^{15}N nuclear magnetic resonance studies of the B domain of *Staphylococcal* protein A: sequence specific assignments of the imide ^{15}N resonances of the proline residues and the interaction with human immunoglobulin G

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^{15}N nuclear magnetic resonance (NMR) studies of the B domain (FB) of *Staphylococcus* protein A, which is uniformly labeled with ^{15}N, are reported. The α CH(i)-^{15}N(i) connectivity in the ^{1}H-^{15}N HMBC spectrum and the ^{13}C(i-1)-^{15}N(i) spin coupling in the ^{15}N spectrum of a ^{13}C-, ^{15}N-doubly labeled FB were used to establish the assignments of the imide ^{15}N resonances for all the three Pro residues that exist in FB. Addition of human IgG caused a significant downfield shift of the Pro-39 resonance. This result is quite consistent with our previous suggestion that a significant conformation change is induced in the Ser-42-Ala-55 helical region of FB when it is bound to human IgG.

^{15}N-labeling; Proline residue; ^{1}H-^{15}N HMBC; Double labeling; Recombinant B domain of protein A; Immunoglobulin G

1. INTRODUCTION

Protein A is a cell wall component of *Staphylococcus aureus*. It binds specifically to the Fc portion of immunoglobulin G (IgG) from various mammals [1]. Protein A contains a tandem of 5 highly homologous domains designated as E, D, A, B, and C, each of which is able to bind to the Fc region of IgG [2–4].

We have examined the solution conformation of the recombinant B domain (FB) comprising 60 amino acid residues [5] by using two-dimensional proton nuclear magnetic resonance (NMR) spectroscopy [6], and shown that the free FB in solution contains three helical regions, Glu-9–His-19, Glu-25–Asp-37, and Ser-42–Ala-55. The three Pro residues exist at positions 21, 39, and 58, flanking each of these three helices [6]. On the basis of a crystallographic stsudy of the complex between FB and the Fc fragment of human polyclonal

IgG [7], it has been suggested that a significant conformation change is induced in the Ser-42–Ala-55 helical region of FB when it is bound to Fc [6]. Further attempts using ^{1}H-NMR faced a serious difficulty due to the large size of the FB-IgG complex, which gave overlapped and inappropriately broad signals.

In the present study we followed the imide ^{15}N signals of the three Pro residues using uniformly ^{15}N labeled FB. The observed ^{15}N resonances are sufficiently sharp presumably due to the low gyromagnetic ratio of the ^{15}N nucleus. All of the three ^{15}N resonances have been assigned. The ^{15}N-NMR data obtained will be used along with our previous ^{1}H-NMR data [6] to discuss the interaction of FB with IgG in solution.

2. MATERIALS AND METHODS

2.1. Preparation of stable-isotope labeled FB

A chemically synthesized gene for FB has been cloned and expressed in *Escherichia coli* by Saito et al. [5]. In order to label FB uniformly with ^{15}N, *E. coli* W3110 (prototroph) containing the expression plasmid for FB was grown in M9 minimal medium [8] using ^{15}NH$_4$Cl (Shoko Co., Ltd.) as the sole nitrogen source. The uniformly labeled FB will hereafter be designated as [^{15}N]FB. *E. coli* GC4670 (lon::Tn10, thr, leu, lacY) harboring the same expression plasmid for FB was also grown in M9 minimal medium using ^{15}NH$_4$Cl and [1-^{13}C]Leu (Cambridge Isotope Laboratories) in order to label FB uniformly with ^{15}N and, at the same time, selectively with [1-^{13}C]Leu. The doubly labeled FB will be designated as [^{13}C,^{15}N]FB. These stable-isotope labeled proteins were purified as described previously by Saito et al. [5] with a slight modification.

2.2. *Preparation of human myeloma protein IgG1(x) Ike-N*

Human myeloma protein IgG1(x) Ike-N was purified from the plasma of a patient with multiple myeloma as described previously by Endo and Arata [9].

