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A ^{13}C NMR study of the hinge region of a mouse monoclonal antibody

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Dedicated to the memory of Professor V.F. Bystrov

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SUMMARY

A ^{13}C NMR study is reported of the hinge region of an intact mouse monoclonal antibody with a molecular weight of 150 K. Cys, Ile, and Pro analogs of the antibody labeled with ^{13}C at the carbonyl carbon were prepared by growing hybridoma cells in the serum-free media. Resonance assignments have been performed as described previously [Kato, K., Matsunaga, C., Igarashi, T., Kim, H., Odaka, A., Shimada, I. and Arata, Y. (1991) *Biochemistry*, **30**, 270–278]. The spectral data obtained show that ^{13}C NMR can give detailed information about the structure of the hinge region of the intact antibody molecule. Prospects for the future role of ^{13}C NMR in the structural analyses of larger proteins are briefly discussed.

INTRODUCTION

Immunoglobulin G (IgG), which is a multi-domain glycoprotein with a molecular weight of 150 K, consists of two identical heavy chains and two identical light chains. The heavy chains are composed of four homology units, V_H, C_H1, C_H2, and C_H3, whereas the light chains are divided into two homology units, V_L and C_L. The C_H1 and C_H2 domains are separated by the hinge region,

Abbreviations: C_L, constant region of the light chain; C_H1, C_H2, C_H3, constant regions of the heavy chain; CPMG, Curr–Purcell–Meiboom–Gill; DTT, dithiothreitol; Fab, antigen binding fragment; Fab*, a three-domain fragment composed of V_H, V_L, and C_L; Fc, fragment composed of the C-terminal halves of the heavy chains; Fv, antigen binding fragment composed of V_H and V_L; IgG, immunoglobulin G; IgG-RA, reduced and alkylated IgG; IgG2a(s), a short-chain mouse monoclonal IgG2a antibody that lacks the entire C_H1 domain; [X]IgG, IgG labeled with [1-^{13}C]X; [X, Z]IgG, IgG that is doubly labeled with [1-^{13}C]X and [^{15}N]Z; V_H, variable region of the heavy chain; V_L, variable region of the light chain.

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which is highly susceptible to attack by proteolyses, giving rise to the Fab and Fc fragments.

The hinge region has a unique structure with the *core*, which consists of two parallel disulfide-linked segments, typically with the sequence Cys-Pro-Pro-Cys. The core hinge is flanked by the *upper* hinge and the *lower* hinge, which are short peptide segments. It has been shown that the hinge region is primarily responsible for the internal flexibility that exists in the IgG molecule (Nezlin, 1990).

It is known that reduction and alkylation of the inter heavy chain disulfide bridges in the core hinge impair effector functions of the antibody (Metzger, 1978). It has also been shown that IgG1 Dob, a human myeloma protein that lacks most of the hinge region, cannot express effector functions (Klein et al., 1981). These results strongly suggest that the hinge region constitutes an essential part of the IgG molecule for the expression of proper antibody functions.

In a previous ^{1}H NMR study, effects of *spin diffusion* were examined using human myeloma IgG1 protein Ike-N along with protein Dob, and resonances originating from the side chains of some of the amino acid residues that exist in the hinge region have been identified (Endo and Arata, 1985). Further analyses have been performed using hinge fragments cleaved by proteolyses out of protein Ike-N (Ito and Arata, 1985). On the basis of the chemical shift and spin coupling constant data observed for the α- and β-protons of the Cys residues in the core hinge, the structure of the hinge region has been discussed. However, for further discussion on the role of the hinge region in the expression of antibody functions, a more direct approach is obviously needed, using the spectroscopic probes that are assigned in a site-specific way to the hinge region of the *intact* IgG molecule.

We have recently reported on a series of ^{13}C NMR studies using switch variant anti-dansyl IgG antibodies developed by Dangl et al. (1982). Each of these antibodies was specifically labeled with ^{13}C at the carbonyl carbon by growing hybridoma cells in the serum-free medium. It has been shown that, even with the *intact* IgG with a molecular weight of 150 K, the line widths of carbonyl carbon resonances are sufficiently narrow and, therefore, a ^{13}C-^{15}N double labeling method developed by Kainosho and Tsuji (1982) can be applicable to site-specific resonance assignments. We have established a general strategy for the ^{13}C resonance assignments by combination of the double labeling method with a variety of techniques (Kato et al., 1989a,1991a). On the basis of the spectral data collected for the IgGs and their proteolytic fragments, we have demonstrated that the carbonyl carbon resonances can be invaluable probes for the structural analyses of antigen binding and also of domain–domain interactions in the antibody molecules (Kato et al., 1991a,b).

