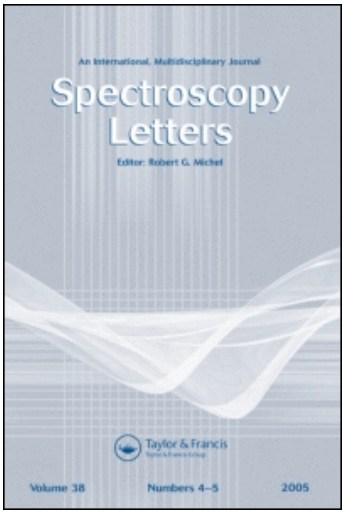

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Assignments of Nonexchangeable Proton Resonances and the Solution Structures of d-TGGGT

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ASSIGNMENTS OF NONEXCHANGEABLE PROTON RESONANCES and the SOLUTION STRUCTURES of d–TGGGT

KEY WORDS: d–TGGGT, 2D–NMR, proton resonance assignment, conformation, single strand, tetramolecular complex

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ABSTRACT

In this paper, we report the studies of the solution structures of synthetic pentadeoxyribonucleotide d–TGGGT(NH_4^+ salt) using 2D–NMR. The 1H–NMR experiments with different temperatures and concentrations reveal an equilibrium between single strand and aggregation. In the experimental condition(22°C, 13mmol / L), the cross peaks in the COSY spectrum are mainly from single strand, and the spin systems of sugar resonances of this component can be assigned. In contrast, the cross peaks in the NOESY spectrum mainly come from aggregation and the sequential assignments of bases, sugar 1′, 2′ and 2″ protons can be carried out. From NOE connectivities, it is obvious that the aggregation adopts a right–handed helix conformation. It is suggested that the aggregation in our experiment corresponds to the tetramolecular complex.

INTRODUCTION

It has been known that the G–rich DNA fragment, through self–association, can form the four–stranded complex stabilized by a full complement of Hoogsteen hydrogen bonding(Fig.1)[1,2]. With nitrogen–15–labeled method, Jones et al[3]found

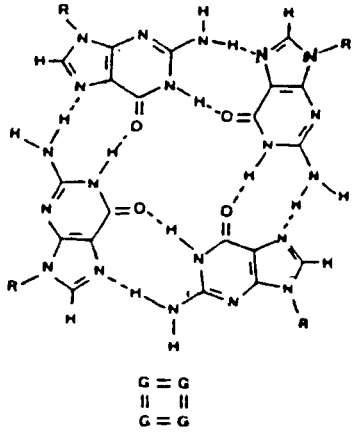

Fig.1 Model structure of a Hoogsteen hydrogen–boned guanine tetrad.

pentadeoxyribonucleotide d–TGGGT is able to form tetramolecular complex but they did not obtained proton resonance assignments and the solution structures of single strand and the tetrad. The purpose of this study is to use 2–dimensional proton NMR to assign the nonexchangable proton resonances and analyse the solution structures of d–TGGGT.

EXPERIMENTAL

The synthesis and purification of pentadeoxyribonucleotide d–TGGGT(NH_4^+) were described elsewhere[4]. The lyophilized sample was dissolved in D_2O(unbuffered, PH5.8) at a concentration 8 or 13mmol / L. All NMR spectra were recorded on a Bruker AM–500 spectrometer. One dimensional NMR spectra were measured with 16K data points. Prior to Fourier transformation, NMR spectra were improved by a sine bell and F1 dimension was zero–fulled. The suppression of residual HOD resonance was carried out by presaturation method. The COSY spectrum was recorded with a sequence: D_1–90°–t_1–60°–t_2, $D_1 = 1.8$s, NS = 32, $t_1 = 256$, $t_2 = 1024$. After Fourier transformation, the spectrum was further improved by symmetrization. NOESY spectrum was recorded by phase sensitive mode(TPPI) with a sequence: D_1–90°–t_1–90°– τ_m–90°–t_2, $D_1 = 2$s, $\tau_m = 500$ms, NS = 48, $t_1 = 230$, $t_2 = 1024$. Chemical shifts are expressed relative to 4,4–dimethylsilapentane– l–sulfonate(DSS).

