SUBSTITUENT EFFECT OF AN *N*-AMINO GROUP ON THE H-D EXCHANGE OF RING HYDROGENS OF ADENINES COMPARED WITH AN *N*-METHYL GROUP

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Abstract–Substituent effects of an *N*-amino group on the H-D exchange of ring hydrogens of adenines were compared with those of *N*-methyl group by treating *N3*-, *N7*-, and *N9*-substituted adenines in phosphate buffered D_2O media at 70°C. *N*-Aminoadenines underwent H-D exchange in position 8 faster than the corresponding *N*-methyladenines. Rates of the exchange of *C8*-hydrogen of *N9*-substituted adenines were independent of pD of the medium in an ordinary pD range above their p*Ka*. In strongly alkaline media, *N9*-aminoadenine underwent H-D exchange at position 2, but *N9*-methyladenine did not.

Purine derivatives may undergo H-D exchange at positions 8 and 2 in neutral to alkaline media.[1-5] The H-D exchange of adenine in an ordinary pD range (in neither strongly acidic nor alkaline media) is considered to proceed in a mechanism where the protonated species of adenine undergoes deprotonation of *C8* hydrogen when attacked by OD^-. The carbanion intermediate might be stabilized by coulombic interaction between an anionic carbon and an adjacent protonated cationic nitrogen, *i.e.*, an ylide structure. Therefore, the rate is expressed by $k \cdot Kw/(Ka + [D^+])$, where *Kw* is the ionic product of D_2O and *Ka* is the acid-dissociation constant of the protonated N atom in question. Hence, in a certain pD range where *Ka* is sufficiently larger than $[D^+]$, the rate is approximated to $k \cdot Kw/Ka$, which is independent of pD of the reaction medium.[1,2] The present paper describes the substituent effect of an *N*-amino group in reference to that of an *N*-methyl group on the H-D exchange of *C8* and *C2* hydrogens of *N*-substituted adenines. The compounds examined were *N3*-, *N7*-, and *N9*-aminoadenines and the corresponding *N*-methyl derivatives. They were treated at 70°C in a phosphate buffered D_2O solvent at pD 8.26. For *p*D-dependence of the reaction rate, *N9*-substituted adenines were treated at various pD's ranging from 5.5 to 13.5. The mechanism involved will be discussed.

RESULTS AND DISCUSSION

All H-D exchange reactions studied here fit the reaction kinetics of pseudo-first order, i.e., the time-course yield of the deuterated product was linear in semi-log plots, all correlation coefficients (r^2) being better than 0.98. The observed rate constants (k_{obs}) and half-lives ($T_{1/2}$) are summarized in Table I and II. The rate was determined by measuring the areal intensities of ^{1}H-nmr signals of the proton in question of the test chemical (*ca.* 3.6 mg/ml) sealed in a tube in reference to the internal standard signal of methyl protons of potassium methanesulfonate ($CH_3SO_3^-K^+$). The chemical shift assignments for C2- and C8-protons have been established from the three-bond coupling between the protons of the methyl groups and the ring carbon atoms.[6,7] For reliability of the observed values, some experimental data are shown in Figure 1. As shown in Table I, most of the adenines examined in this study underwent H-D exchange at position 8 when they were treated at pD 8.26 at 70°C. The rate is appreciably dependent on the position where the *N*-substituent is located. In both series of *N*-amino and *N*-methyl derivatives, the fastest rates were shown by *N7*-substituted adenines, followed by *N9*-substituted ones and then by adenine which has no *N*-substituent. *N3*-Substituted derivatives underwent slowest exchange. *C8*-Hydrogen of *N3*-methyladenine did not exchange under the conditions employed in this study. When the rates for H-D exchange at position 8 were compared between *N*-amino and *N*-methyl derivatives in each pair of the positional isomers, the rates of *N*-amino derivatives were several times higher than those of the corresponding *N*-methyl derivatives in all pairs. With regard to the hydrogen at position 2, only *N3*-substituted adenines underwent H-D exchange in an ordinary pD range.

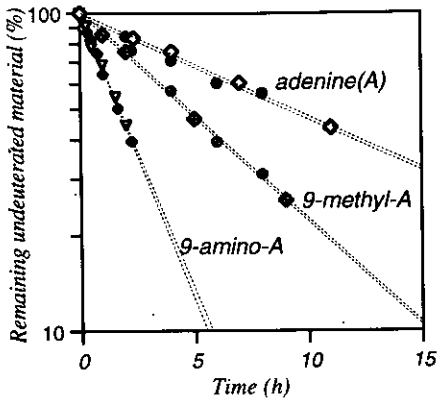


Figure 1. Some Rate-Measurement Data for 8- H's of Adenine (pD 7.23 and 9.35), 9-Methyladenine (pD 5.43 and 7.23) and 9-Aminoadenine (pD 7.23 and 9.40), correlation coefficients r^2 ranging from1.00 to 0.98

pD-Dedendence of H-D Exchange of *N9*-Substituted Adenines Dependence of the H-D exchange rate on pD of the medium was examined using *N9*-substituted adenines and adenine itself. As shown in Table II, the rate for H-D exchange of *C8*-hydrogen of *N9*-aminoadenine (p*Ka*(N1) 3.7) was more or less independent of pD in a range from pD 5 to 9, as previously evidenced for adenine (p*Ka*(N1) 4.2) and *N9*-methyladenine (p*Ka*(N1) 3.9).[2,7] The averaged values of k_{obs} (±SD) in this

