NMR Study of Spontaneous Degradation of Penicillin G in Aqueous Solution

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The spontaneous degradation of penicillin G in aqueous solution has been studied using NMR and thin layer chromatography. Correlation NMR spectroscopy was used to follow products which appear at the early stage of degradation. It has been found that in 0.3 M phosphate buffer (pH 7.0, 30 °C) a successive first order degradation

$$\text{penicillin G} \xrightarrow{k_1} \text{penicilloic acid} \xrightarrow{k_2} \text{secondary product}$$

is dominant with kinetic constants of $k_1=0.7\times10^{-2}$ h^{-1} and $k_2=6\times10^{-2}$ h^{-1}.

It is well known that penicillin G is labile in aqueous solution, and degraded to give a number of products. Several pathways of degradations have already been proposed.[1] However, until now kinetic studies of the degradation of penicillin G[2-4] have been based on the observation of a decreasing time-course of undegraded penicillin G, and information from a number of degradation products has never been used quantitatively; only an overall rate of degradation of penicillin G was reported, which actually consists of several consecutive steps.

In the present work, in order to clarify the elementary process of degradation of penicillin G, a degradation product such as penicilloic acid has been followed quantitatively using NMR spectroscopy. Correlation NMR spectroscopy[5] was employed to follow products which appear at the early stage of degradation.

Experimental

Penicillin G potassium salt for injection (Takeda Chemical Industries, Lot No. 0021) and penicillinase (Calbiochem., Lot No. 400960) were used without further purification. All other reagents are of analytical grade. The concentration of penicillin G is 10 mg/ml which is approximately equal to that used for intramuscular injection. Penicillin G was dissolved in 0.3 M deuterated phosphate buffer (pH 7.0). The sample temperature was maintained at 30 °C throughout the experiments.

NMR measurements were performed using a JEOL PS-100 spectrometer. Chemical shift values reported are from external TMS (10% (v/v) in CCl_4). The concentrations of penicillin G and its degradation products were determined by comparing the peak height of each signal with that of TMS sealed in a coaxial tube. Correlation NMR technique was employed to follow degradation products which give small signals near the strong HDO peak.[5]

Degradation of penicillin G was also monitored by thin layer chromatography (TLC), the procedure of Vandamme and Voets[1] being followed with a slight modification. TLC plates are Merck $60F_{254}$ (5×20 cm with a thickness of 250 μ). 5—6 μl of penicillin G solution (4 mg/ml, 10 mg/ml) was applied and developed at 21 °C using an 85% aqueous solution of acetone. The solvent was allowed to rise to a height of 17 cm. Each TLC plate was sprayed consecutively with 2 M NaOH, 1% starch gel and iodine azide reagent. The R_f values of degradation products were standardized by that of penicillin G.

Results and Discussion

First, a decreasing time-course of degradation of penicillin G was followed using NMR spectroscopy. Figure 1 shows a time change in the NMR peak intensity of α-methyl, $-CH_2-$ and 3-H of penicillin G dissolved in 0.3 M phosphate buffer (pH 7.0). This result indicates that the overall degradation process of penicillin G is of a pseudo-first order reaction with a half life of 85 h. From this result the kinetic constant of overall degradation process, $k_{overall}$, can be estimated at 0.8×10^{-2} h^{-1} at 30 °C in the phosphate buffer solution. A kinetic constant of 1.4×10^{-3} h^{-1} has been reported at 35 °C, pH 7.0 using UV spectroscopy and other methods.[2,3] The phosphate buffer solution used in the present experiment is known to accelerate the degradation of penicillin G.[4] In view of this, the kinetic constant obtained using NMR is consistent with the previously known value.