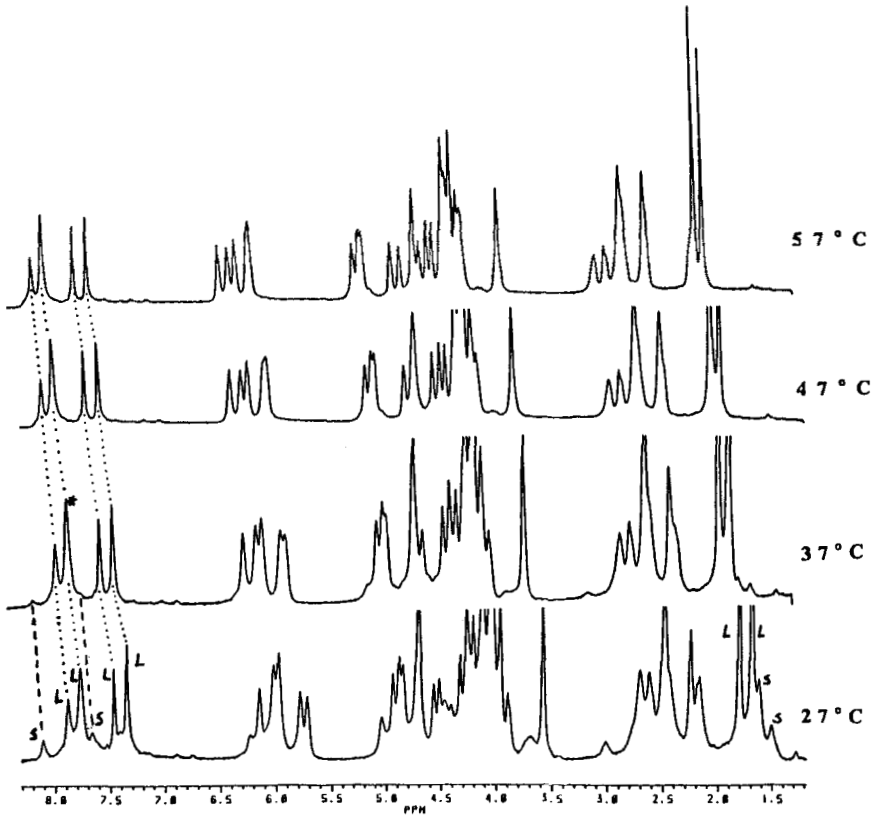

Fig.2 500MHz 1H–NMR Spectra of d–TGGGT (D_2O, PH5.8, 8mmol / L) at various temperatures. L and S correspond to the resonances from "L" and "S" components respectively.

RESULTS and DISCUSSION

Figure 2 shows one–dimensional spectra at various temperatures(8mmol / L). Because there are five residues(three G and two T), there should be five proton peaks betwen 7.2 and 8.3 ppm. In figure 2 , the four peaks, one of which (labelled with asterisk) has a intensity corresponding to two protons, are observed obviously when the temperature is raised to 37°C or higher. However, at 27°C, besides the above four peaks(subscripted L), there are some peaks with less intensity(subscripted S). Figure 3 shows the changes of spectra with temperature when the concentration is up to 13mmol / L. It is seen that two components("L" and "S") coexist in

