CONFORMATIONAL RECOGNITION OF RA-XII BY 80S RIBOSOMES: A DIFFERENTIAL LINE BROADENING STUDY IN ^{1}H NMR SPECTROSCOPY

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^{1}H NMR spectroscopy has been used to demonstrate specific binding of rat 80S ribosomes to the major conformer of an antitumor bicyclic hexapeptidic glucoside, RA-XII, isolated from *Rubia cordifolia*, in a fast exchange process.

KEYWORDS RA-XII; conformation; 80S ribosome; line broadening; ^{1}H NMR

Antitumor bicyclic hexapeptides of RA series, RA-I - RA-XVI, isolated from *Rubia cordifolia* (Rubiaceae)[1] and the related compounds, bouvardins,[2] have been shown to inhibit protein synthesis through binding to eukaryotic 80S ribosomes, resulting in inhibition of aminoacyl-tRNA binding formation and peptidyl-tRNA translocation, which is presently considered to be the major cause of the antitumor activities of these agents.[3] In apolar solvents, such as $CDCl_3$, the major active principle of the RA series, RA-VII, takes two stable conformational states resulting from isomerization about the N-methyl amide bond between Ala-2 and Tyr-3, each conformer taking stable antiparallel conformation with two intramolecular hydrogen bondings between Ala-4 and D-Ala-1.[4]

The conformation of RAs in physiological aqueous solutions, however, has not been studied yet. Thus, we performed full assignments of ^{1}H NMR signals in aqueous sodium phosphate buffer (PH 7.4) of water-soluble hexapeptidic glucoside, RA-XII, which showed that RA-XII exists as a mixture of two conformers in a ratio of 7:3 at 303K. A combination of DQF-COSY, HOHAHA[5] and NOESYPH[6] spectra at 500MHz showed that the major conformer contains type II β-turn at Ala-2 and Tyr-3, as in solid state, and the minor conformer contains type VI β-turn with cis N-methyl amide bond between residues 2 and 3 (Fig. 1).

It has been known that in the presence of a minute amount of ribosome, the proton NMR signals of chloramphenicol and erythromycin A, well known as protein biosynthesis inhibitors, tend to become broad.[7] This is considered to be due to fast exchange of protons between the bound drug and the free drug. More extensive broadening was observed in the signals of protons involved in drug-ribosome binding.

In the present work, various amounts of washed rat liver ribosomes[8] were added to a 2 mM solution of RA-XII in deuteriated sodium phosphate buffer. The spectra of these solutions were taken at PH 6.0, 7.4 and 8.0. All experiments were repeated at least twice and were found to be perfectly reproducible.

The ^{1}H NMR spectra of RA-XII singly and of RA-XII in the presence of 1 μM ribosomes are shown in Figure 2. Figure 3 shows the signal profiles in the region δ 2.5

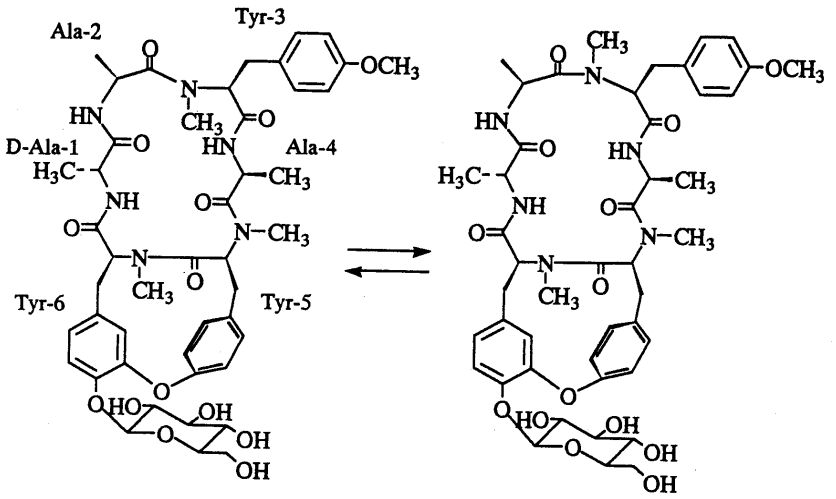

Fig. 1. Structures of RA-XII in Deuteriated Sodium Phosphate Buffer (PH 7.4); Left: major conformer, Right: minor conformer.