2.3. *NMR measurements*

For NMR analyses, purified and lyophilized stable-isotope labeled FB and human myeloma protein IgG1(x) Ike-N were dissolved in 90% H_2O/10% D_2O, or 99.8% D_2O, at pH 5.0 for NMR analyses. Each NMR sample had a total volume of 0.4 ml in a 5 mm NMR tube. One dimensional ^{15}N-NMR experiments were performed at 30°C on a JEOL JNM-GSX 400 or a Bruker AM 400 spectrometer. Two dimensional ^{1}H-detected ^{15}N multiple bond correlation (HMBC) experiment was performed at 30°C on a Bruker AM 400 spectrometer with 256 t_1 increments of 2K data points each. The delay time set to 70 ms, slightly less than $1/2(^2J_{NH})$. Nitrogen chemical shifts were measured using formamide as a secondary standard (112.4 ppm). Proton chemical shifts were determined relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

3. RESULTS AND DISCUSSION

Fig. 1A shows part of the ^{15}N-NMR spectrum of [^{15}N]FB measured at 30°C in 90% H_2O/10% D_2O, pH 5.0. Three singlet peaks a, b, and c, which are located at 138.68 ppm, 137.40 ppm, and 136.98 ppm, respectively, are due to the imide ^{15}N resonances of the three Pro residues that exist in FB. Due to the absence of the amide proton in the Pro residue, sequence specific assignments of these resonances cannot be achieved by measurements of ^{1}H-detected ^{15}N multiple quantum coherence (HMQC) spectra [10–15]. Therefore, ^{1}H-
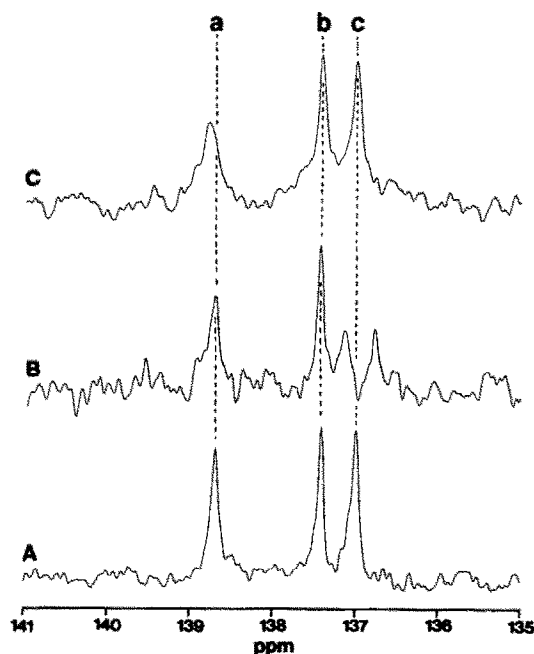
detected ^{15}N multiple bond correlation (HMBC) spectroscopy [16] was used in combination with a double labeling method [17] for the assignments of the imide ^{15}N resonances.

Fig. 2 shows part of the ^{1}H-^{15}N HMBC spectrum of [^{15}N]FB, in which the intraresidue two-bond scalar connectivities between CH(i) and ^{15}N(i) atoms of the Pro residues can be observed. The ^{15}N chemical shifts of cross peaks a and b are identical with those of peaks a and b shown in Fig. 1A, respectively. Analyses of proton two-dimensional spectra of FB have shown that Pro-21, Pro-39, and Pro-58 gave the αCH proton resonances at 4.38 ppm, 4.48 ppm, and 4.41 ppm, respectively [6]. Thus the ^{1}H-^{15}N HMBC data establish sequence-specific assignments of resonances a and b to Pro-39 and Pro-58, respectively. This also means that resonance c originates from Pro-21. In the HMBC spectrum, however, the cross peaks of the αCH-^{15}N connectivity for Pro-21 cannot be observed, probably due to the broad linewidth of the αCH proton resonance. The connectivities between δCH_2(i) and ^{15}N(i) atoms for the three Pro residues cannot be observed. This is probably because the δCH(i) and δ'CH(i) proton resonances are low intensity due to the complex spin-coupling pattern.

