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The Hydrolysis of Several Purine Nucleosides with Strong Hydrochloric Acid or Cation Exchange Resin

Yoshihisa Suzuki

Central Research Laboratories, Ajinomoto Co., Inc., Suzuki-cho, Kawasaki 210

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Synopsis. The rates of hydrolysis were determined for several purine nucleosides with aq. HCl(0.1—4 M) or cation exchange resins(H-form) at 30 °C. The hydrolysis of adenosine with Diaion SK-1B is slower than guanosine while that with HCl is faster. This discrepancy was discussed.

Purine nucleosides are known to be susceptible to acid, giving nucleic bases and D-ribose. This property is based on the hydrolysis of the glycosidic bond. However, the treatment with cation exchange resin in the H-form has been found to be useful for the industrial isolation or purification of purine nucleosides.[1] This paper will describe their rates of hydrolysis with strong acids including cation exchange resins at room temperature.

Experimental

Materials. The guanosine, inosine, and adenosine used were commercial products of the Ajinomoto Co., Inc. Diaion SK-1B (Mitsubishi Chem. Ind. Ltd.), Duolite C-3 and Duolite ES-63 (Diamond Alkali Co.) were of 20—50 mesh and were conditioned to the H-form.

Paper Chromatography (PC). One-dimensional ascending PC was carried out on Toyo-Roshi No. 51A, the following two solvent systems being used: Solvent A, *n*-butanol–acetic acid–water(4 : 1 : 1); Solvent B, *n*-propanol–28% ammonia–water (20 : 15 : 3). Solvent A was used for the analysis of experimental runs involving inosine and adenosine (R_f, inosine 0.25, hypoxanthine 0.42, adenosine 0.46, adenine 0.58), and Solvent B, for guanosine (R_f, guanosine 0.50, guanine 0.41). The UV-absorbing spots of the samples and authentic standard references were cut off, together with their appropriate blanks, and eluted in 0.1 M HCl. The optical densities of the eluents at the wavelength of the maximum absorption of each component were measured. The percentages of the remaining original nucleosides and the nucleic bases thus produced were calculated from the optical densities.

Hydrolysis with HCl. Guanosine(0.1 g), inosine(0.25 g) or adenosine(0.25 g) was added to an Erlenmeyer flask which contained 50 ml of an HCl solution with a definite concentration (0.1—4.0 M) and kept at 30 °C. Each nucleoside was dissolved with vigorous stirring as soon as it was added. After the solution had been allowed to stand for a definite period (30 min-216 hr), it was cooled on an ice bath and neutralized with a cold NH_3 aqueous solution in order to stop the reaction. The resultant solution was analyzed with PC.

Hydrolysis with Cation Exchange Resins. Each nucleoside (0.3 g) was dissolved in a 0.15 M acetic acid solution(300 ml). Then, each wet resin(10 ml) was put into a stoppered Erlenmyer flask which contained the acetic acid solution described above. The resultant mixture was shaken for a definite period (20 min—48 hr) at 30 °C. The resin was filtered, washed with 20 ml of water, and then put into another Erlenmeyer flask which contained 50 ml of a 2 M NH_3 aqueous solution. After the mixture had been shaken for 30 min in order to elute the nucleoside and the base formed on the resin, the resin was put in a column and eluted with 350 ml of the 2 M NH_3 aqueous solution for complete elution. The eluates were then mixed and analyzed with PC. In the case of the experimental run using Duolite ES-63, 2M NaOH was used for the elution in place of the NH_3 aqueous solution because resin is not a strong cation-exchange resin.

Results and Discussion

Guanosine, inosine, and adenosine were hydrolyzed to give guanine, hypoxanthine, and adenine respectively. The decrease in the mole amount of nucleoside was equal to the amount of the nucleic base produced within the range of experimental error, and no side reaction was observed. In all the cases in the present study, first-order linear plots were obtained for the logarithm of the concentration of nucleoside with the reaction time. The observed first-order rate constants, *k*, were determined by the linear plots given by Eq. (1) or Eq. (2);

$$\ln([N_0]/[N]) = kt \qquad (1)$$

$$\ln\{([N]+[B])/[N]\} = kt \qquad (2)$$

where $[N_0]$ is the initial concentration of nucleoside, $[N]$ is the concentration of it at time t, and $[B]$ is that of the nucleic base.