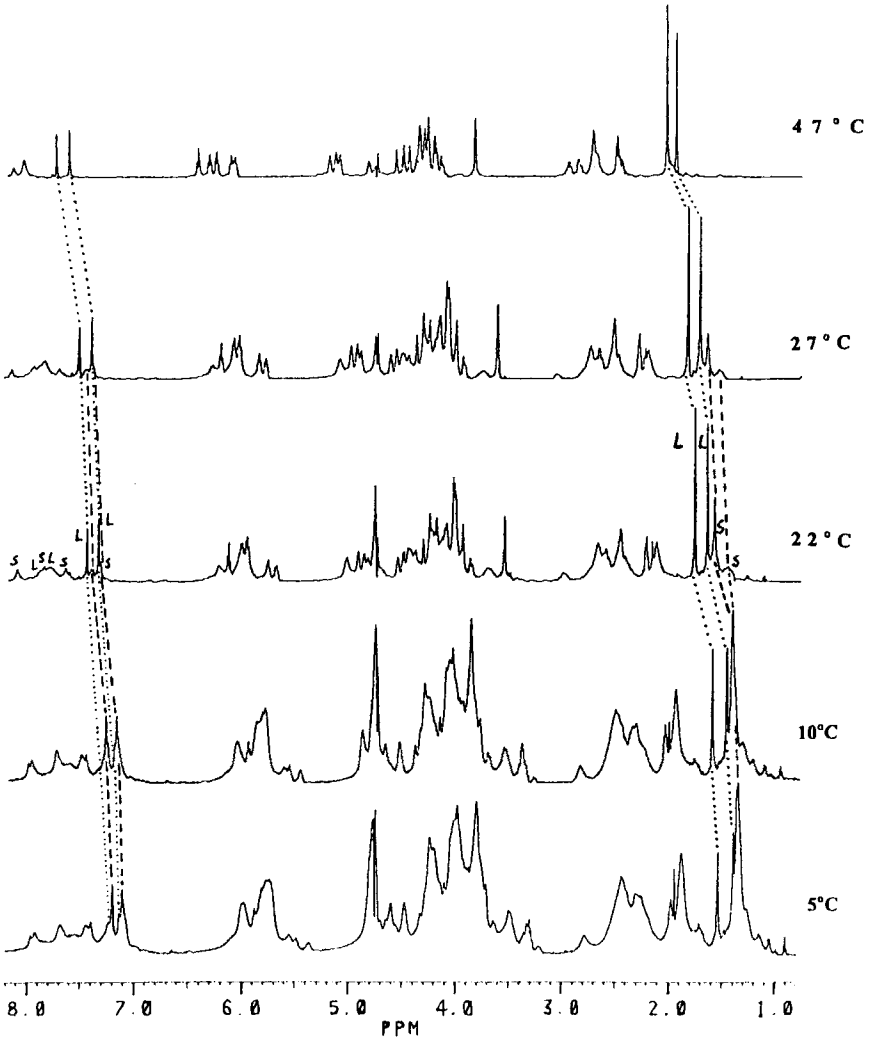

Fig.3 500MHz 1H–NMR spectra of d–TGGGT (D_2O, PH5.8, 13mmol / L) at various temperatures

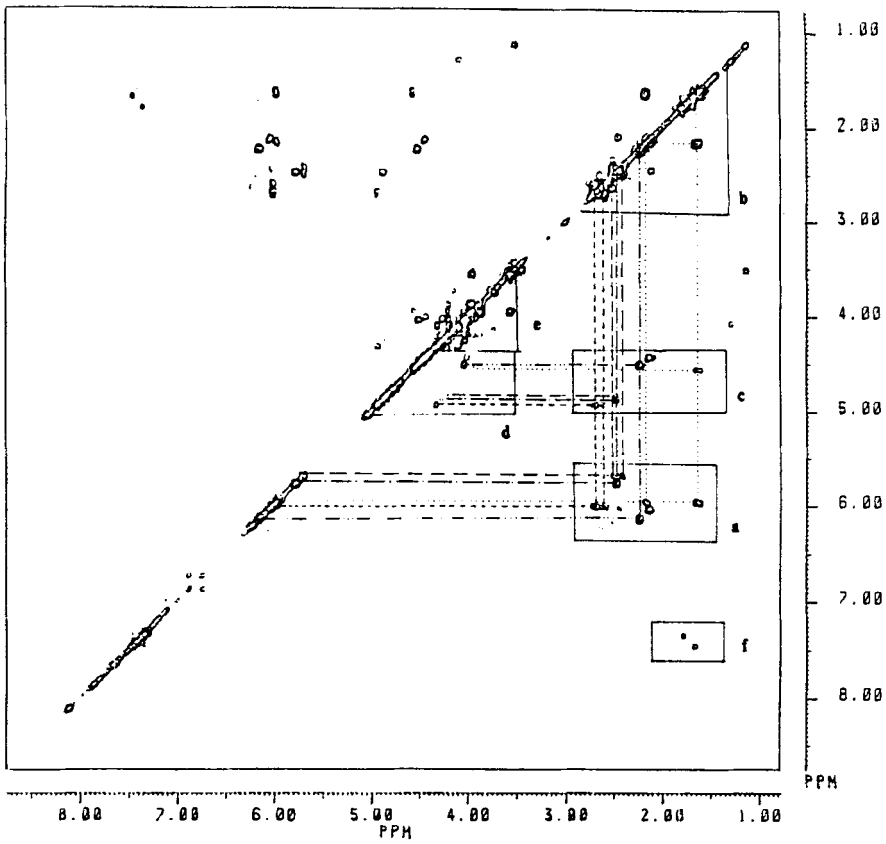

Fig.4 500MHz COSY spectrum of d–TGGGT(22°C, 13mmol / L) The cross peaks corresponding to the H1′–H2′ / H2″, H2′–H2″, H2′ / H2″–H3′, H3′–H4′, H4′–H5′ / H5″, CH_3–H6 of T connectivities are indicated by boxed regions marked a–f respectively.

