C ^{31}P indicates rapid motions (nsec) in backbone + bases.

   b. Time resolved decay of fluorescence anisotropy of bound dyes (ethidium) or spin labelled molecules points to nsec relaxation processes

   c. Hydrodynamics : flexing in torsion / bending exists.

2. DYNAMICS MAY MEDIATE ACCESS TO H-BONDS IN INTERIOR OF DNA AND RNA.

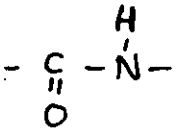
   a. RNA polymerases act as melting protein in forming open complex (initiation of transcription).

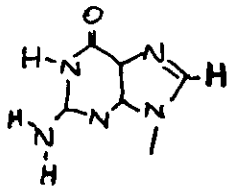
   b Nucleases may require opening of duplex to cut.

   c. Opening reactions can mediate drug binding in principle.

B. EXCHANGE REACTIONS . MONITOR ACCESSIBILITY OF INTERIOR DOMAIN IN PROTEINS + NUCLEIC ACIDS, TO SOLVENT OR IONS.

1. N-H and O-H protons occur frequently in biopolymers; they can exchange with solvent protons in general.

   a Peptide bonds : 

   b Nucleobases :  (C_8) * Some C-H are exchange [Varshavsky, Schimmel]

2. AS index of interior vs. exterior, exchange reactions are least perturbative chemically.

   a. Thus, N-H ↔ N-D / $N-{}^{3}H$ is minimal substitution

   b. 

Native structure generally retards exchange reactions

3. In nucleic acids exchange reactions detect an "open state" with novel properties.

a. Exchange in principle can occur via direct penetration of interior by solvent.

b. Alternatively, exchange requires opening = breakage of H-bonds.

c. In the last case, the state that is responsible for exchange is not necessarily denatured.

$$N \longleftrightarrow I \longleftrightarrow D$$

Native (**Intermediates**) **Denatured**

d. Exploring these issues requires rate measurements at different I, pH, ... in order to provide a means of distinguishing cases. This is not easy or straightforward!

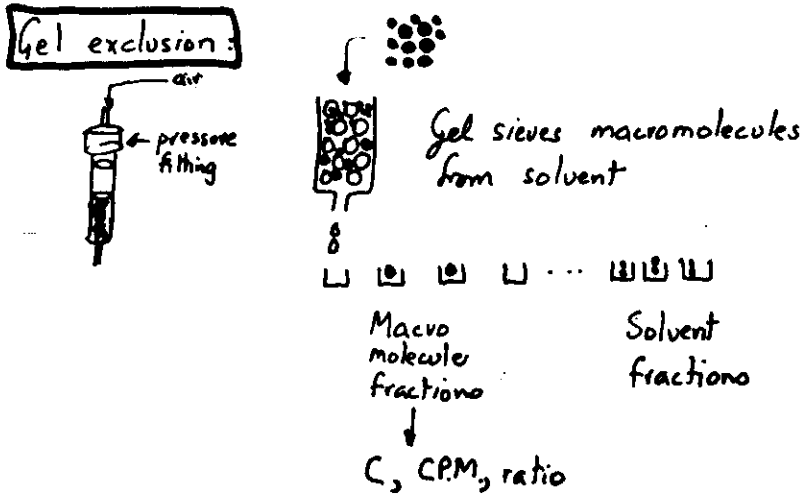
C. RATES OF HYDROGEN EXCHANGE ARE MEASURED BY ISOTOPIC SUBSTITUTION OR DIRECTLY IN NMR

1. ^3H-^1H EXCHANGE BY GEL EXCLUSION OR ABSORBION CHROMATOGRAPHY.

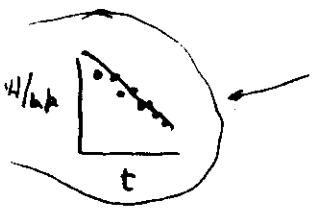
a. Principle: (1) Molecule is equilibrated in ^3H_2O labelled H_2O (radioactive!)
(2) At time t=0, labelled molecule is separated from solvent.
(3) At time t (>10"), macromolecule & separated from solvent is counted (→CPM) and Concentration determined (e.g. IR, A_{260}, P, N)
(4) Profile of H / molecule (or base pair, etc) is constructed from doing this at many times t.

Each time point ↔ one column run!

b. Gel exclusion:



c. Absorption :



↓

C, CPM, ratio

d. **Drawbacks**

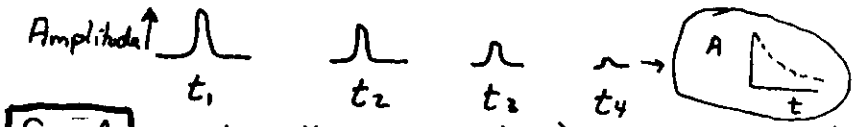
Slow > 10^4 sec.