On Diaion SK-1B, 93% of the guanosine or adenosine was adsorbed within 1 hr. It increased up to 97% within 6 hr. However, 50% and 47% of the inosine remained in the solution unadsorbed on the resin after the contact times of 6 hr and 16 hr respectively. This was because of the small pK_a value[2] of inosine(1.2) compared with that of guanosine(2.2) or adenosine(3.5). On Duolite ES-63, only 50% of the guanosine was adsorbed during the contact time of 48 hr, while the figure was above 95% within 1 hr on Duolite C-3. This was because of the small dissociation of the cation exchange group, phosphonic acid, in the former resin compared with that of methylenesulfonic acid in the latter.

More than 95% of the adsorbed substances were eluted in all the cases. There were some nucleic bases produced on the resin. The amount of the bases increased with the increase in the contact time, and the rate constants of hydrolysis were determined by Eq. (2).

Figure 1 shows the observed rate constants summarized with the acidity function, H_0. The acidity function represents, in short, the strength of the acidity of the solvent. The H_0 values of HCl solutions were determined from the literature.[3] Linear plots were obtained for the logarithm of the rate constant, log *k*,

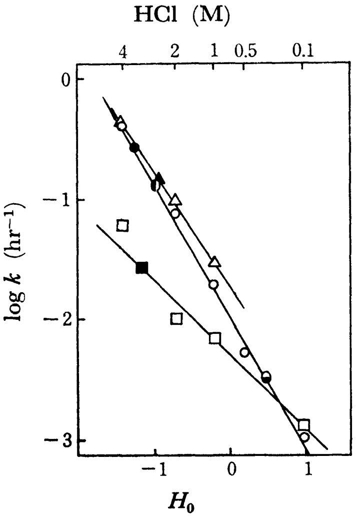

Fig. 1. Rate constants of acid hydrolysis for some purine nuclesides at 30 °C. The scale of the upper abscissa is given for convenience to compare the strength of cation exchange resins with that of HCl solutions. Adenosine with HCl (△) and with Diaion SK-IB (▲); guanosine with HCl (○), with Diaion SK-1B (●), with Duolite C-3 (◐) and with Duolite ES-63 (◒); inosine with HCl (□) and with Diaion SK-1B (■).

with the acidity function of H_0 from the experimental results for hydrolysis with an HCl solution. The results for hydrolysis with cation-exchange resins were placed on the lines corresponding with the individual nucleoside.

The rate constant with Diaion SK-1B for guanosine is equal to that with 3.2 M HCl. Hydrolysis with Duolite C-3 was equivalent to that with 2.5 M HCl. It is known that the hydrogen-ion concentration in cation-exchange regins is a few mol per liter. The above results are consistent with this knowledge, and the difference in the acid strength between Diaion SK-1B (sulfonic acid group, R–SO_3H) and Duolite C-3 (methylenesulfonic acid group, R–CH_2SO_3H) was quantitatively determined. The hydrogen-ion concentration in Duolite ES-63(phosphonic acid group, R–P(O)$(OH)_2$) was estimated to be equivalent to 0.3 M HCl from the rate of hydrolysis for guanosine, as above.

The hydrogen-ion concentration in Diaion SK-1B, as estimated from the rate constant for the acid hydrolysis of inosine, is in good agreement with that estimated from guanosine, while that estimated from adenosine is somewhat different from them. In relation to this estimation, a contradiction has been observed in this study in that hydrolysis with Diaion SK-1B for adenosine is slower than for guanosine, whereas hydrolysis with HCl is slightly faster. These facts strongly suggest that there is some difference between the position of the protonation in the acidic forms of adenosine (N-1)[4] and of guanosine (N-7).[5] This difference must affect the stereospecific binding of the nucleosides with the fixed cation-exchange group, the sulfonic acid in the resin, and the susceptibility of cleavage at the glycosyl bond. Therefore, the apparent values of the hydrogen-ion concentration were different between them. Jones and Robins[6] suspected that the position of the protonation in the acidic form of inosine was probably N-7, the same as that of guanosine.

In strong acid, the rate constant of hydrolysis for inosine is smaller than for guanosine, while the reversal is observed in 0.1 M HCl (Fig. 1). It is presumed that inosine will be hydrolyzed faster than guanosine in the weaker-acid or in the acidic pH region.

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