Reactivity of Nucleosides Toward Hydroxyl Radicals in Aqueous Solution

H. SHINOHARA, T. MASUDA and M. KONDO

Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, Setagaya, Tokyo 158, Japan
(Received May 31, 1976; Revised version received June 29, 1976)

Rate constants for reactions of hydroxyl radicals with nucleosides and related compounds were determined by a p-nitrosodimethylaniline method. The obtained rate constants of nucleosides were found to be generally smaller than the sum of those for free bases and ribose, of which the nucleosides are composed. An attempt was made to explain the reduced reactivity of nucleoside in terms of steric hindrance of reaction sites due to other part of the same molecule. The steric hindrance was estimated by solid angle admitted to the radical attack. The observed reactivity was found to be well interpreted by the calculated reactivity according to the following equation,

\[ k_{\text{nucleoside}} = f_1 k_{\text{base}} + f_2 k_{\text{ribose}} \]

where \( f_1 \) and \( f_2 \) are steric factors estimated from the solid angles.

INTRODUCTION

Reactivity of nucleic acid bases toward hydroxyl radicals has been studied from the view-point of fundamental understanding of radiation-induced damage in nucleic acids. In the succeeding investigation, nucleosides were chosen as the subject compounds which were expected to give the information as to an effect of ribose moiety on reactivity toward hydroxyl radicals. In the reaction of hydroxyl radicals with a nucleoside, hydroxyl radicals are expected to react not only with base moiety but also ribose moiety, since ESR study suggested a radical intermediate in which a hydrogen atom was detached from C-5 of ribose moiety and furthermore our determination of rate constant gave \( 1.4 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1} \), a comparable value with that for bases, for the reaction between ribose and hydroxyl radicals. However, the determined rate constant of a nucleoside is generally smaller than the sum of those for free base and free ribose, of which the nucleoside is composed. The decrease in reactivity may be explained by the following reasons; change in frequency of encounter, change in electronic structure which affects the reactivity toward hydroxyl radicals and/or steric hindrance.

The frequency of encounter between a hydroxyl radical and a nucleoside molecule is expected to be almost the same as that for base, because the diffusion constants...
of nucleoside were reported to be as large as those of bases and the diffusion constant of hydroxyl radical, which is approximately two times greater than that for bases, governs the frequency of encounter predominantly. Furthermore, enlarged reaction radius of nucleoside increases the frequency of encounter.

Electronic structure will be affected to some extent by formation of bond between N-1 of pyrimidine base (or N-9 of purine base) and C-1 of ribose, but such a change in electronic structure may not cause a significant decrease in the reactivity, because the rate of addition of hydroxyl radical on pyrimidine (or purine) ring depends on the state of \( \pi \)-electron which is hardly affected by the bond formation and also C-5'-H of ribose moiety, another reaction site, is remote from the bond between base and ribose.

In the present investigation, an attempt was made to explain the reduced reactivity of nucleosides in terms of steric hindrance which decreases the collision frequency between hydroxyl radical and the reaction sites of nucleoside. For estimation of degree of steric hindrance, calculation of solid angle, which represents the three-dimensional range admitted to the radical attack to each reaction site, was carried out. The obtained solid angles were found to interpret well the decrease in reactivity toward hydroxyl radicals.

MATERIALS AND METHOD

Adenosine, uridine, thymine and d-ribose were obtained from Wako Pure Chemical Industries, Ltd. Purine riboside and cytidine were obtained from Sigma Chemical Corp. All compounds were purchased as a purest grade preparation.

Solutions for irradiation were prepared with triply distilled water and adjusted to neutral pH with sodium hydroxide when considered necessary.

Irradiation was carried out with gamma rays from \(^{137}\text{Cs}\) source at about 17°C. The dose rate was determined to be \(1.92 \times 10^6 \text{ eV g}^{-1} \text{ min}^{-1}\) by a Fricke dosimeter taking \(G(\text{Fe}^{3+})=15.5\). Rate constants for reactions of nucleosides and related compounds with hydroxyl radicals were determined by competition kinetics using p-nitrosodimethylaniline as a reference compound. The rate constant for reaction of p-nitrosodimethylaniline with hydroxyl radicals was taken to be \(1.25 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}\).