Tedious

Not selective unless coupled with mass spectroscopy

2. ^2H - ^1H EXCHANGE BY STOPPED FLOW OR DIRECT MEASUREMENT OF $[^2H]$ as $f(t)$.

a. **Slow exchange**: t=0 dissolve or dilute sample in H_2O (D_2O) detect a proton say:

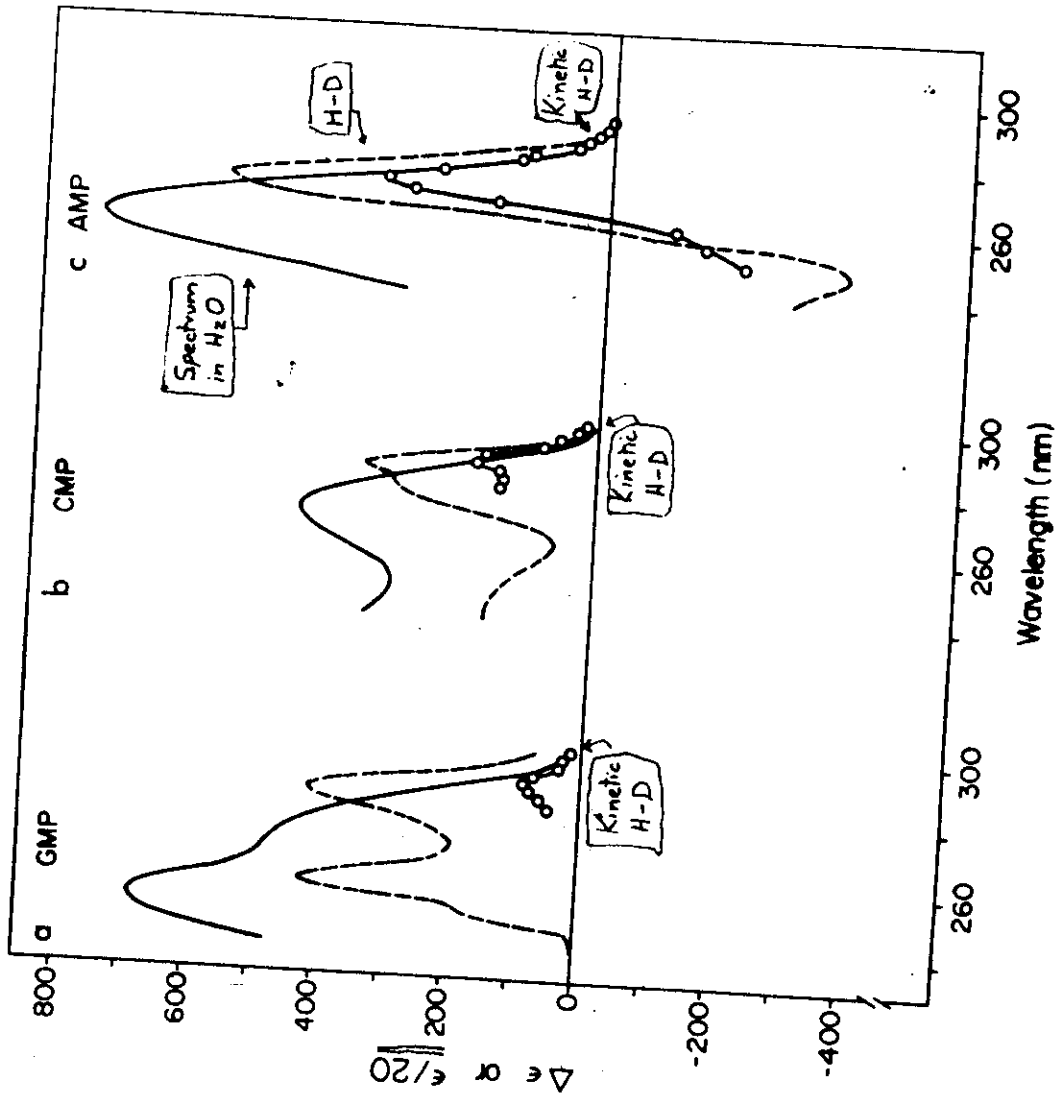


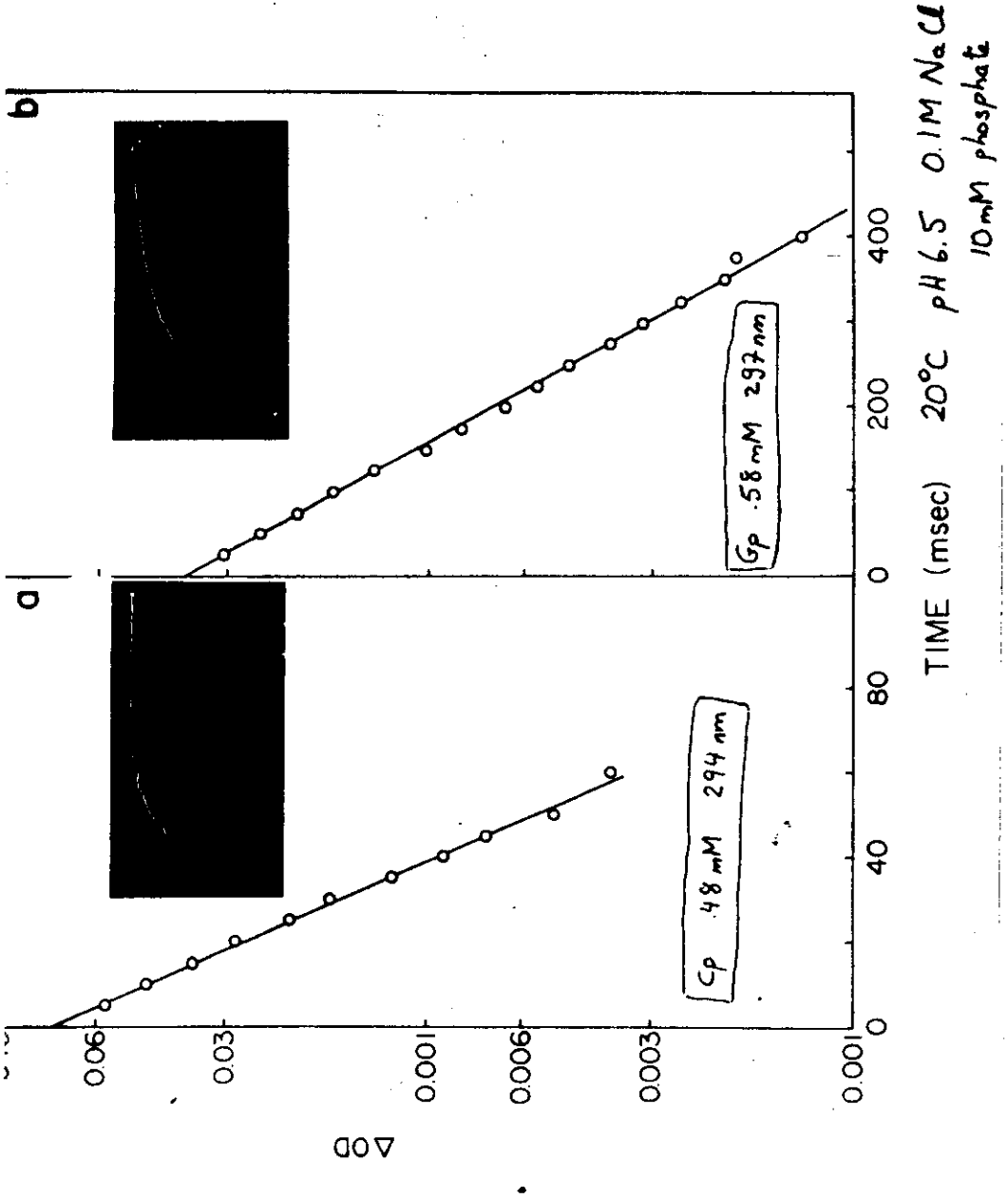
Signal can be ^1H resonance, (^{15}N), IR or Raman band. This is the basis for HX work in most proteins (BPTI)

b. Bases exchange too fast generally (not proteins!)

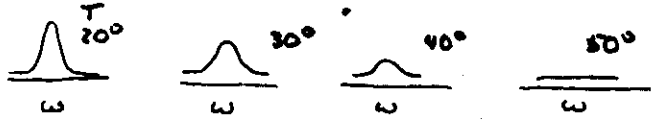
Absorbance or fluorescence of a chromophore often depends on H vs. D substitution. Zero point vibrational $\Delta E \rightarrow$ difference in electronic signal.