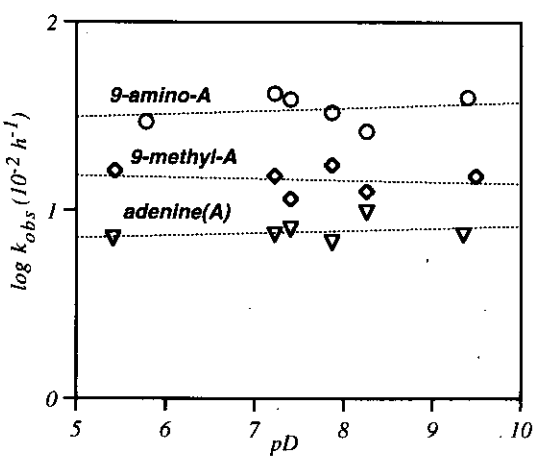


Figure 2. H-D Exchange Rate of C8-H of N9-Subatituted Adenines

Table I Pseudo-first order rate constants for H-D exchange of adenine derivatives at pD 8.26 at 70°C

	2-H		8-H	
	kobs (h^{-1})	*half-life* (h)	*kobs* (h^{-1})	*half-life* (h)
adenine	*NE**	∞	9.7×10^{-2}	7.2×10^{0}
N3-amino-	1.3×10^{-2}	5.2×10^{1}	5.0×10^{-3}	1.4×10^{2}
N3-methyl-	4.4×10^{-3}	1.6×10^{2}	*NE*	∞
N7-amino-	*NE*	∞	4.2×10^{0}	1.7×10^{-1}
N7-methyl-	*NE*	∞	1.3×10^{0}	5.5×10^{-1}
N9-amino-	*NE*	∞	2.6×10^{-1}	2.6×10^{0}
N9-methyl-	*NE*	∞	1.3×10^{-1}	5.5×10^{0}

**NE*: Not exchanged under the condition employed.

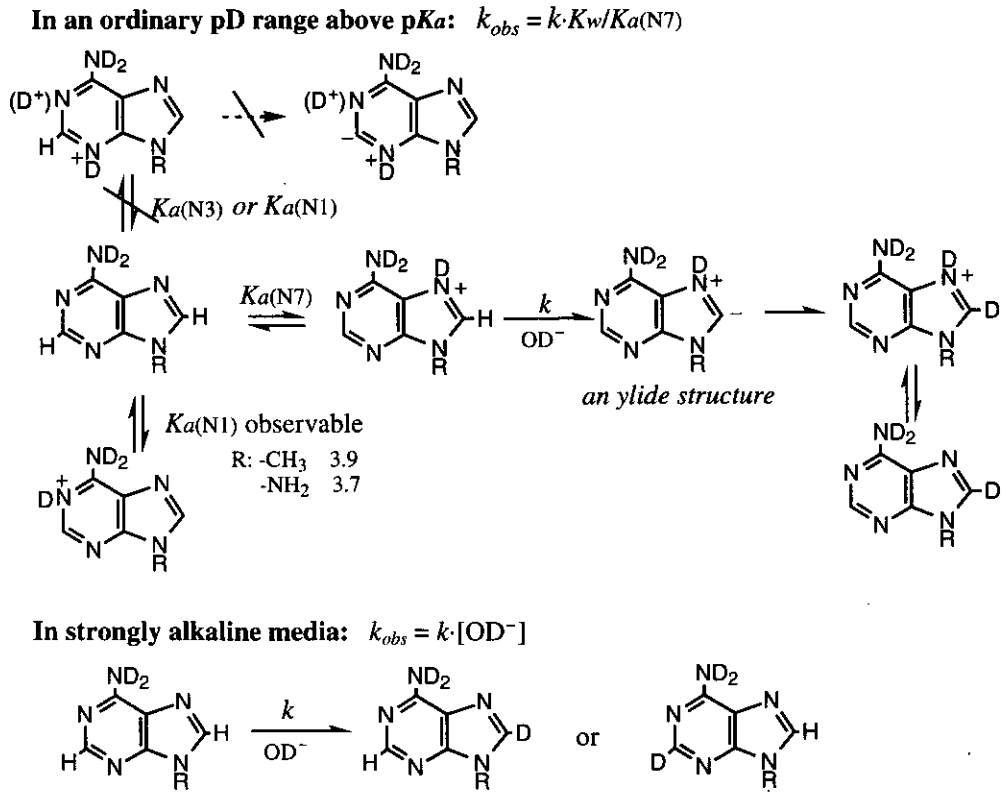
pD range were 35.0 (±5.7) x $10^{-2}h^{-1}$, 14.6 (±2.0) x $10^{-2}h^{-1}$, and 7.73 (±1.0) x $10^{-2}h^{-1}$ for *N9*-aminoadenine, *N9*-methyladenine, and adenine, respectively. *C8*-Hydrogen of *N9*-aminoadenine was more readily replaced than that of either *N9*-methyladenine or adenine in an ordinary pD range. Since p*Ka* of the N7 atom should be much less than observable *pKa* (N1) in each derivative, *Ka* (N7) values of these adenine are significantly larger than $[D^+]$ of the medium; hence the rate is expressed as $k \cdot Kw/Ka$ which is independent