FACOM-230-45S computer was used for calculation of solid angles.

Calculation of Solid Angles

To simplify a model for calculation, it is assumed that the reaction sites of nucleosides are C-5 of base moiety and C-5'-H (or C-5'-H') of ribose moiety. Although for free base or free ribose, the steric hindrance due to the other part of the same molecule should be considered, it is reasonably assumed in the present estimation of reactivity of nucleoside that free base or ribose has a solid angle of \(4\pi\) steradian, because such steric hindrance is inevitably involved in the observed rate constants, \(k_{\text{base}}\) or \(k_{\text{ribose}}\). The solid angle for reaction site of base moiety is reduced by steric
hinderance due to ribose moiety and vice versa.

Solid angle of a circular cone which opens unsymmetrically is described as follows,
\[ \omega = \frac{s}{r^2} \]  
where \( \omega \) represents the solid angle and \( s \) is the surface area where a spherical surface, which has the center at the vertex of the circular cone and a radius \( r \), intersects the unsymmetrical cone (Fig. 1). The area \( s \) in the region of \( F \) on the spherical surface is expressed by
\[ s = \iint_D ds \]  

In practice, this calculation is carried out according to the following equation, when spherical surface is expressed by \( x = f(y, z) \) in Cartesian co-ordinates and the projection of region \( F \) on the \( y-z \) plane is region \( D \).
\[ s = \iint_D \sqrt{1 + \left( \frac{\partial x}{\partial y} \right)^2 + \left( \frac{\partial x}{\partial z} \right)^2} \ \mathrm{dy} \ \mathrm{dz} \]  

In calculation of solid angle, we use the unit spherical surface which has a radius

![Fig. 1. Schematic representation of solid angle for steric hinderance due to ribose moiety. C-5; assumed reaction site on base ring. s; surface area on sphere with radius of unit length. Atoms of ribose moiety are indicated by spheres, radii of which are equal to sum of van der Waals contact distances for hydroxyl and each atom.](image)

![Fig. 2. The decrease in solid angle around C-5 of base moiety due to an atom of ribose moiety situated on (1, 0, 0) for approach of hydroxyl radicals. The point O is the co-ordinate of C-5 atom of pyrimidine base and also the center of spherical surface (radius of unit length). The integration region D on the y-z plane, which is represented as p-p' on x-y plane, is a circle of radius r with the center on the x axis. ab=cp=r.](image)
of unit length and the center at C-5 of base or at C-5'-H of ribose. The distance $d_i$ between C-5 of base moiety (or C-5'-H of ribose moiety) and atoms of ribose moiety (or base moiety) was calculated by using the co-ordinates of nucleosides reported by Lakshminarayanan and Sasisekharan and normalized to unit length, so as to put each atom of ribose moiety (or base moiety) on the unit spherical surface centered at C-5 of base (or ribose) moiety. Therefore, $r_i$ equals to $R_i/d_i$, where $R_i$ represents van der Waals contact distance between hydroxyl radical and each atom of ribose moiety. The values of $R_i$ were calculated from van der Waals radii proposed by Pauling as follows; C=1.7Å, N=1.5Å, O=1.4Å, H=1.2Å and OH=1.6Å, respectively. By equation (1), the hindered solid angle for C-5 of base moiety due to ribose moiety is calculated from the area on the unit spherical surface occupied by a small sphere which has a radius $r_i$ and the center on the unit spherical surface (Fig. 2). The small spheres on the unit spherical surface correspond to atoms of ribose moiety through the normalization procedure as mentioned above. Therefore, the co-ordinate $(x_i, y_i, z_i)$ of the centers of the small spheres is expressed by the following relation,

$$x_i^2 + y_i^2 + z_i^2 = 1$$

Since the unit spherical surface is expressed by $x = \sqrt{1 - y^2 - z^2}$,

$$1 + \left(\frac{\partial x}{\partial y}\right)^2 + \left(\frac{\partial x}{\partial z}\right)^2 = \frac{1}{\sqrt{1 - y^2 - z^2}}$$

$D$ is a circle of radius $r$ centered at the origin, then,

$$s = \int_0^{2\pi} \int_0^r \frac{r}{\sqrt{1 - r^2}} dr d\theta = 2\pi(1 - \sqrt{1 - r^2})$$

where $\theta$ is the angle on circle $D$ between the radius vector and the axis.