D. Cross (1975) reported this for adenine derivatives





2c. ^1H-^1H rates can be measured in NMR. Oligonucleotide [See Hilbert Lecture



D. PROTON TRANSFERS IN FREE BASES.

1. General $AH + B \rightleftarrows A + BH$

Mechanism (Eigen 1964):

$$AH + B \underset{k_D}{\rightleftharpoons} [AH \cdots B \overset{K}{\rightleftharpoons} A \cdots HB] \rightleftharpoons A + HB \qquad \rightarrow \Delta pK$$

a. Diffusion controlled encounter
b. H bonded encounter complex

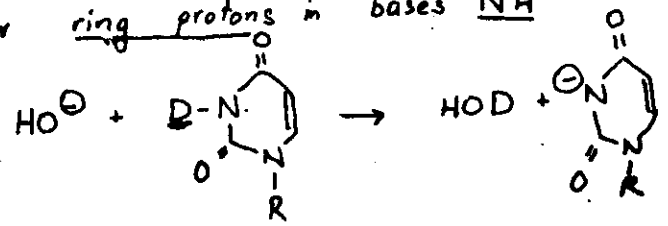
Rapid equilibrium in complex is controlled by $K = 10^{\Delta pK}$ $\Delta pK = pK_{acc} - pK_{donor}$

So if B is stronger base, every collision succeeds, rates approach k_D ($\sim 10^{10}\ M^{-1}s^{-1}$)

If not, only a fraction of encounters succeed because equilibrium in complex lies to left.

$$k_{ex} = \frac{k_D [B] K}{1+K} \quad ; \quad K = 10^{\Delta pK}$$

2. For ring protons in bases NH



$\Delta pK = pK_{OH^-} - pK_{\text{ring NH}}$

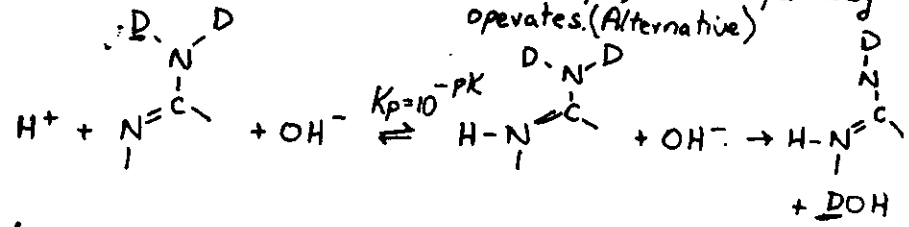
$\sim 15.7 - (9.5/10) \gg 0$

$\therefore$ Diffusion controlled.

3. NH_2 protons more complex because pK is very high (~ 19).

$K \sim 10^{-3}$

At high pH, this still works. At low pH, another pathway operates. (Alternative)



In addition, direct catalysis by H_2O itself can operate, as a third alternative.

At any pH

$\therefore$ $k_{ex} = I + II + III$

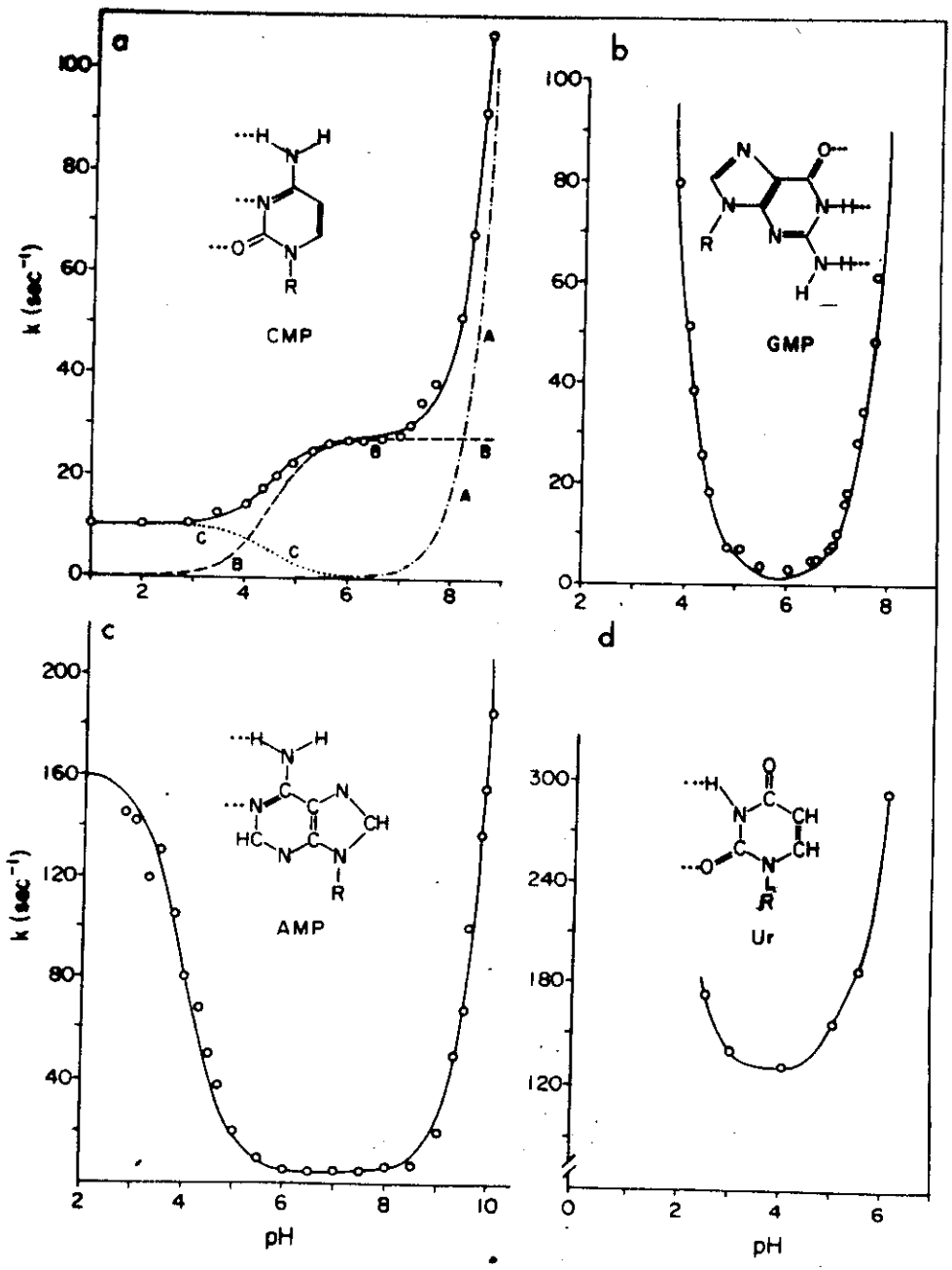
$k_I = \dfrac{k^{unprot.}(OH^-)\,K}{1+K}$