In order to assign resonance c more directly, we used a doubly labeled protein, [^{13}C,^{15}N]FB. Fig. 1B shows the same region as Fig. 1A of he ^{15}N-NMR spectrum of [^{13}C,^{15}N]FB. Resonance c is split into doublet, due to the ^{13}C-^{15}N coupling between the carbonyl ^{13}C of Leu and the imide ^{15}N of Pro ($^1J_{CN}$ = 15 Hz). Since there

Fig. 1. The imide resonance region of the ^{15}N-NMR spectra measured at 30°C in 90% H_2O/10% D_2O, pH 5.0. (A) 4 mM [^{15}N]FB; (B) 2 mM [^{13}C,^{15}N]FB; (C) 3.6 mM [^{15}N]FB and 0.3 mM human myeloma protein IgG1(x) Ike-N. These spectra were obtained with a spectral width of 20 kHz and a data point of 64K. The acquisition time was 1.64 s. Chemical shifts are referenced to formamide.
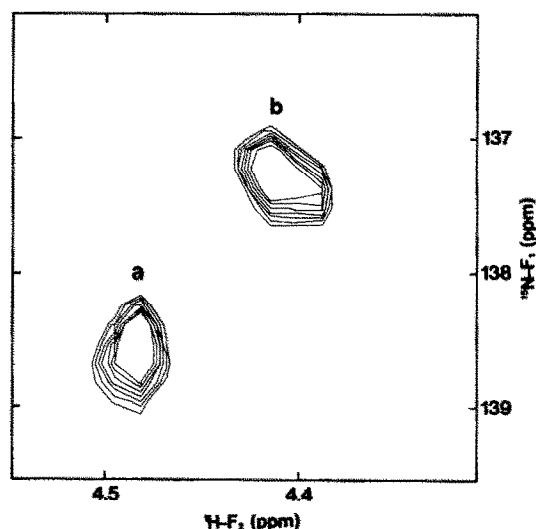
Fig. 2. The aliphatic (F_2 axis)-^{15}N F_1 axis) region of the ^{1}H-^{15}N HMBC spectrum of [^{15}N]FB measured at 30°C in D_2O, pH 5.0. The spectral width in F_2 dimension was 5620 Hz, and the spectral width in F_1 dimension was 850 Hz. ^{1}H chemical shifts are referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). ^{15}N chemical shifts are referenced to formamide. Cross peaks arise from the two-bond correlation peaks. Two peaks labeled a and b are due to αCH(i)-^{15}N(i) connectivities for Pro-39 and Pro-58, respectively.

is only one Leu-Pro combination in FB, resonance c can unambiguously be assigned to Pro-21.

Interactions between FB and IgG have been investigated by ^{15}N-NMR on the basis of the assigned imide ^{15}N resonances of the three Pro residues. Fig. 1C shows the ^{15}N-NMR spectrum of [^{15}N]FB in the presence of a human myeloma protein IgG1(x) Ike-N. This experiment was performed at 30°C using 3.6 mM [^{15}N]FB and 0.3 mM IgG, pH 5.0 at 30°C. Under these conditions, [^{15}N]FB exists in excess, and chemical exchange of [^{15}N]FB between free and bound states is fast on the ^{15}N chemical shift time scale. Addition of IgG caused a downfield shift of 0.09 ppm for the Pro-39 resonances, whereas the Pro-58 and Pro-21 resonances did not show any significant shift in the presence of IgG. It has been shown that there is a close similarity in conformation in the Glu-9–His-19 and Glu-25–Asp-37 helical regions between the free and IgG-bound FB, and that Pro-58 is located in the C-terminal segment and flexible both in the free and bound states [6,7]. The present ^{15}N data are quite consistent with our previous suggestion [6] that a significant degree of conformation change is induced in the Ser-42–Ala-55 helical region of FB when it is bound to the Fc portion of IgG.

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