the systems at 27°C or lower temperature. When the temperature was lowered to 5°C, "S" component become major.

The two–dimensional COSY spectrum of 13mmol / L D_2O solution at 27°C is shown in figure 4 and permits the grouping of suger resonances to spin systems along the intranucleotide pathway H1′—H2′ / H2″—H3′—H4′—H5′ / H5″[5,6]. In addition, the connectivities between the H6 and methyl protons of the T residues via their four bond spin–spin coupling is readily observed in the COSY spectrum. Comparing the COSY spectum with the one–dimensional

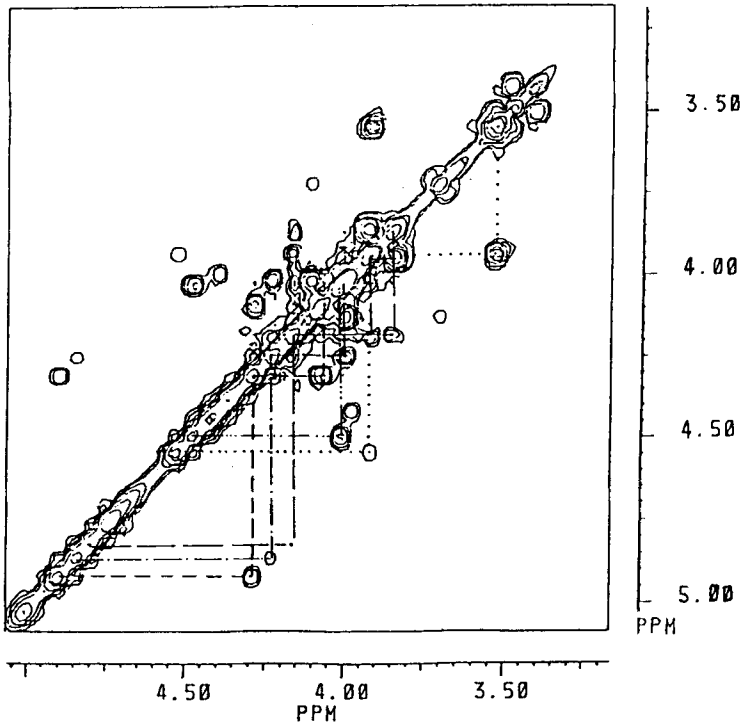

Fig.5 Expanded spectral regions d and e of figure 4. The experiment condition is same as in figure 4.

spectrum, we found the cross peaks in the COSY spectum are mainly from the major component "L". Figure 4 may be divided to a–f six regions containing the following connectivities:

a: H1'—H2' / H2", b: H2'—H2", c: H2' / H2"—H3'
d: H3'—H4', e: H4'—H5' / H5", f: CH_3—H6 of T

First, the spin systems of two thymine bases can be assigned from the cross peaks in f region, but we cannot distingush that which of the spin systems belongs to T1 or T5, and their distinction can only be made from the cross peaks in NOESY spectrum(vide infra).

Based on tne connectivities in a–e regions, the assignments of the five spin systems of sugars can be carried out. Figure 4 shows the connectivities between H1' and H2' / H2" and H3' protons of "L" component. The connectivities between H4' and H5' are shown in figure 5 .