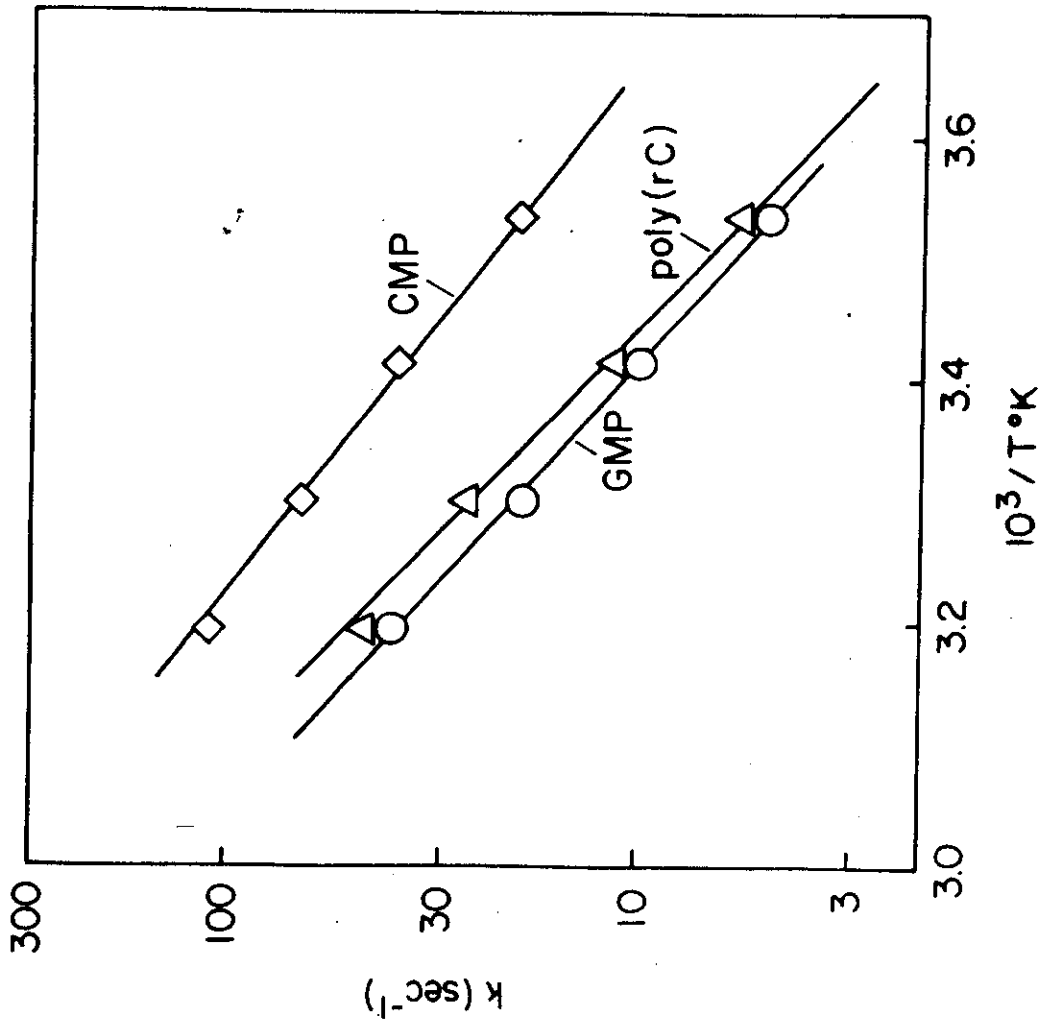
$k_{II} = k^{prot}(OH^-)\,\dfrac{(H^+)K_p}{1+(H^+)K_p}$