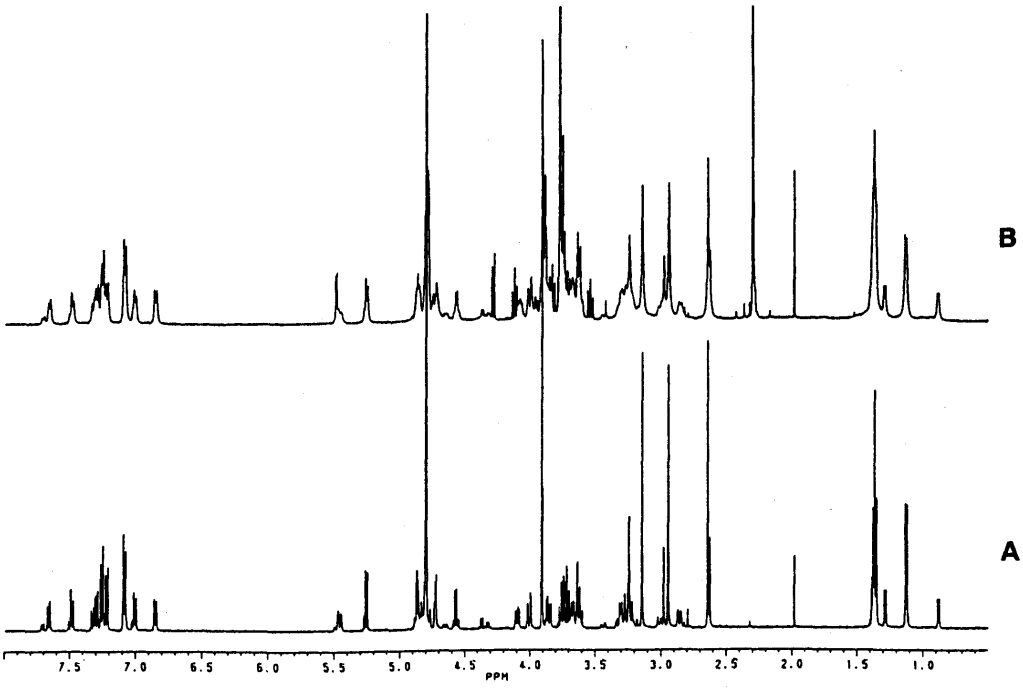

Fig.2. ^{1}H NMR Spectra of RA-XII (2 mM) in Deuteriated Sodium Phosphate Buffer, PH 7.4 (500MHz, 303K)
A:singly; B: in the presence of 1μM rat liver ribosomes.

- 3.4, in which are observed the singlets of three N-methyl groups of Tyr-3, -5 and -6. All the spectra were processed without weighting. The signals tend to broaden in the presence of ribosomes. Although the heights and widths of the signals due to the N-methyl groups of three Tyr of the major and minor conformers change with the addition of ribosomes, the ratios between the two signals in each pair in the presence of ribosome are about the same as those in the corresponding signal pairs in the absence of ribosome. The signal line broadening and height decrease were more obvious in the signals of the major conformer than in those of the minor conformer. However, the area of the Tyr-5 signal of the minor conformer could not be accurately estimated as it is overlapped by other signals, as shown in Fig. 3. The heights and linewidths (Hz) at half height of the three N-methyl signals are shown in Table 1. Furthermore, the broadening degrees could also be estimated by the proton T1 value[9] of each signal, calculated by using the inversion-recovery pulse sequence. The T1 values of the signals corresponding to the major conformer were apparently more affected than those of the minor conformer, as shown in Table I. Apparently, PH values, within the range tested, had little effect on the broadening of the signals.

