THE CONFORMATIONAL STUDIES OF PLIPASTATIN A1 BY 400 MHz PROTON MAGNETIC RESONANCE

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Plipastatin A1 has been isolated from the culture of *Bacillus cereus* BMG302-fF67 and is an analog of plipastatins, acylpeptide inhibitors of phospholipase A_2[1~3]. Plipastatin A1 is a cyclic acyldecapeptide consisting of 3(*R*)-hydroxyhexadecanoic acid, L-Glu, D-Orn, L-Tyr, D-allo-Thr, L-Glu, D-Ala, L-Pro, L-Gln, D-Tyr and L-Ile. A lactone linkage is proved between the carboxyl of *C*-terminal L-Ile and the phenyl hydroxyl of L-Tyr.

In this paper, we propose the conformational model of plipastatin A1 (Fig. 3) by means of recent NMR techniques, high field NMR system, COSY and NOESY. The temperature dependence and hydrogen-deutrium exchange of amide protons are also studied. There are two β-turns formed from D-Orn to L-Tyr and L-Pro to L-Gln.

The elucidation of the solution structure of small biological polypeptides using ^{1}H NMR spectroscopy have been attempted by many workers[4~9]. This analysis involves identification of the $J_{NC}\alpha$ coupling constant with individual amino acids in the peptide and correlating these with the peptide dihedral angle ϕ. The hydrogen bond and/or solvent-shielded nature of peptide amide protons are derived from exchange and temperature-dependent studies.

Recent ^{1}H NMR studies have shown that the β-turn associated with antiparallel pleated sheet structures is a common conformation feature of several cyclic polypeptide antibiotics. In ^{1}H NMR parameters, the β-turn is characterized by an amide proton resonance that is shifted to high field because of the magnetic anisotropy of the vicinal peptide moiety, and has a low temperature coefficient due to intramolecular hydrogen bonding. Signals with a small $J_{NC}\alpha$ coupling constant, reflecting the vicinal dihedral angle at the corner, are also observed.

The spectrum of plipastatin A1 at 400 MHz (50°C, 17 mM in DMSO-d_6, Jeol JNM-GX400 NMR spectrometer) is shown in Fig. 1. Nine amide proton resonances are observed in the region 6.60~8.87 ppm downfield from internal TMS. Three of these are shifted to high field and superimposed on the tyrosine ring proton resonances. The assignments indicated in the spectrum are based on the results of the following 2D-NMR (^{1}H-^{1}H and ^{1}H-^{13}C COSY) and decoupling experiments. The respective amide proton resonances were linked and correlated with the corresponding α-CH protons (the $J_{NC}\alpha$ are 7.5, br d, 10.0, 6.8, 8.4 and 8.1 Hz from lower to higher field respectively for the six lower field resonances). All amide protons were then exchanged by deuterium to eliminate α-CHNH splitting. Spin decoupling between α-CH and β-CH protons was also used to identify specific residues. The β-CH resonances of fatty acid and allo-threonine were confirmed by this procedure. The assignments of two COOH resonances of glutamic acids and NH_2 of ornithine remains to be solved. The other assignments are illustrated on the spectrum.

The temperature dependence of amide proton chemical shifts (Fig. 2) shows that the five resonances (allo-Thr, ^{9}Tyr, Gln, ^{1}Glu and Ala) are less temperature dependent, whereas the remaining four (Orn, ^{5}Glu, ^{3}Tyr and Ile) have approximately the same dependence as that of the amide proton of *N*-methyl acetamide (NMA). The temperature coefficients ($\times 10^{-3}$ ppm/°C) were allo-Thr 0.3, ^{9}Tyr 1.5, Gln 2.4, ^{1}Glu 2.8, Ala 1.9, Orn 3.3, ^{5}Glu 4.4, ^{3}Tyr 4.1, Ile 4.3 and NMA 4.8. This indicates that the amide protons of the former five amino acid residues are intramolecularly hydrogen bonded or buried. Dreiding model examination indicated that the stable conformation due to the hydrogen bonds between the NH of allo-Thr and the CO of ^{1}Glu and between NH of ^{9}Tyr and CO of Ala enable the formation of characteristic β-loops. Hydrogen bonds between the NH of ^{1}Glu and the CO of allo-Thr and between NH of Ala and CO of ^{9}Tyr are then formed possibly to stabilize the respective β-loops. The NH of Gln may be shielded by the CO of Gln and the aromatic portion of ^{9}Tyr.