$k_{III} = k^{H_2O}(H_2O)\cdot\dfrac{(H^+)K_p}{1+(H^+)K_p}$

Data at different pH (next page) give:

	pK_a	$k_{OH}^{unp.}$	k_{OH}^{prot}	$k_{H_2O}^{prot}$ ($M^{-1}s^{-1}$)
C_p	4.5	1.6×10^7	28×10^{10}	.2
G_p	2.4	9×10^7	–	40
A_p	4.0	$.9\times10^7$	19.8×10^{10}	3





4. OTHER BASES THAN OH^- ?

a. Ring protons sensitive to concentration of base added (Imidazole Tris etc). Effect $\propto pK$.

b. Amino protons respond to concentration of catalyst acid form:

$$\therefore k_{ex} = k_{base}(Base)\frac{(H^+)K_P}{1+(H^+)K_P}$$

(small)

Above pH 6:

$$\approx k_{base}(Base)\frac{(H^+)K_P}{(BH^+)K_a}$$

$$k_{ex} = k_{base}K_P K_a [BH^+]$$

$$= (\text{constant}) =$$

E. Examples of exchange data from nucleic acid polymers: DNA, tRNA, rA·rU rI·rC. (Data from ^3H-^1H column method) 0°C slow reaction

1. DNA: several rates
2. tRNA: folding in Mg^{2+} produces new H-bonds.
   NMR: ring N-H downfield / amino's nearer H_2O, aromatic
   ∴ Harder to resolve.
3. rA·rU: Detect only 2 H/b.p. Originally these
4. rI·rC: were assigned as Watson-Crick H-bonds protons; but they turn out to be the I,A -NH

5 dG·dC: All 5 N-H show up at 0°C.

Some Points: 1. G·C → 5H/b.p. at t=0 extrapolated
2 A·U → 2H/b.p. but rAU gives 3.

∴ DNA (50% GC) → ½(5+3) = 4

3. Cloverleaf structure in tRNA alone → k100 (fmet)
More protons than this / molecule occur.
Recently applied to 5S, other species.

4. Note that exchange rate is a property characteristic of a polynucleotide. rG:rC exchanges slowly compared to dG:dC, for example.

F Structurally Limited Exchange.

Consider the model in which H-bond breakage necessarily precedes exchange.

$$k_{ex} = k_c(C)\,(\text{open})$$

$$\frac{d}{dt}(\text{open}) = k_1(\text{closed}) - k_{-1}(\text{open}) - k_c(C)(\text{open})$$

Steady state: $$(\text{open}) = \frac{k_1}{k_{-1} + k_c(C)}$$

$$\boxed{k_{ex} = \frac{k_1 k_c(C)}{k_{-1} + k_c(C)}}$$ ← Similar to Michaelis Menten eqn.

Simple limiting cases:

(I) $k_c(C) \gg k_{-1}$: transfer rate higher than structural closing

$$\boxed{k_{ex} = k_1}$$

(II) $k_c(C) \ll k_{-1}$ open/close repeatedly before exchange

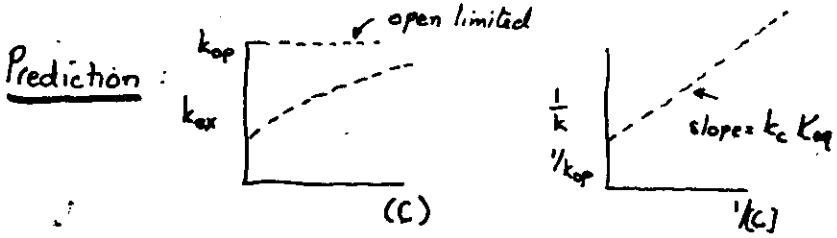
$$k_{ex} = \frac{k_1}{k_{-1}} \cdot k_c(C) = \boxed{K_{eq}\, k_c(C)}$$

opening equilibrium constant

Note Reciprocal Plot (Lineweaver):

$$\frac{1}{k_{ex}} = \frac{1}{k_{op}} + \frac{1}{k_c K_{eq}} \frac{1}{(C)}$$

Prediction:



6. Case of Poly(A)·Poly(U)

Ref: C. Mandal et al. J. Mol. Biology 135 391 (197

1. Chemistry 3H / b.p. : U N_3H
   A NH_2

Expect : 6 < pH < 8	Rate	Response to base catalyst Tris, e.g.
U N_3H	Fast	$\propto$ (B)
A N-H_2	Slow	$\propto (BH^+)$

2. Observe Two rates : Fast; Slow

Fast : no response to base
Slow : responds to base, $\propto (BH^+)$!