TABLE 1 Spin system assignments for "L" component of d–TGGGT[a](ppm)

	H1′	H2′	H2″	H3′	H4′	H5′	H5″	H6	CH_3
T1	5.97	1,61	2.13	4.48	3.87	3.48	3.48	7.33	1.78
G_A	5.75	2.45	2.45	4.80	4.17	3.95	3.95		
G_B	5.67	2.49	2.38	4.75	4.11	3.87	3.80		
G_C	6.01	2.61	2.54	4.87	4.23	4.02	4.02		
T5	6.12	2.23	2.23	4.42	3.95			7.43	1.64

a: D_2O, PH5.8, 13mmol / L, 22°C

However, it is impossible to assign the spin systems to particular residue from the COSY spectrum alone. Some rules can be used to aid the resonance assignments[7,8]. When the phosphate group is bonded to a carbon of deoxyribose, the corresponding proton is shifted to lower fields. The initial residue(T1) is characterized by H5′ / H5″ resonances which is located upfield from the other H5′ / H5″ resonances and the terminal residue(T5) by H3′ upfield from the other H3′ resonances. The H2′and H2″ resonances can be distinguished on the basis of its chemical shifts: $\delta_{H2''} > \delta_{H2'}$(pyrimidine residue), $\delta_{H2''} < \delta_{H2'}$(purine residue). The assignments of spin systmes of "L" component are listed in table 1. Here, any one of three G residue spin systems has not been given a precise assignment to G2, G3 or G4. In c region of figure 4, the three residues T1, G_B, G_C are characterized by that H3′–H2′ cross peaks are more intense than H3′–H2″, therefore, the sugars of the three residues adopt $C_{2'}$–endo pucker conformations[5].

NOESY spectrum can provide the intra– and internucleotide proton–proton distances and the NOE connectivities in NOESY spectrum have much to do with DNA conformation.Figure 6 lists all interproton connectivities(distance $<$ 5Å) which are applicable to right–handed DNA[6]. Figure 7 shows the part of NOESY spectrum(22°C, 13mmol / L). In contrast to the condition in the COSY spectrum, the cross peaks in the NOESY spectrum are mainly from "S" component. It is necessary to make a distinction between the H2′ and H2″ resonances before the sequential resonance assignments. The intranucleotide NOEs between the H1′ and H2″ is usually larger and can never be smaller than that between the H1′ and H2′ for all sugar pucker conformations. Once H2″ have be assigned, the sequential resonance assignments can be made on the basis of NOEs connectivities between the H8 / H6 and H2″ protons(Fig. 7). Methyl protons of T1 and T5 can be distigushed from NOEs between them and H8 / H6. For the terminal residue(T5), there are two NOE cross peaks, one is from intranucleotide and another is from internucleotide between methyl proton of T5 residue and H8 proton of G4 residue. However, for the initial residue(T1), there is only one NOE cross peak that is from intranucleotide. Table 2 lists the proton resonance assignments of "S" component. In figure 7, the NOE connectivities between H2″ and H8 / H6 are consistent with the NOE connectivities criteria of right–handed

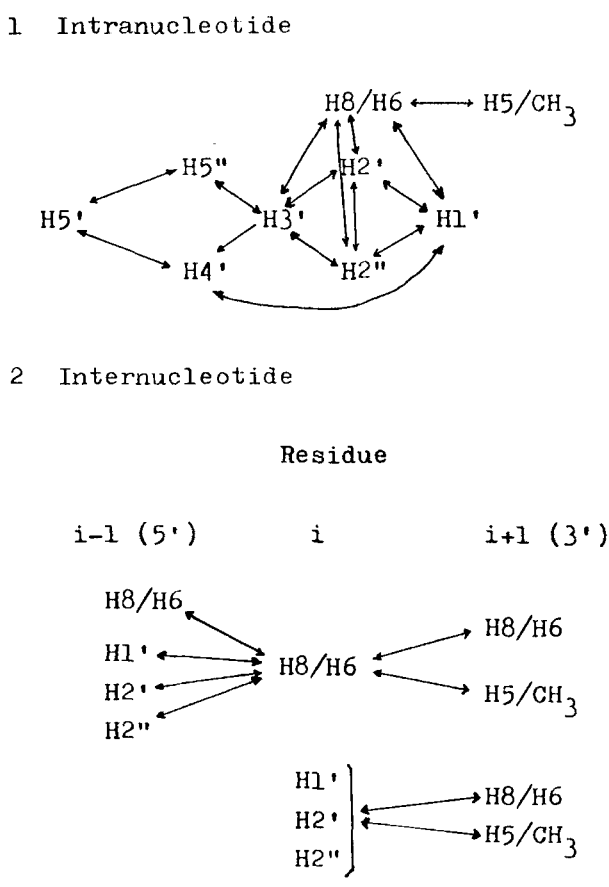

Fig.6 Schematic illustration of intra– internucleotide interproton distances with values of < 5Å in right–handed DNA which form the basis of the sequential resonance assignment procedure.

helix conformation in figure 6, therefore, "S" component adopts a right–handed helix conformation.

