PENICILLIN INTERACTION WITH GUANOSINE

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Summary: Because of the widespread use of penicillins as antibacterial agents, the question of how penicillin affects the function and structure of nucleic acids becomes of biological importance. This communication reports a nuclear magnetic resonance study which shows that penicillin-G interacts with guanosine in dimethyl sulfoxide and can break the strong guanosine-cytidine pairing by forming a binary hydrogen-bonded complex of penicillin-guanosine. The binding sites in penicillin are the carboxylate and the carbonyl groups, while the NH and NH_2 groups of guanosine act as hydrogen donors.

Because of the widespread use of penicillins as antibacterial agents, the question of how penicillins affect the function and structure of nucleic acids becomes of biological significance. We wish to report a nuclear magnetic resonance (nmr) study which shows that penicillin-G interacts with guanosine and can break the strong guanosine-cytidine pairing[1,2] by forming a binary complex of penicillin-guanosine. Although we have used both 60- and 100- MHz NMR spectrometers, we find that only by using a higher-field NMR spectrometer (in this case, 250 MHz) can a well-resolved spectrum of penicillin be obtained. Figure 1 gives the nmr spectrum of 0.2 M penicillin (250 MHz) in D_2O, and shows that the phenyl peak is a multiplet and the CH_2 peak an AB quartet, $J \sim 15$ Hz. Both the phenyl and CH_2 signals appear as singlets at 60- and 100- MHz.

In unbuffered aqueous solution, penicillin decomposes in less than 24 hours, whereas in dimethyl sulfoxide (DMSO), we have found it to be stable for more than seventeen weeks at 30°. For this

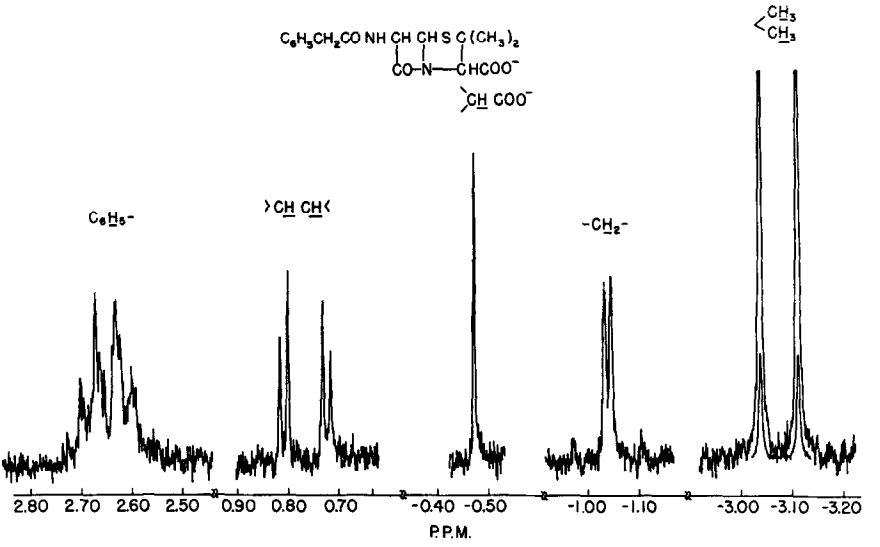

Figure 1. Nmr spectrum of 0.2 M penicillin-G (250 MHz) in D_2O.

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reason, and because guanosine is not sufficiently soluble in D_2O at neutral pH for nmr measurements, we have studied the interaction between penicillin and guanosine in DMSO-d_6. The CH_A - CH_B - NH_X signals in penicillin represent an ABX system, with $J_{AX} \cong 0$, $J_{AB} \sim 4$ Hz, $J_{BX} = 9$ Hz. The C<u>H</u>COO$^-$ signal is a singlet, and the two methyl groups are nonequivalent, giving rise to two separate singlets.

