Isotopic Hydrogen Exchange in Purine, Adenine, Adenosine, and Benzimidazole

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Summary Measurements are reported on the solvent-catalysed rates of ionisation of purine, adenine, adenosine, and benzimidazole in aqueous media at 85°.

THE acidities of carbon acids have been well studied with the result that the wide range of values ($-8 < pK_a > 40$) is at least qualitatively understood in terms of the nature of adjacent substituents. However, the acidity of ring protons in heterocyclic compounds has been less well investigated. Here we present preliminary results from a study of the rates of ionisation of such compounds of biological interest, these being precursors or base analogues of the nucleic acids.

There are several reports that isotopic hydrogen exchange in the 8-position of purines takes place under neutral conditions[1] but no quantitative data are available. The Table shows that the tritium atom in the 2-position of adenine (Ib) undergoes exchange nearly 2000 times as slowly as that in the 8-position which in turn exchanges at the same rate as that in the 8-position of purine (Ia). Substitution in the six-membered ring of the purine structure seems, therefore, to have no great influence on the acidity of the C-8 proton. The difference in rates between the 2- and 8- positions then allows one to label, selectively, compounds containing purine rings.

The tritium–hydrogen exchange for [8-^{3}H]adenine was followed by three different methods and these gave results which were self-consistent. Firstly, advantage was taken of the fact[2] that adenine forms a complex with 2-ethylhexanoic acid which is toluene soluble and can therefore be extracted into the scintillator. Secondly, the initial rate method[3] which is particularly suitable for measurement of very slow reactions was used. (Sufficient points can be obtained in 20 min to obtain a satisfactory rate constant in contrast to the detritiation procedure for which *ca.* 3 h are required.) Finally, the more conventional method was adopted where the rate of appearance of HTO was followed for more than 75% of the reaction.

The adenosine (Ic) contained tritium in the 2- and 8-positions but because of the large differences in rates of isotopic exchange from the 2- and 8-positions in adenine the rate of isotopic exchange from the 8-position alone could be determined. By comparing the total radioactivity of a solution with that of the HTO formed after *ca.* 24 h at 85° it was possible to calculate the percentage tritium in the 8-position; our value was 83 ± 3%. The tritium in the 8-position of adenosine exchanges twice as fast as in adenine in line with the fact that the ribofuranosyl group is electron withdrawing. The result for adenosine can be compared with a value of $1{\cdot}3 \times 10^{-4}$ s^{-1} for $k^{T}_{H_2O}$ at 92° for adenosine-5′-monophosphate.[4]

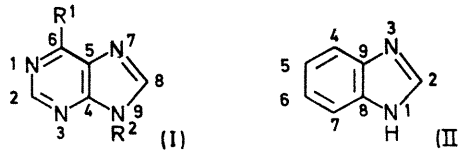
Both the solvent isotope effect and Arrhenius parameters (E = 22·3 kcal mol^{-1}, $A = 1{\cdot}3 \times 10^{9}$ s^{-1}) for [8-^{3}H]adenine are similar in magnitude to values obtained for some of the more extensively studied carbon acids of similar acid strengths.[5] The extrapolated rate constant of $5{\cdot}8 \times 10^{-8}$ s^{-1} at 25° suggests a pK_a of between 17 and 19. The reported activation energy for adenosine-5′-monophosphate is 25—26 kcal mol^{-1}.

Rates of detritiation at 85°

Compound	Position of label	Catalyst	k_T(s^{-1})	k_H/k_T[a]	k_{H_2O}/k_{D_2O}
Purine	8	H_2O	$3{\cdot}2_5 \times 10^{-5}$	3·8 ± 0·2	$1{\cdot}1_5$
	8	D_2O	$2{\cdot}8 \times 10^{-5}$		
Adenine	2	H_2O	*ca.* 5×10^{-8}		
	8	H_2O	$3{\cdot}2 \times 10^{-5}$		1·2 ± 0·08
	8	D_2O	$2{\cdot}7 \times 10^{-5}$		
Adenosine	2,8	H_2O	$6{\cdot}3 \times 10^{-5}$		
Benzimidazole	2	H_2O	$8{\cdot}1 \times 10^{-4}$		

[a] Measured in D_2O; rate of disappearance of the 8-H n.m.r. signal gave k_H; appearance of DTO gave k_T.

The primary isotope effect for purine is consistent with a rate-determining proton transfer and if our estimate of the pK_a for adenine and therefore purine is correct a relatively symmetrical transition state is involved. Consequently, and in view of the often stated importance of proton tunnelling in biochemical reactions the system seems to be worthy of further study.



a; $R^1 = R^2 = H$
b; $R^1 = NH_2, R^2 = H$
c; $R^1 = NH_2$ R^2 = β-D-ribofuranosyl

[2-^{3}H]Benzimidazole, [2-^{3}H]-(II), undergoes exchange 25 times faster than purine even though nitrogen is known to be more electronegative than carbon. Further studies

of the role of heteroatoms in stabilising anions are in progress. Such compounds may have an important role to play in the further formulation of the mechanism of proton transfer reactions in aqueous media.

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