NMR SATURATION TRANSFER AND LINE SHAPE ANALYSES OF CYCLIC TETRADEPSIPEPTIDE AM TOXIN II

Conformational equilibrium with very unequal populations

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1. Introduction

AM toxin II (fig.1) is a host-specific phytotoxin produced by *Alternalia mali*, a fungus causing leaf-spot disease of apples [1–3]; in particular, some varieties of apple, such as Indo and Delicious, are highly susceptible to this disease. For the hope of elucidating the structure–toxicity relationship of this AM toxin II and related peptides, we have measured the 270 MHz ^{1}H NMR spectra of AM toxin II and have found several unusually broad resonances affected by chemical exchange. Here we report the successful combination of the NMR saturation transfer and line shape analyses for elucidating the conformational equilibrium with very unequal populations and conformational transition rates of AM toxin II in solution.

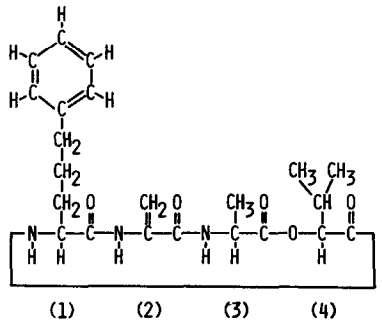


Fig.1. The primary structure of AM toxin II: (1) L-App (2-amino-5-phenyl pentanoic acid); (2) Dha (dehydroalanine); (3) L-Ala (alanine); (4) L-Hyi (hydroxyisovaleric acid).

2. Materials and methods

AM Toxin II was isolated and purified from *Alternalia mali* (strain number I-716) as in [1,2]. The sample for NMR measurements was dissolved in the mixed solvent $C^2HCl_3/(C^2H_3)_2SO$ (9/1, v/v) at ~10 mM, so that NMR measurements were possible at temperatures as low as –37°C. 270 MHz ^{1}H NMR spectra were recorded on a Bruker WH-270 spectrometer equipped with Bruker B-ST-100/700 temperature control unit. 100 MHz ^{1}H NMR spectra were recorded on a Jeol FX-100 spectrometer.

3. Results and discussion

The 270 MHz ^{1}H NMR spectrum of AM toxin II at 23°C is shown in fig.2, together with the complete assignments as confirmed by the spin-decoupling technique. It is remarkable to observe several very broad peaks, including the C_β proton (H_B) peak of Dha (5.33 ppm), the NH proton peaks of Dha (8.97 ppm) and App (8.06 ppm) and the C_α proton peak of L-Ala (4.57 ppm). All these proton resonances are sharper at 100 MHz (fig.2, inserts (a,b)) than at 270 MHz, indicating the chemical exchange between different conformations. For obtaining