When guanosine is added to 0.1 M penicillin in DMSO-d_6, the penicillin proton peaks are shifted, and the extent of downfield shift decreases in the order: C<u>H</u>COO$^-$ > CH_B > NH, CH_3, CH_3 > CH_A > CH_2, C_6H_5, as shown in Figure 2A. If penicillin is added to 0.1 M guanosine in DMSO-d_6, the NH_2 signal in guanosine is shifted downfield and slightly broadened, the G-NH signal is shifted downfield

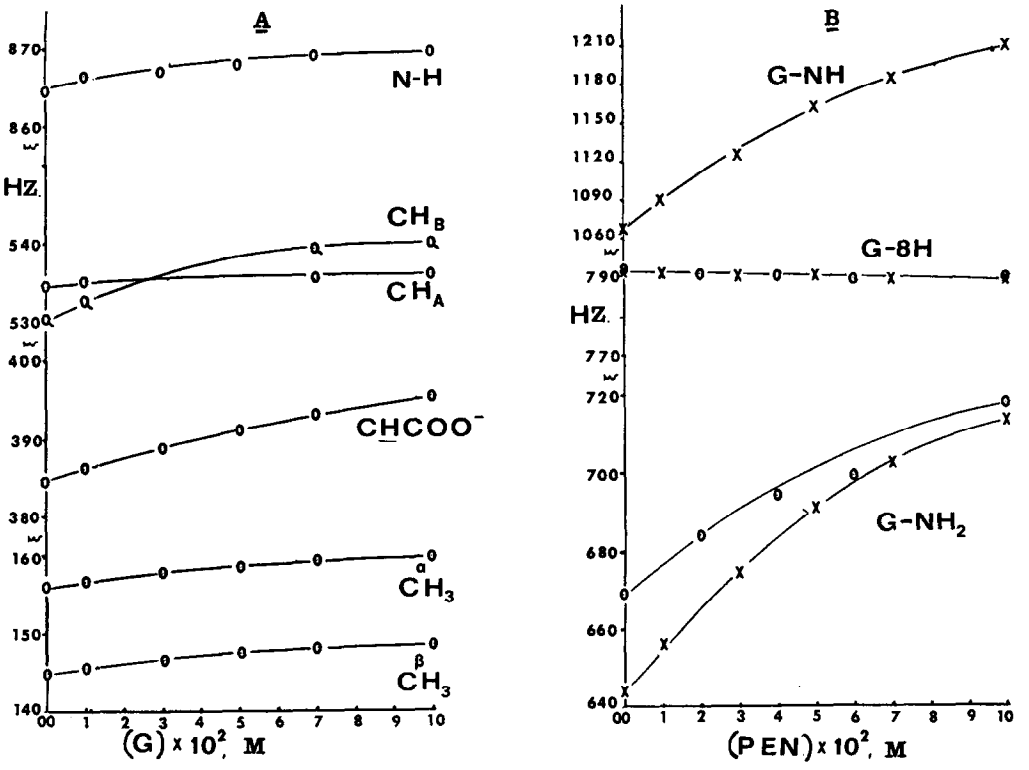

Figure 2A. Chemical shifts of penicillin-G protons at 100 MHz (TMS as internal reference) in DMSO solutions containing 0.1 M penicillin and varying concentrations of guanosine.
Figure 2B. Chemical shifts of guanosine protons vs. penicillin concentration in DMSO (x, with 0.1 M guanosine; o, with 0.1 M guanosine and 0.1 M cytidine present). For numbering scheme, see Figure 3. Note the different scales used for G-NH and for $G\text{-}NH_2$.

at a pace double that of $G\text{-}NH_2$ and greatly broadened, and the G-8H signal is only slightly affected by the addition of penicillin (see Figure 2B). The 100% increase in the rate of downfield shift of G-NH compared with that of $G\text{-}NH_2$ is understandable, because of the two NH_2 protons, only one is involved in hydrogen bonding with penicillin. The other proton is hydrogen bonded to DMSO. These nmr data lead to the conclusion that a penicillin-guanosine complex is formed in DMSO, in which two hydrogen bonds are involved, and the binding sites are as shown in Figure 3. Construction of molecular models has shown that this is reasonable. The G-8H is at a