Conclude:
U-N_3H (?
A-NH

Detailed catalysis shows slow ⟶ fast with catalysis.

3. What is Opening Process?

a. Fluctuation must break H-bond for A N-H_2 in W.C. pair, otherwise no 'encounter complex can form.

b. Same step seems to allow U N-H to exchange; its chemistry is faster — hence it appears open-limited.

c. Under maximum catalysis A N-H_2 (both) accelerate only to F rate, not faster.

d. Open state can be defined energetically, as well as kinetic.
$\Delta H_F^+ = +15$ kcal/mole (mole opening).
$\Delta H_S^+ = +17.6$ kcal/mole
($\Delta H^+_{AMP} = 12$; $\Delta H^+_{pA} = 14$).

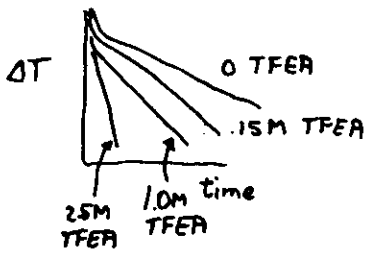
e. Calculation of K_{eq} from reciprocal plot yields $K = .05$ (polyA nf) at 20°C.

	ΔG_{25}°	ΔH_{25}°	ΔS° e.u.	
rA·rU	+1.8	+4	7	polyA
	+2.4	+6	12	Amp

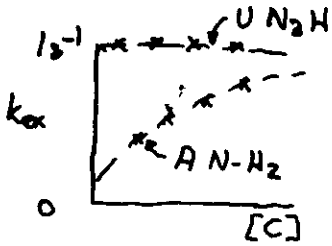
Comparison numbers:

rA·rU end base unpair	+1.1	+7.1	+20	calorimetry
rA·rU, int base loopout	+1.5-4	+6-+9	+13-17	(Fink + Crothers 1972; Lomant + Fresco 1975)

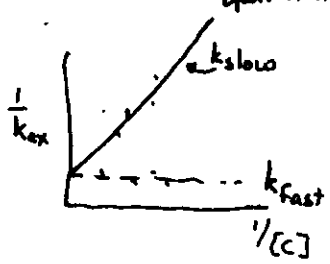
Data on poly(rA) poly(rU)

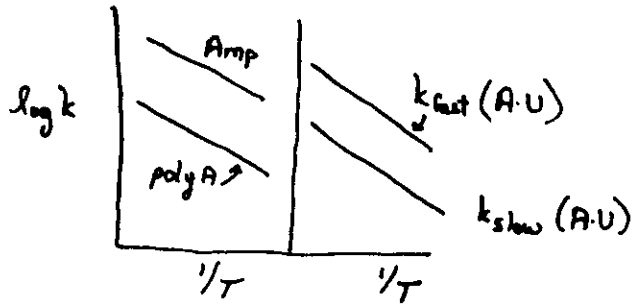


Catalysis of exchange in poly(rA)·poly(rU) by trifluoroethylamine pH 6.2 20°C .1M NaCl.



Rates obey reciprocal eqn. for open limited process:





N.B. All rates are insensitive to salt (0.01 - 1M).

TABLE I

Kinetic and Thermodynamic Parameters of Hydrogen Exchange and Base Pair Oepning[a]

Property	poly (rA):poly (rU)	poly (rI):poly (rC)
k_{ex} (fast phase), sec^{-1} at 20 C	1.1	12
k_{ex} (slow phase), sec^{-1} at 20 C	0.14	0.13
Polynucleotide standard		
K_{op} at 20°C	0.05	0.01
ΔH^c, kcal/mole	3.8	.3.7
ΔS°, cal/mole-°K	6.7	3.3
T_m, °C	290	840
Mononucleotide standard		
K_{op} at 20°C	0.02	0.004
ΔH°, kcal/mole	6.1	6.3
ΔS°, cal/mole-°K	12	2.5
T_m, °C	240	360

[a]Kinetic measruements were performed in 100mM NaCl, 10mM NaH_2PO_4, pH 7.0.

H. Does the HX open state relate to <u>equilibrium</u> premelting?

Small extent of change may be detectible spectroscopically. <u>Obvious approach</u>: follow T dependence of A_{260} CD Raman or IR bands, verify if same ΔH° as HX open state.