Consistent with this interpretation is the hydrogen-deuterium exchange of the amide protons at 23°C in 10% D_2O - DMSO. The order

Fig. 1. ^{1}H NMR spectrum of plipastatin A1 in DMSO-d_6.

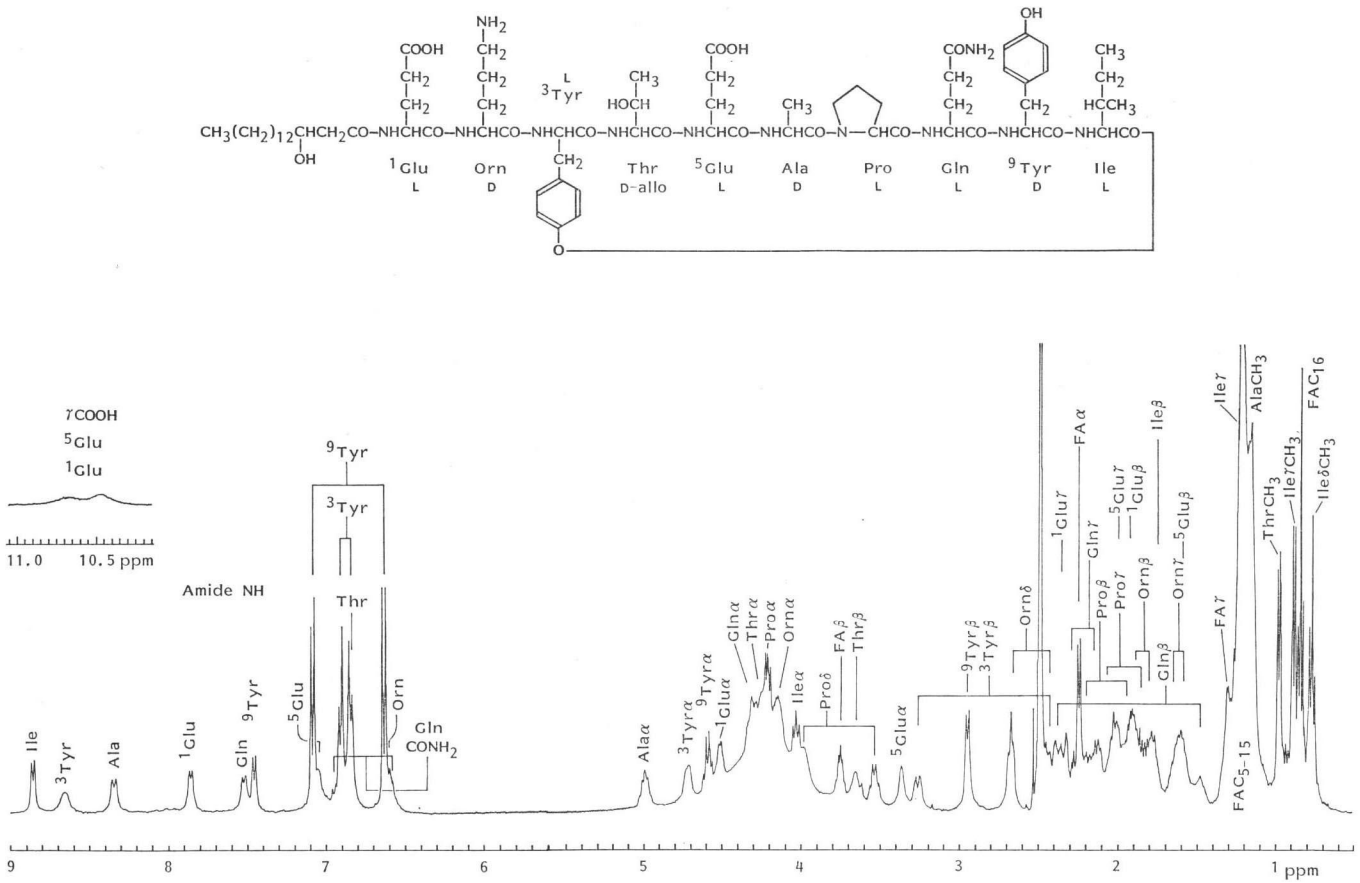
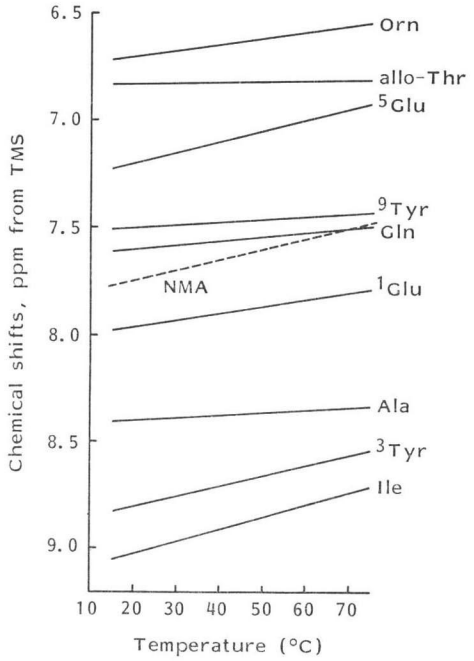