Consequently, the surface area hindered by all atoms of ribose moiety is calculated by integrating the equation (3) over the region of $D$, which is obtained by projecting each circular cross section (radius $r_i$, center at $(x_i', y_i', z_i')$) to the $y$-$z$ plane (Fig. 3). Generally the circular cross section centered at $(x_i', y_i', z_i')$ becomes an ellipse, when projected to the $y$-$z$ plane, except the case where the center is found on the $x$ axis. The short axis of the ellipse equals to $rx_i'$. Thus the integration region $D$ is the superposition of these ellipses. As shown in Figure 3, the integration region $D$ for calculation of the equation (3) is not always a circle and the integration is analytically impossible. However, when the region is approximated to be a circle, the integration gives an approximate value for $s$.

As shown in Figure 4, the area $s$ composed of two circular cross section A and B is equal to the area $s'$ calculated from the region $D'$ which closely resembles a circle (center at the origin) and therefore the area $s$ is calculated conveniently by using the circle approximated to region $D'$.

For a better approximation, the region $D'$ is separated to several parts and
convex parts of the region are put between the circumscribing and inscribing circles as shown in Figure 5. The area $s$ should be found between the "small "approximate" value $s_s$ and the large, $s_l$, when $s_s$ is calculated by using $D_i$ obtained from the inscribing circle and $s_l$ by $D_i$ obtained from the circumscribing one.
RESULTS AND DISCUSSION

Second order rate constants for reactions of hydroxyl radicals with nucleosides and related compounds determined by a $p$-nitrosodimethylaniline method are shown in Table 1. Values cited as reference were corrected for the recent rate constant of thiocyanate ion of $1.1 \times 10^{10} \text{M}^{-1} \text{sec}^{-1}$ when thiocyanate ion was used as a reference compound in pulse radiolysis.

The ratios of $k_{\text{nucleoside}}$ to $(k_{\text{base}} + k_{\text{ribose}})$ are found in the region of 0.7-0.8. The reactivity of purine is about one tenth of those of other bases. Obviously the rate constant of purine riboside indicates that hydroxyl radicals react with not only with base moiety but also with ribose moiety. It is necessary to know the accurate conformation of nucleosides in aqueous solution for the argument on reactivity from a view-point of steric hindrance. With nucleotides, Barry et al.\textsuperscript{5} reported that there is some movement of phosphate in aqueous solution compared with crystalline state but the orientation of base ring to sugar in aqueous solution is very similar to that...
in crystal, where conformation is recognized as anti-type. Unfortunately paper on the conformation of nucleosides in aqueous solution was not available. However, it is reasonably assumed that the conformation of nucleoside is anti-type similarly to nucleotide. The solid angle was, therefore, calculated as anti-type. In Table 2, the ratios of solid angles for base moiety or ribose moiety of nucleosides to those for free base or ribose are listed. Since reaction sites of purine base are not yet clarified, it is assumed that hydroxyl radicals attack to C-5 atom similarly to the case of pyrimidine base. With ribose moiety, solid angles were calculated for two reactive hydrogens (C-5'-H and C-5'-H'), respectively.