I. Two state analysis for ΔH°:

$$A \overset{K_{eq}}{\rightleftharpoons} B$$

At temp T

$$\ln K_{eq} = \ln \frac{(\alpha_B - \alpha)}{(\alpha - \alpha_B)} = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT}$$

α = any parameter sensitive to A/B diff.

α_A = value of α for 100% A state

α_B = " " " " " B state

$$\ln\left(\frac{\alpha_A - \alpha_i}{\alpha_i - \alpha_B}\right) = \frac{\Delta H^\circ}{R}\left(\frac{1}{T_m} - \frac{1}{T_i}\right) \qquad i = 1, \ldots, N_{obs}$$

<u>Strategy</u> Trial ΔH° T_m

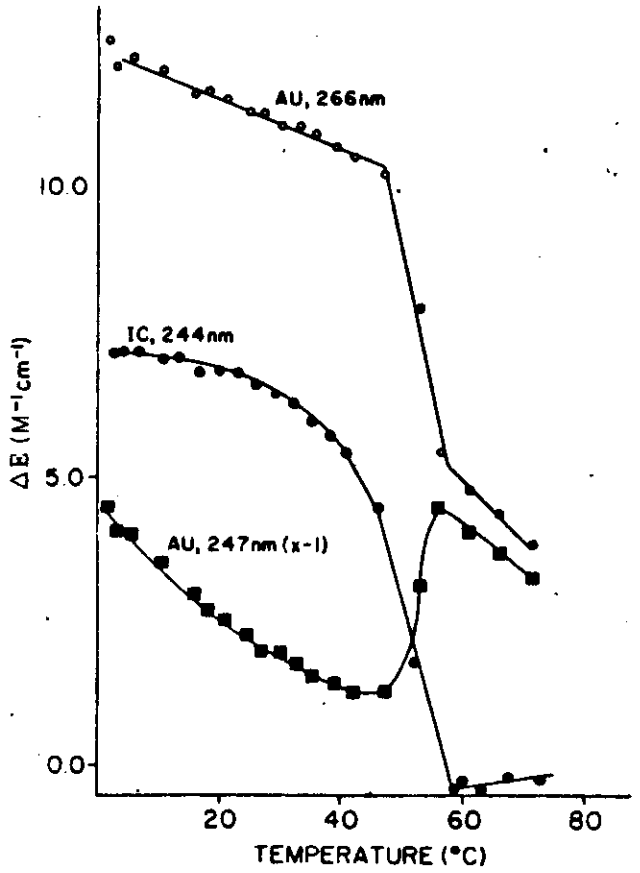
↓

Calculate α_A α_B K

↓

minimize $S = \sum_i^N (\alpha_i^{calc} - \alpha_i)$ (α_i^{calc})

Preisler Fig. 10.



Preisler Fig. 12

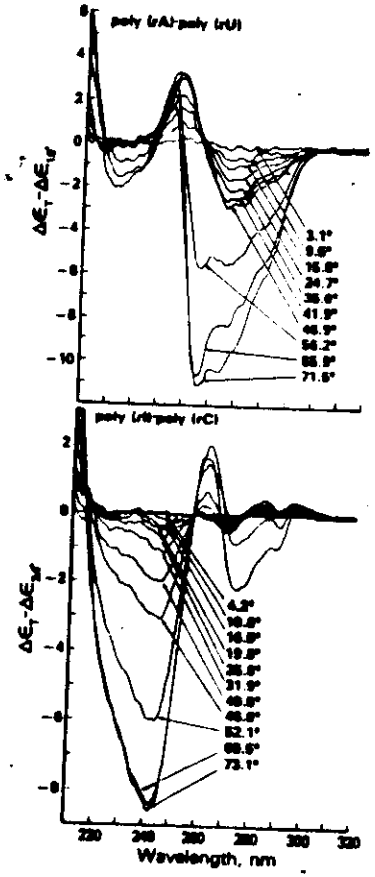


TABLE V
Calculated Enthalpies of Premelting Transitions

	Technique	Number of Data Sets	H^c, kcal/mole
poly (rA) : poly (rU)			
	HX	1	3.8[a]
			6.1[b]
			5.5[c]
	IR	5	16
			25
			22
			17
			9.4
		(Average)	18 ± 5.4
	CD	2	9.4
			6.3
		(Average)	7.9
poly (rI) : poly (rC)			
	HX	1	3.7[a]
			6.3[a]
			4.3[c]
	IR	5	39
			14
			8.9
			17
			7.0
		(Average)	17 ± 11
	CD	2	14
			14
		(Average)	14

[a]From van't Hoff plot based on equation (1); polynucleotide standard.
[b]From van't Hoff plot based on equation (1); mononucleotide standard.
[c]From program 1; polynucleotide standard.