With different mixing times, the sufficient NOE connectivities of "L" component have not been obtained and the NOEs in NOESY spectrum are mainly from the minor component "S". It is because that "L" corrosponds to single strand and the larger flexibility and various conformations of "L" component make NOEs appear difficutly. Besides the NOEs of "S" component, the

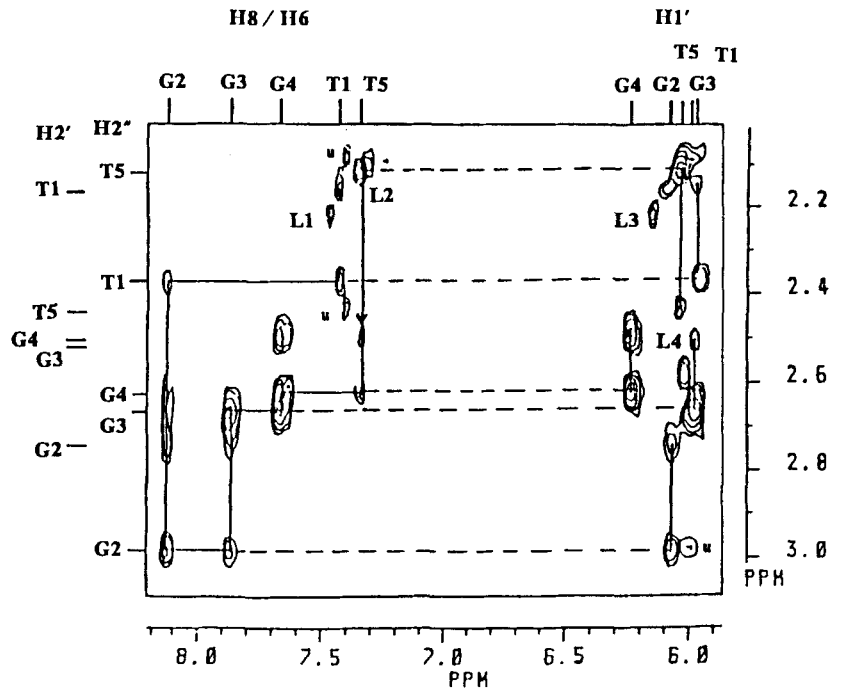

Fig.7 Sequential resonance assignments using NOESY connectivities between H2′ / H2″ and H8 / H6 or H1′. (D_2O, PH5.8, 13mmol / L, 22°C, $\tau_m = 500$ms). The NOE cross peaks from "L" component and very minor component "u" are labelled L and u respectively. L1:H6–H2″(T5), L2:H6–H2″(T1), L3:H1′–H2″(T5), L4:H1′–H2″(G_C)

TABLE 2 Nonexchangable base and sugar proton chemical shifts for "S" component (ppm)[a]

	H8 H6	CH_3	H1′	H2′	H2″
T1	7.42	1.49	5.96	2.15	2.37
G2	8.13		6.07	2.75	2.98
G3	7.87		5.98	2.50	2.66
G4	7.67		6.24	2.49	2.62
T5	7.32	1.57	6.04	2.43	2.11

a: D_2O, PH5.8, 13mmol / L, 22°C

NOEs from "L" component and very minor component "u" which disappears on raising the temperature to 47°C are also observed. As noted in figure 7 caption , the cross peaks labelled L1 and L2 are from the NOEs connectivities between H6 and H2" protons of T5 and T1 residues of "L" component respectively, therefore the assignments of the spin systems in f region of figure 4 can be made(Table 1).

The G-rich DNA fragment can form tetramolecular complex in which G binds to each other through Hoogsteen hydrogen bonding(Fig.1). Recently, R. A. Jones et al [3]have used 15N-NMR to find the formation of tetraplx in d-TGGGT. In this paper, the 1D, 2D proton NMR experiments confirm an equilibrum between single strand and aggregation in d-TGGGT solution. It seems that "S" component in our experiment corresponds to the tetramolecular complex.

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