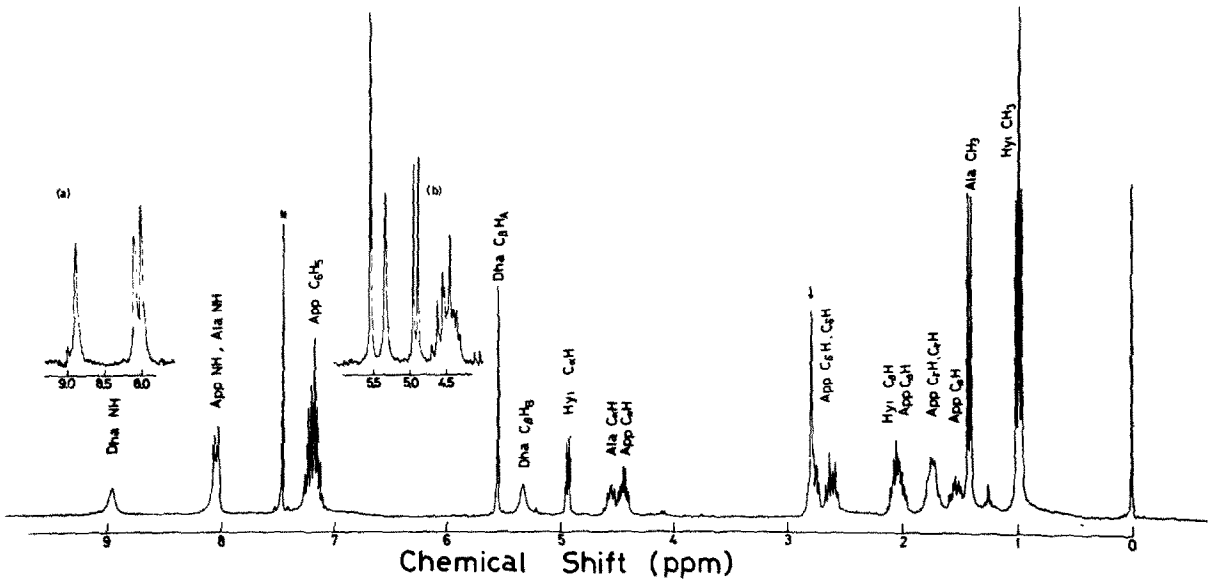


Fig.2. 270 MHz ^1H NMR spectrum of AM toxin II in $C^2HCl_3/(C^2H_3)_2SO$ (9/1, v/v) at 23°C; (a,b) 100 MHz ^1N NMR spectra. The chemical shifts (ppm) are measured downfield from the internal standard of 2,2-dimethyl-2-silapentane-5-sulfonate. The resonances due to residual water and $CHCl_3$ are marked with arrow and asterisk, respectively.

some detailed information on this conformational equilibrium, the temperature dependences of the line profiles of these peaks were observed. However, even at −37°C, the counterpart resonances did not appear and the area intensitites of the two C_β proton resonances (H_A and H_B) were nearly equal. These observations indicate that the equilibrium is one-sided and the populations of the two conformers are very unequal, at least, at low temperatures.

Accordingly, saturation transfer experiments were made of the C_β proton resonance (H_B) of Dha at −37°C and the intensity decrease of the H_B proton resonance of Dha was found to depend upon the irradiating frequency. Upon irradiation at 6.36 ppm, the intensity of the major resonance (5.29 ppm) was decreased to the minimum value of 67% (fig.3). Thus the chemical shift of the Dha H_B proton of the minor

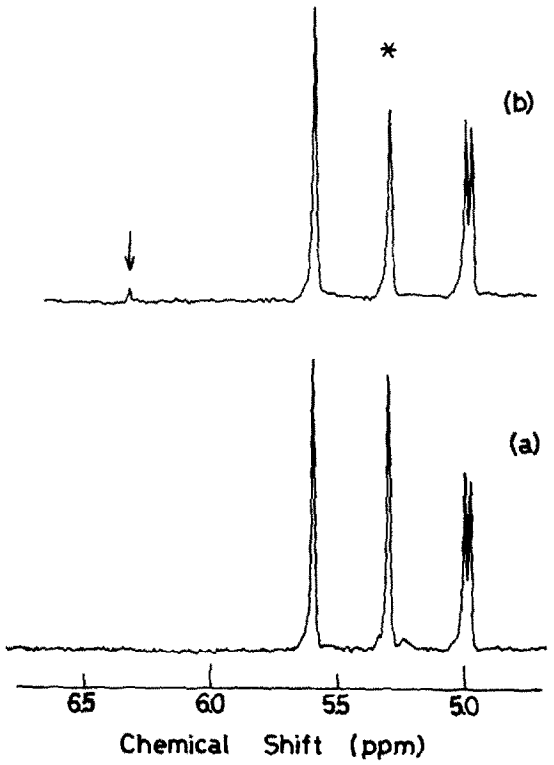


Fig.3. 270 MHz ^1H NMR spectrum of AM toxin II (6.5–5.0 ppm) at −37°C: (a) normal spectrum; (b) saturation transfer spectrum with the irradiation at 6.36 ppm. The major peak of the C_β proton resonance (H_B) of Dha is marked with asterisk.

conformer (minor peak) was located at ~6.36 ppm and the chemical shift difference between the two conformers was found to be δ = 290 Hz. Similarly for the major peaks of the Dha NH proton (9.43 ppm), L-Ala C_α proton (4.59 ppm) and L-App NH proton (8.25 ppm) at −37°C, the counterpart minor resonances were located at about 8.69, 4.33 and 7.88 ppm, respectively.

Subsequently, for the major peak of Dha C_β resonance (H_B), the line width (Δ, full width at half height) was measured at various temperatures (fig.4). This width was found to reach maximum (Δ^{max} = ~17.5 Hz) at 13°C, as compared with the width (~2.5 Hz) of the other C_β proton resonance (H_A, at 5.57 ppm) of Dha. The exchange broadening effect is estimated as 15.0 Hz.

The fractional population (p) of the minor conformer may be estimated from the maximum width (Δ^{max}) and the chemical shift difference (δ) between the two conformers [4,5]:

$$p = (\Delta^{max})/\delta$$

Thus, from the Δ^{max} and δ values for the C_β proton (H_B) of Dha, the fractional population of the minor conformer is found to be 5.2% at 13°C. The enthalpy difference between the minor and major conformers is estimated to be ΔH = 1.65 kcal/mol (the entropy difference being assumed to be zero), and for example, the fractional population of the minor conformer at −37°C and 63°C are calculated as 29% and 7.8%, respectively.