Table II pD-Dependence of kobs for H-D Exchange of N9-Substituted Adenines at 70°C

***N9*-aminoadenine**							
	pD	*5.78*	*7.23*	*7.41*	*7.87*	*8.26*	*9.4*	*11.82*
2-H	*kobs* ($10^{-2}h^{-1}$)	*NE*	*NE*	*NE*	*NE*	*NE*	3.7	28
	half-life (h)	∞	∞	∞	∞	∞	*19*	*2.5*
8-H	*kobs* ($10^{-2}h^{-1}$)	30	42	39	33	26	40	63
	half-life (h)	*2.3*	*1.7*	*1.8*	*2.1*	*2.6*	*1.7*	*1.1*
***N9*-methyladenine**							
	pD	*5.43*	*7.23*	*7.41*	*7.87*	*8.26*	*9.49*	*13.01*
8-H	*kobs* ($10^{-2}h^{-1}$)	16	15	11	17	13	15	16
	half-life (h)	*4.3*	*4.6*	*6.1*	*4.0*	*5.5*	*4.6*	*4.3*
adenine							
	pD	*5.41*	*7.23*	*7.41*	*7.87*	*8.26*	*9.35*	*10.73*
8-H	*kobs* ($10^{-2}h^{-1}$)	7.0	7.5	8.0	6.7	9.7	7.5	5.0
	half-life (h)	*9.9*	*9.3*	*8.6*	*10.3*	*7.2*	*9.3*	*14.0*

**NE*: Not exchanged under the condition employed.

of [D^+]. Although p*Ka* (N7) values are not observable, it is assumed that *N9*-amino adenine has p*Ka*(N7) similar to that of the corresponding *N*-methyladenine, when one takes into account that the observable *pKa* (N1) values of the *N*-amino derivatives are almost the same as those of the corresponding *N*-methyl derivatives.[7] Therefore, the rate constant *k* for the ylide formation from the *N7*-protonated species (Scheme 1) must be larger in the amino derivative than in the methyl derivative; in other words, the *N*-amino group facilitates deprotonation at position 8 more readily than does the *N*-methyl group, probably due to a more electron-withdrawing effect of an amino group compared with a methyl group.[8,9] With regard to H-D exchange at position 2, *N9*-substituted adenines did not underwent H-D exchange in an ordinary pD range at all. As illustrated in Scheme 1, it is likely that the mechanism involved in ylide formation does not work, i.e., *Ka* (N3) is too large or *k* is too small for this mechanism to operate.



Scheme 1 H-D Exchange in N9-Ssubstituted Adenines in Neutral and Alkaline Media

At pD higher than 10, the rates for H-D exchange of both *C8*- and *C2*-hydrogens in *N9*-aminoadenine, but not in *N9*-methyladenine, increased abruptly, probably because another mechanism must have operated, i.e., a direct attack of OD^- to *C8*-hydrogen of non-protonated neutral molecules. Hydrogen exchange in strongly alkaline media is common for aromatic hydrogens in general, as reported especially for

heterocyclic aromatics.[3,10,11] In these alkaline media, it is apparent that an *N*-amino group may also facilitate H-D exchange to a greater extent than an *N*-methyl group like in a neutral pD range. In contrast, the rate of adenine itself at pD 10.73 was appreciably lower than those in the neutral pD range. *N9* Hydrogen of the adenine molecule is deprotonated to an anionic structure in alkaline media (*pKa* (N9) 9.8) to interfere with the carbanion formation as already reported.[2]

EXPERIMENTALS

Materials All *N3*- and *N7*-aminoadenines were synthesized as we previously reported.[7] *N9*-Aminoadenine[12] and the corresponding *N*-methyladenines[13] were prepared by known methods. Heavy water (D_2O) of 99.8% isotope purity was purchased from Merck and Co. Inc. (Rahway, NJ).

Preparation of buffered D_2O solvents

The D_2O buffers at a pD range of 5 to 10 were prepared by mixing appropriate volumes of 1/10 M KH_2PO_4 and 1/10 M Na_2HPO_4 (50 ml in total). The mixture was lyophilized, dissolved in a small amount of D_2O, and lyophilized again. After repeating the dissolution/lyophilization procedure one more time, the residual solid was redissolved in 50 ml D_2O. Reaction solvents at pD above 10 were prepared by diluting NaOD in D_2O. The buffers thus prepared were subjected to pD measurement using a regular *pH* meter and calibrated with the read on the *pH*-meter as follows:

pD (in D_2O) = pH (read on pH-meter) + 0.4.[14]

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