However, the result of TLC indicates that the process of spontaneous degradation of penicillin G is actually not so simple as described above. The R_f values of degradation products at several different stages are

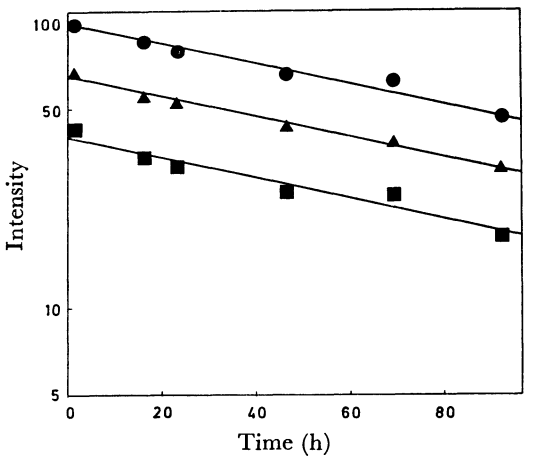

Fig. 1. Semilog plots of intensity of α-CH_3 ●, $-CH_2-$ ▲ and 3-H ■ of penicillin G *vs.* time at 30 °C in the phosphate buffer solution (pH 7.0). Initial value of α-CH_3 intensity of penicillin G is arbitrarily taken to 100.

Table 1. R_f values ($\times 100$) of degradation products of penicillin G at several different stages in the phosphate buffer solution
Penicillin G was used as a standard.

Time(h)	0	2.5	7	17	30	70	118	240	Product
	56	56	56	56	56	56	56	56	Penicillin G
						49			
					37	40	43	40	
R_f values $\times 100$			31	32	27	31	33	32	Secondary product
		16	17	17	15	15	17	14	Penicilloic acid

summarized in Table 1. Only one species of degradation products can be observed at 2.5 h after penicillin G is dissolved. After 7 h, the second species becomes observable, and more than three species can be observed after 17 h. This result indicates that from the observation of the decreasing time-course of penicillin G, only an overall degradation rate of penicillin G can be obtained. Therefore, in order to study the actual complicated process of degradation of penicillin G, the formation process of the degradation products was followed quantitatively.

In order to identify the initial product of spontaneous degradation using NMR, the chemical shift values of the initial product were compared with those of penicillinase-degraded products.[6] This result clearly indicates that the initial product is penicilloic acid.

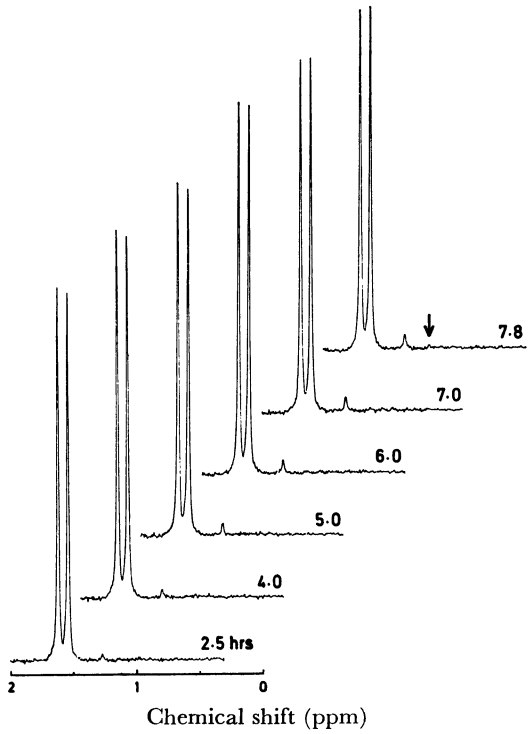

Fig. 2. Correlation NMR spectra of methyl region of penicillin G and penicilloic acid at early stages of degradation. Number of accumulations: 128. Two large peaks at $\delta=1.54$ and 1.62 ppm result from penicillin G and a small peak at $\delta=1.27$ ppm results from penicilloic acid. The signal due to the secondary product ($\delta=1.07$ ppm) is marked by an arrow.