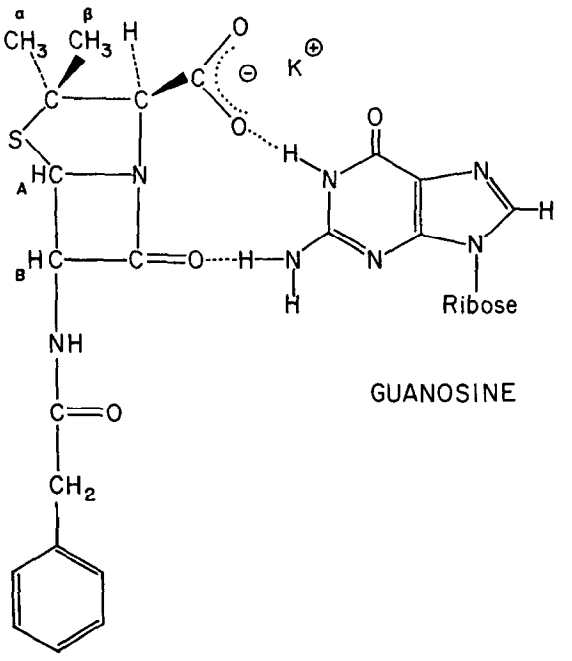

Figure 3. Schematic representation of penicillin-guanosine complex.

distance away from the hydrogen-bonding sites, and therefore its frequency is only slightly affected by the addition of penicillin.

Figure 2B further shows that the G-NH_2 signal in a solution containing 0.1 M guanosine, 0.1 M cytidine in DMSO is downfield from that in a solution 0.1 M guanosine in DMSO. This is understandable because of hydrogen-bonding between guanosine and cytidine[1,2]. On the addition of penicillin to a solution containing 0.1 M guanosine and 0.1 M cytidine, the G-NH_2 signal shifts downfield; however, the rate of downfield shift is not as great as the rate observed on adding penicillin to a solution containing guanosine alone. Furthermore the trend is that the G-NH_2 signal frequency in a guanosine-cytidine mixture approaches that of a solution containing guanosine alone, at high concentration of penicillin.

These results lead us to conclude that penicillin disrupts the guanosine-cytidine pairing, a binary complex penicillin-guanosine is formed.

From the chemical shift data for G-NH_2 in solutions containing 0.1 M guanosine and varying concentrations of penicillin-G, as shown in Figure 2B, we have determined the formation constant of 1:1 penicillin-guanosine complex, in the manner previously described for 1:1 guanosine-cytidine complex[2]. The formation constant of the penicillin-guanosine complex is about 30 l./mole, and is considerably higher than the value K = 4 l./mole for the guanosine-cytidine complex[1,2], also determined in DMSO. The penicillin-guanosine interaction therefore is strong enough to disrupt the guanosine-cytidine pairing.

We have also obtained nmr spectra for DMSO solutions containing 0.1 M penicillin-G and 0.1 M nucleoside (nucleoside = cytidine, adenosine, uridine, and thymidine). The data obtained for both penicillin and nucleoside protons show that the interaction between penicillin-G and cytidine or adenosine is significantly weaker than with guanosine. The reason can very well lie in the fact that cytidine and adenosine, unlike guanosine, do not have both NH and NH_2 groups in the same molecule. With uridine and thymidine, the carboxylate group appears to be the only binding site in penicillin-G, with the NH group in uridine and thymidine as the hydrogen donor.

Using an infrared method, Pitha, et al.[3], have reported a formation constant of over 10^5 for hydrogen-bonding between guanosine and cytidine derivatives in chloroform. Since DMSO is a strong proton acceptor, the formation constant of the guanosine-cytidine complex is only about 4 in this medium[1,2]. In water vertical stacking occurs for mononucleosides[4], and there is little

evidence for formation of specific hydrogen-bonded complexes between mononucleosides in water. According to Newmark and Cantor[1], the structure of the guanosine-cytidine pair in DMSO is almost certainly the same as the base-pairing scheme known to occur in double stranded DNA, since the interior of the double stranded DNA molecule is largely hydrophobic and is therefore "nonaqeous." The experiments reported in this communication therefore were carried out in DMSO medium, and have relevance to behavior in biological systems. Certainly if one compares the formation constants of guanosine-cytidine complex in chloroform ($K \backsim 10^5$) and in DMSO ($k=4$), one has to conclude that the degree of hydrogen-bonding in DMSO is far more similar to interactions in water than in chloroform.

The nmr results described in this communication were obtained with a Varian A-60, a Varian HA-100 or a spectrometer incorporating a superconducting solenoid, at a frequency of 250 MHz for protons. The data illustrated in Figure 2 were obtained with a Varian Ha-100 spectrometer. Sample preparation was the same as described previously.[5]

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