With the calculated fractional populations of the minor conformer over the temperature range −37°C to +63°C, the mean life time (τ_{major}) of the major conformer was estimated as shown in fig.4, by the line-shape analysis [6] of the C_β proton resonance (H_B) of Dha. From the Arrhenius plot (fig.4), the enthalpy of activation ($\Delta H^{\ddagger}$) and frequency factor for the major conformer were obtained as 12.9 kcal/mol and 8.3×10^{11} s^{-1}, respectively.

The validity of all these procedures was confirmed by the examination of the exchange broadening and saturation transfer. Firstly, the exchange broadening is expected to reach the maximum at the temperature where $2\pi\delta = \tau_{major}^{-1}/P$ [4]. For the C_α proton resonance of L-Ala (δ = 70 Hz), as an example, the temperature for maximum broadening is estimated as

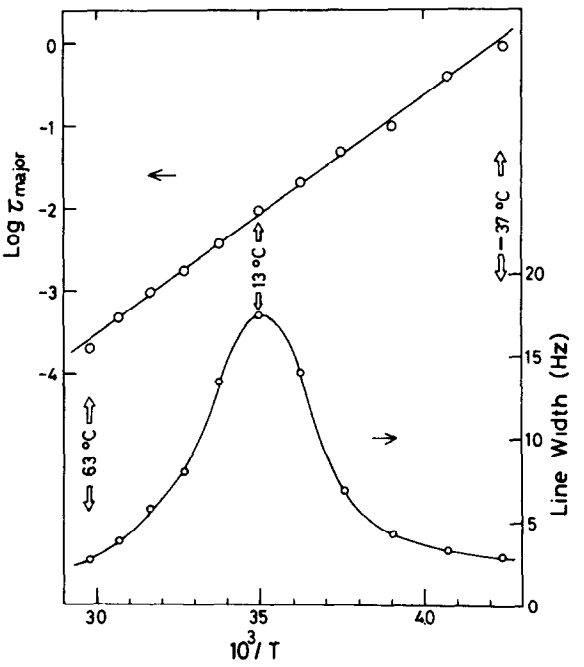
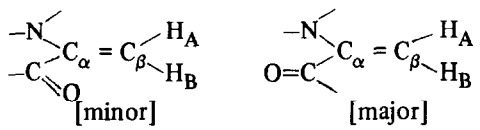


Fig.4. The observed line width of the major peak of the C_β proton resonance (H_B) of Dha and life time (τ_{major}) of the major conformer at various temperatures T (K).

−9°C, in good agreement with the observed temperature of −7°C. Secondly, the intensity ratio of the major peak with and without saturation transfer may be calculated [7] by $\tau_{major}/(\tau_{major} + T_1)$ where T_1 is the spin-lattice relaxation time of the major peak. For the C_β proton resonance (H_B), T_1 was determined as 0.33 s (at −37°C) by the inversion recovery method and the life time τ_{major} was estimated as 0.90 s (fig.4) so that the intensity ratio of the major peak with and without saturation transfer is calculated as 0.73, in good agreement with the observed ratio of 0.67 (fig.3).

Finally it may be noted that the minor resonance of the Dha C_β proton resonance (H_B) is shifted downfield by as much as 1.1 ppm from the major peak (5.29 ppm). This large shift is probably due to the anisotropic deshielding effect [8] of the carbonyl group of Dha in the minor conformer.



Accordingly, the internal rotation about the C_α–CO bond of Dha, at least, is involved in the conformational change of the cyclic tetradepsipeptide ring of AM toxin II.

In conclusion, the combination of the saturation transfer analysis and line shape analysis as carried out in the present study on AM toxin II will also be useful for the analyses of conformational equilibria with very unequal populations and conformational transitions of other cyclic peptide molecules.

Acknowledgements

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References

[1] Ueno, T., Nakashima, T., Hayashi, Y. and Fukami, H. (1975) Agr. Biol. Chem. 39, 2081–2082.
[2] Ueno, T., Nakashima, T., Hayashi, Y. and Fukami, H. (1975) Agr. Biol. Chem. 39, 1115–1122.
[3] Okuno, T., Ishita, Y., Sawai, K. and Matsumoto, T. (1974) Chem. Lett. 635–638.
[4] Anet, F. A. L. and Basus, V. J. (1978) J. Mag. Res. 32, 339–343.
[5] Okazawa, N. and Sorensen, T. S. (1978) Can. J. Chem. 56, 2737–2742.
[6] Gutowsky, H. S. and Holm, C. H. (1956) J. Chem. Phys. 25, 1228–1234.
[7] Forsén, S. (1963) J. Chem. Phys. 39, 2892–2901.
[8] Jackman, L. M. (1959) in: Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, Pergamon, London.