These data clearly indicated that rat liver ribosomes form binding with RA-XII, preferentially of the major conformer, in a fast exchange process. Line broadening may be due to the rapid exchange between two states having protons of different

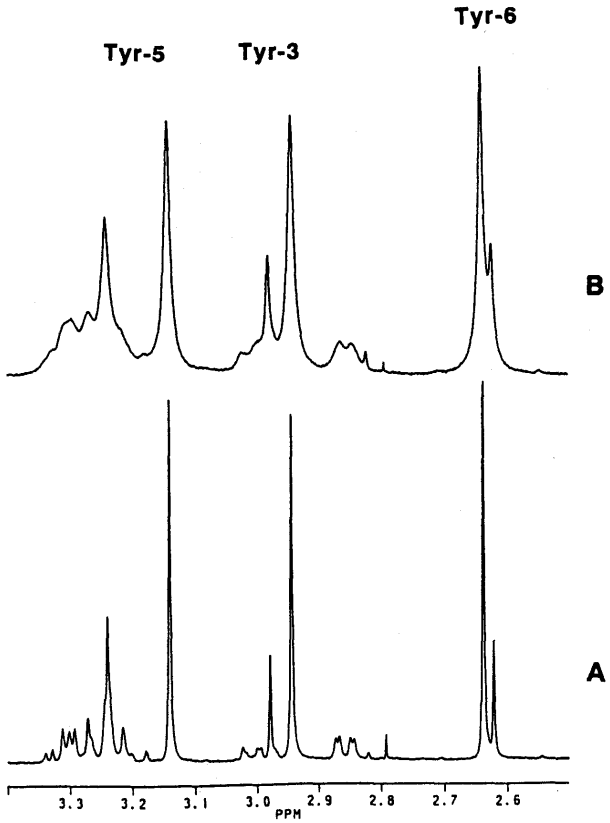

Fig. 3. ^{1}H NMR Spectra of RA-XII (δ2.5 - 3.4) Showing the Singlets of Tyr-3 N-Me, 5 N-Me and 6 N-Me
A: singly; B: in the presence of 1μM rat liver ribosomes.

chemical shifts, and line broadening is considered to be correlating with bind formation.

The present study allowed us to demonstrate the different conformational recognition of RA-XII by rat 80S ribosomes. More precise conformational analysis around the site of RA-XII major conformer, to which ribosome is attached, is now in progress.

Table I. ^{1}H NMR Signal Heights and Linewidths, and T1 Values of RA-XII Singly and in the Presence of Ribosomes

			Heights (cm)					
	δ (ppm)		Major			Minor		
Position	Major	Minor	A	B	A/B	A	B	A/B
Tyr-3 N-Me	2.94	2.98	13.8	11.1	1.24	4.1	4.0	1.03
Tyr-5 N-Me	3.14	3.24	14.5	11.4	1.28	5.6	5.0	1.12
Tyr-6 N-Me	2.64	2.62	15.2	14.5	1.05	4.8	6.1	0.79

			Linewidths at half height (Hz) spectrum					
	δ (ppm)		Major			Minor		
Position	Major	Minor	A	B	ΔHz	A	B	ΔHz
Tyr-3 N-Me	2.94	2.98	1.6	5.4	3.8	1.4	3.3	1.9
Tyr-5 N-Me	3.14	3.24	1.6	5.6	4.0	2.0	5.4	3.4
Tyr-6 N-Me	2.64	2.62	1.6	5.1	3.5	1.4	4.0	2.6

			T1 values (ms)					
	δ (ppm)		Major			Minor		
Position	Major	Minor	A	B	A/B	A	B	A/B
Tyr-3 N-Me	2.94	2.98	848	602	1.41	1078	819	1.31
Tyr-5 N-Me	3.14	3.24	830	597	1.39	580	576	1.00
Tyr-6 N-Me	2.64	2.62	1000	646	1.55	1029	773	1.33

A: RA-XII singly B: RA-XII in the presence of ribosomes.

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9) The longitudinal relaxation times (T1) reported in Table I were obtained by using six τ values (100, 200, 400, 600, 1000 and 3000 ms), and standard deviations were in the range of 0.004 to 0.057 ms.

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