Table 1
Rate constants for reactions of hydroxyl radicals with nucleosides and the related compounds

| Compound           | k (10^8 M⁻¹ sec⁻¹) | Reference       |
|--------------------|--------------------|-----------------|
| Cytosine           | 4.0^a              | 4.9 (pH 7.4)^b, 4.5 (pH 5.6)^b |
| Cytidine           | 4.0                | 4.9 (pH 5.2)^b, 4.6 (pH 7.2)^b |
| Thymine            | 4.7^a              | 5.3 (pH 7.2)^c, 5.1 (pH 7)^c |
| Thymidine          | 4.2                | 4.6 (pH 7.4)^c, 5.0 (pH 5)^c |
| Uracil             | 4.5^a              | 5.3 (pH 7.3), 5.2 (pH 5)^b |
| Uridine            | 4.7                | 4.3 (pH 6.5)^b, 4.2 (neut.)^b |
| Adenine            | 5.8^a              | 5.0 (pH 7.3), 3.8 (pH 5-5.5)^b |
| Adenosine          | 5.4                | 4.2 (pH 7.6)^c, 3.8 (pH 5.2)^b |
| Purine             | 0.3^a              |                 |
| Purine riboside    | 1.2                |                 |
| D-ribose           | 1.4                | 2.1 (pH 9)^c    |

a. These values were reported in the previous paper.¹
b. Reference (9).
c. Reference (10).
d. Reference (11).
e. Reference (12).

Since solid angle is hardly affected by the change in θ₂ as indicated by the results for cytidine (Table 2), θ₂ observed in crystal state was used for the present calculation of thymidine, uridine or adenosine.

When the steric hindrance estimated from the decrease in solid angles for the reaction sites of nucleoside is taken as correction factor, rate constant of nucleoside is expressed by the following relation,

\[ k_{\text{nucleoside}} = f_1 k_\text{base} + f_2 k_\text{ribose} \]  \hfill (7)

where \( f_1 \) and \( f_2 \) are correction factors obtained from the steric hindrance for reaction site on base moiety due to ribose moiety and that for ribose due to base, respectively. For thymidine, deoxyribose should be considered as sugar moiety instead of ribose. However, there is no positive reason for significant difference in reaction site or reaction rate between ribose and deoxyribose. Therefore, \( k_\text{ribose} \) was taken as the rate constant of deoxyribose and the calculation of solid angle was carried out by
the same procedure with the case of ribose moiety.

Rate constants of bases are generally three or four times larger than that of ribose except purine, and the contribution of \( k_{\text{ribose}} \) to \( k_{\text{nucleoside}} \) is small. With ribose moiety, change in solid angle is not significant whatever approximation is applied for \( r \) in the equation (6). Therefore, the solid angle for ribose moiety was calculated by using the large approximate of \( r \) in the estimation of integration region. The approximation may be practically sufficient for the present argument.

### Table 2

Ratio of solid angle for base or ribose moiety of nucleoside to that for free base or free ribose

| Nucleoside | \( \theta_1 \) | \( \theta_2 \) | Ratio of solid angle | Base moiety | Ribose moiety |
|------------|---------------|---------------|----------------------|-------------|---------------|
|            |               |               |                      | 5-H | 5-H' |
| Cytidine   | 90 315        | 0.67-0.70     | 0.92                 | 0.89        | *             |
|            | 180 45        | 0.67-0.70     | 0.76                 | *           | 0.85          |
|            | 180 135       | 0.67-0.71     | 0.79                 | *           | 0.79          |
|            | 180 315       | 0.67-0.69     | 0.80                 | 0.80        | 0.80          |
|            | 270 45        | 0.62-0.67     | 0.92                 | 0.92        | 0.88          |
|            | 270 135       | 0.62-0.65     | 0.83                 | 0.83        | 0.93          |
|            | 270 225       | 0.63-0.66     | 0.83                 | 0.93        | 0.93          |
|            | 270 315       | 0.66-0.69     | 0.89                 | 0.89        | *             |
| Thymidine  | 45 60         | 0.70-0.73     | 0.90                 | 0.90        | 0.90          |
|            | 60 60         | 0.71-0.73     | 0.92                 | 0.92        | 0.92          |
|            | 0 60          | 0.68-0.70     | 0.85                 | 0.85        | 0.85          |
| Uridine    | 45 60         | 0.72-0.73     | 0.89                 | 0.89        | 0.89          |
|            | 60 60         | 0.73-0.75     | 0.91                 | 0.91        | 0.91          |
|            | 0 45          | 0.70-0.72     | 0.92                 | 0.92        | *             |
| Adenosine  | 15 45         | 0.69-0.71     | 0.92                 | 0.92        | 0.81          |
|            | 60 45         | 0.65-0.67     | 0.93                 | 0.93        | 0.85          |