In order to follow the formation of penicilloic acid at the early stage, 32—128 accumulations were performed in the correlation mode. Each accumulation was completed within 10 min. Figure 2 gives NMR spectra of the methyl region of penicillin G and penicilloic acid at the early stage of degradation. The α-methyl peak of penicilloic acid appears at $\delta=1.27$ ppm. Although NMR is less sensitive than TLC, a signal due to the secondary product ($\delta=1.07$ ppm) can be clearly seen in Fig. 2, where the signal is marked by an arrow. This signal becomes much stronger at a later stage of degradation. An aqueous solution containing only penicilloic acid also gives a product which is identical with this secondary product. This result demonstrates that the secondary product is produced from penicillin G through penicilloic acid, *i.e.* the process of formation of penicilloic acid and the secondary product is a successive degradation reaction. When pH is not regulated, penicilloic acid is known to undergo degradation forming penilloic acid by decarboxylation.[1] In the present experiment, the secondary product is presumably penilloic acid, judging from the formation of bubbles in the solution during the course of incubation. No attempt has been made to identify this product. The order of appearance of the degradation products observed by the correlation NMR method is consistent with that observed by TLC; as shown in Table 1, penicilloic acid with an R_f value of 0.15 appears first, followed by the secondary product which gives an R_f value of 0.3. Products whose R_f values are 0.4 and 0.49 cannot be assigned to any distinct NMR peaks due to a low concentration of these products.

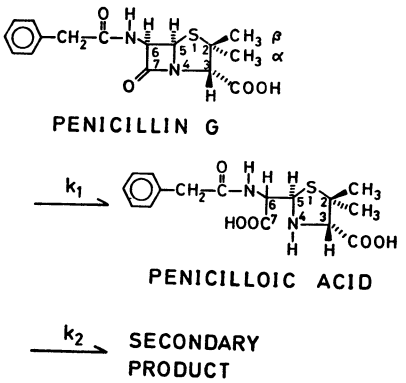

Fig. 3. A degradation process of penicillin G.

A computer simulation of the process of formation of penicilloic acid and the secondary product was performed by assuming that the reaction is successive first order reaction. The result is illustrated in Fig. 3. For the simulation, the yields for these products observed within 17 h after the addition of penicillin G were used; no other products exist during this period judging from the TLC result. The computer simulation gives kinetic constants of $k_1=0.7\times10^{-2}\,h^{-1}$ and $k_2=6\times10^{-2}\,h^{-1}$. Figure 4 illustrates observed and calculated formation processes for penicilloic acid and the secondary product. In the case of penicilloic acid the calculated curve is in good agreement with the observed yield over the entire range of the reaction. On the other hand, the observed

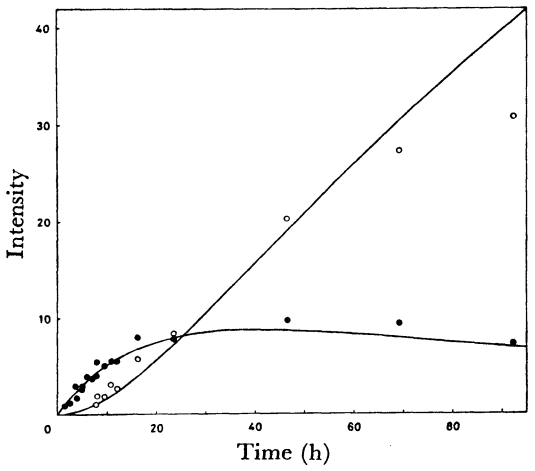

Fig. 4. Plots of intensity of the methyl group of penicilloic acid ● and the secondary product ○ *vs.* time at 30 °C in the phosphate buffer solution. Solid lines indicate the calculated values. Initial value of α-CH_3 intensity of penicillin G is arbitrarily taken to 100.

value for the secondary product does not agree well with the calculated value at a later stage of the reaction. This suggests that the secondary product is labile and further degraded.

A kinetic constant of the first step, k_1 obtained here is in good agreement with $k_{overall}$ which is obtained from the decreasing time-course of penicillin G. From this result it was found that the successive first order reaction given in Fig. 3 is dominant in 0.3 M phosphate buffer solution (pH 7.0) at 30 °C with kinetic constants of $k_1=0.7\times10^{-2}\,h^{-1}$, $k_2=6\times10^{-2}\,h^{-1}$.

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