Fig. 2. Temperature dependence of amide proton resonances for plipastatin A1.



for exchange was ^{3}Tyr=Orn=Ile=^{1}Glu>^{5}Glu≫^{9}Tyr>Gln>Ala; protons of ^{3}Tyr, Orn, Ile and ^{1}Glu exchanged within 4 minutes, ^{5}Glu took 10 minutes, ^{9}Tyr took 40 minutes, and Gln and Ala took approximately 60 minutes. The exchange of allo-Thr proton was obscure because of overlapping on the tyrosine ring proton resonances.

In the NMR study of antamanide in solution, PATEL[8] summarized, for a conformation containing *cis* and *trans* X-Pro peptide bonds, that the proline H^{α} resonance is a doublet at 4.26~4.75 ppm ($J_{H^{\alpha}H^{\beta}} = 7\sim8$ and ~1 Hz) for the *cis* X-Pro peptide bond and a multiplet at higher field, 4.2~4.4 ppm, for the *trans* X-Pro peptide bond. The proline H^{α} resonance of plipastatin Al was a multiplet at 4.22 ppm which suggested a *trans* Ala-Pro peptide bond.

In the case of gramicidin S-A[4], hydrogen bonding of the valine amide proton to the carbonyl oxygen of leucine forms a β-loop from D-Phe to L-Pro. The $J_{NC}\alpha$ value of the Phe residue was small (<1 Hz) indicating that the dihedral angle (ϕ) was small (about $-30°$, nearly *cis*). In plipastatin Al, hydrogen bonding of the allo-Thr-NH to ^{1}Glu-CO supported to form a β-loop from D-Orn to L-Tyr. Because the $J_{NC}\alpha$ value of the Orn residue was small (<1 Hz at 31°C in DMSO-d_6) showing that the ϕ angle is small (about $-30°$, nearly *cis*) and the $J_{NC}\alpha$ value of ^{3}Tyr residue was broad (likely large) suggesting a large ϕ angle (about $-150°$, nearly *trans*), the ϕ angle of ^{3}Tyr must be about $-30°$ (nearly *cis*). In addition, hydrogen bonding of the ^{9}Tyr-NH to Ala-CO supported to form a β-loop from L-Pro to L-Gln. Since ϕ angle of Pro is fixed at $-60°$ (nearly *cis*) and the $J_{NC}\alpha$ value of Gln residue was large (8.4 Hz) indicating a large ϕ angle (about $-150°$, nearly *trans*), the ψ angle of Gln must be about $-30°$ (nearly *cis*). Furthermore, the $J_{NC}\alpha$ value of ^{5}Glu residue was small (<1 Hz at 31°C in DMSO-d_6) suggesting that the dihedral angle is small (about $+30°$, nearly *cis*).