* The solid angle could not be calculated because of close contact distance between base and C-5'-H (or C-5'-H').

a. \( \theta_1 \) or \( \theta_2 \) is the torsional angle about the bond between C-1' and N-1 (or N-9) or the bond between C-4' and C-5'. Clockwise rotation is positive and the eclipsed (cis) position is taken as zero.\(^4\)
In Table 3, the values of $k_{nucleoside}$ calculated according to the equation (7) are compared with the observed ones. The fair agreement between the calculated and the observed values indicates that the reduced reactivity of a complex molecule can be explained by steric hindrance of the reaction sites due to other part of the same molecule and the degree of steric hindrance can be estimated by calculation of solid angle admitted to attack of reactant.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Professor Akira Imamura of Shiga University of Medical Science for his helpful discussion on theoretical treatment and acknowledge the grant for Scientific Research from the Ministry of Education, Science and Culture.

REFERENCES

1. T. Masuda, H. Shinohara and M. Kondo (1975) Reactions of hydroxyl radicals with nucleic acid bases and the related compounds in gamma-irradiated aqueous solution. *J. Radiat. Res.*, 16: 153-161.

2. C. Lagercrantz (1973) Trapping of radicals formed in the photochemical reaction between hydrogen peroxide and some pyrimidine bases, nucleosides, and yeast nucleic acid. *J. Am. Chem. Soc.*, 95: 220-225.

3. W. J. Bowen and H. L. Martin (1964) The diffusion of adenosine triphosphate through aqueous solutions. *Arch. Biochem. Biophys.*, 107: 30-36.

4. I. Kraljic and C. N. Trumbore (1965) p-Nitrosodimethylaniline as an OH radical scavenger in radiation chemistry. *J. Am. Chem. Soc.*, 87: 2547-2550.

5. R. L. Willson, C. L. Greenstock, G. E. Adams, R. Wageman and L. M. Dorfman (1971) The standardization of hydroxyl radical rate data from radiation chemistry. *Int. J. Radiat. Phys. Chem.*, 3: 211-220.

6. A. V. Lakshminarayanan and V. Sasisekharan (1970) Stereochemistry of nucleic acids and polynucleotides. II. Allowed conformations of the monomer unit for different ribose puckerings. *Biochem. Biophys. Acta.*, 204: 49-59.
7. L. Pauling (1960) The Nature of the Chemical Bond (3rd ed.) Cornell University Press, New York. pp. 257-264.
8. C.D. Barry, A.C.T. North, J.A. Glasel, R.J.P. Williams and A.V. Xavier (1971) Quantitative determination of mononucleotide conformations in solution using lanthanide ion shift and broadening NMR probes. *Nature*, 232: 236-245.
9. G. Scholes, P. Shaw, R.L. Willson and M. Ebert (1965) Pulse radiolysis studies of aqueous solutions of nucleic acid and related substances. *Pulse Radiolysis* (ed. M. Ebert et al.) Academic Press, London and New York. pp. 151-164.
10. C.L. Greenstock, M. NG and J.W. Hunt (1968) Pulse radiolysis studies of reactions of primary species in water with nucleic acid derivatives. *Adv. Chem. Ser.*, 81: 397-417.
11. C.L. Greenstock, J.W. Hunt and M. NG (1969) Pulse radiolysis studies of uracil and its derivatives. *Trans. Faraday Soc.*, 65: 3279-3289.
12. I. Kraljic (1967) Kinetics of hydroxyl radical reactions in radiolysis, photolysis and the Fenton system. *Chem. Ioniz. Excitation, Proc. Radiat. Chem. Photochem.*, Univ. Newcastle-upon-Tyne. pp. 303-309.