2D-NMR NOESY spectrum of plipastatin Al showed an obvious NOE (nuclear Overhauser effect) between δ 8.70 (^{3}Tyr-NH) and δ 6.87 (allo-Thr-NH), δ 7.54 (Gln-NH) and δ 7.47 (^{9}Tyr-NH), and other adjacent protons represented by dotted lines in Fig. 3. In proposed conformational model of plipastatin Al (Fig. 3), all amide configurations are *trans*. There are β-turns with hydrogen bonding between the amide proton of D-allo-Thr and the carbonyl of L-^{1}Glu and between the amide proton of D-^{9}Tyr and the carbonyl of D-Ala. One corner of the turn is occupied by D-Orn and L-Tyr; L-Pro and L-Gln are positioned at the other turn. The high field shift of the amide proton of Gln can be explained from this structure in terms of the ring current effect of the phenyl ring of D-^{9}Tyr and the interaction with the carbonyl of Gln. Such an interaction could cause the high field shift of this amide proton compared to the other non-hydrogen bonded protons and the slower rate of H-D exchange. The amide protons of ^{5}Glu and Ile which are not involved in either a β-loops or a hydrogen bond cannot be predicted by such a preliminary observation.

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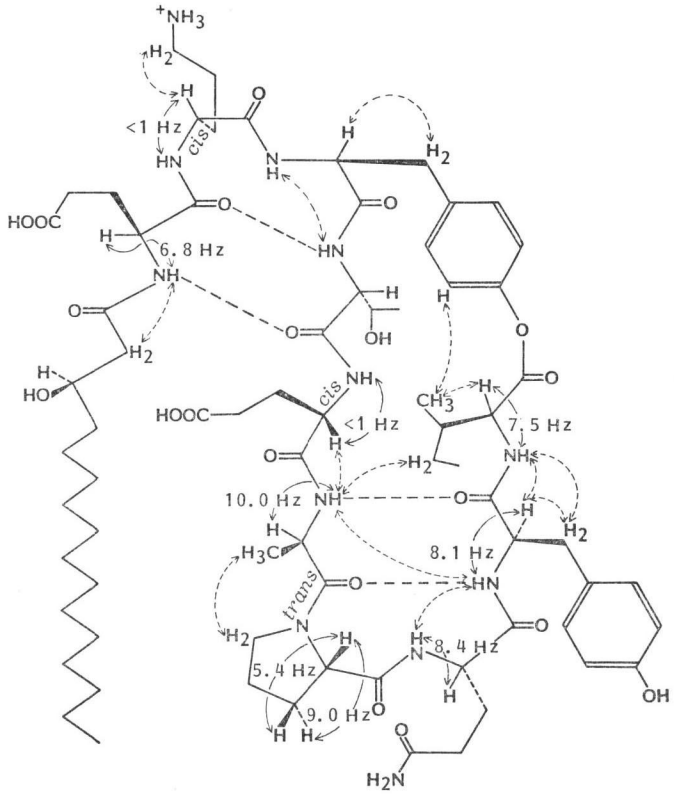
Fig. 3. A proposed conformational model of plipastatin A1.

Schematic drawing of model with ω angles all 180° except Ile CO-O≅−90°. ϕ angles of allo-Thr, ^{9}Tyr and Ile≅+150°, ^{5}Glu≅+30°, Orn≅−30°, Pro≅−60°, ^{1}Glu, ^{3}Tyr and Gln≅−150°. ϕ angles of ^{1}Glu and Ile≅+150°, ^{5}Glu≅+90°, Pro≅+60°, Orn≅+80°, ^{3}Tyr and Gln≅−30°, allo-Thr and ^{9}Tyr≅−150°.



O---H Hydrogen bonds

H<---->H Adjacent protons observed by 2D-NMR NOESY

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