In the present paper, a ^{13}C NMR study is reported of the hinge region of an intact IgG2a antibody, which is a member of the switch variant IgG family described above. Hereafter the IgG2a antibody will simply be referred to as IgG2a. The switch variant family also contains a short-chain IgG2a antibody, in which the entire C_H1 domain is deleted (Igarashi et al., 1990). The short-chain IgG2a antibody will be designated as IgG2a(s). For spectral analyses, a variety of proteolytic fragments were prepared from IgG2a and IgG2a(s). Fab and Fc fragments were prepared by papain digestion of IgG2a (Kato et al., 1991a). The Fc fragment obtained by papain digestion will be referred to as Fc(papain). It was confirmed that most of the hinge region including the core hinge had been cleaved off from Fc(papain). It has been shown that clostripain digestion of IgG2a(s) gives in high yield the Fv fragment, which is a heterodimer of the V_H and V_L domains (Takahashi et al., 1991a). Clostripain digestion of IgG2a(s) under mild conditions gave Fab*, which is a three-domain fragment comprising the V_H, V_L, and C_L domains, and Fc (Kato et al.,

1991a). In contrast to Fc(papain), the Fc fragment obtained by clostripain digestion of IgG2a(s) retains most of the hinge region including the core hinge, and will simply be referred to as Fc.

The IgG2a and IgG2a(s) antibodies labeled with ^{13}C at the carbonyl carbon of Cys, Ile, or Pro were prepared and used along with their Fv, Fab*, Fab, Fc, and Fc(papain) fragments for the NMR analyses. Spectral assignments will be made by following the general strategy that has been established in our previous work (Kato et al., 1989a,1991a). On the basis of the spectral data obtained, the structure of the hinge region will be discussed. Prospects for the application of ^{13}C NMR to larger proteins will also be briefly discussed.

MATERIALS AND METHODS

Materials

L-[α-^{15}N]Lys·2 HCl was purchased from Isocommerz GmbH, Germany. L-[1-^{13}C]cysteine was a gift from Dr. N. Sugita. All other ^{13}C- and ^{15}N-labeled amino acids were purchased from ICON Service Inc., U.S.A. The isotope enrichment is 95% or higher for each of these amino acids. Gly-L-Gln was kindly supplied by Dr. Y. Minamoto. Clostripain, papain, and bis-(dansyl)cadaverine (N,N'-bis-dansyl-1,5-diaminopentane) were purchased from Sigma. All other chemicals were of reagent grade and used without further purification.

Cell lines and sample preparations

Switch variant cell lines 27-13.6 (IgG2a) and 27-1B10.7 (IgG2a(s)) (Dangl and Herzenberg, 1982; Dangl et al., 1982) were kindly provided by Professor L.A. Herzenberg and Dr. V.T. Oi. Hybridoma cells adapted to a serum-free medium (Nissui NYSF 404) were grown in tissue culture flasks at 37°C in a humidified atmosphere of 5% CO_2/95% air.

Protocols for the preparation of singly and doubly labeled antibodies have been described previously (Kato et al., 1989a,1991a,b). After cell growth, the cell supernatant was concentrated with a Millipore Minitan Ultrafiltration system and then applied to an Affigel protein A column (Bio-Rad). A typical yield was 10–40 mg of purified antibody per liter of the cell culture.

IgG2a was reduced by 10 mM DTT at room temperature for 1 h in 1.5 M Tris/HCl, pH 8.5, containing 2 mM EDTA. For alkylation, 22 mM iodoacetic acid or iodoacetamide was added to the above reaction mixture, which was incubated in the dark for 20 min at room temperature. The reduced and alkylated IgG2a preparations thus obtained were dialyzed against 10 mM phosphate buffer, pH 7.4, containing 0.15 M NaCl and 3 mM NaN_3, and were subjected to NMR measurements. Reduced IgG2a was prepared in situ by adding 10 mM DTT to the NMR sample, which was kept under nitrogen throughout the measurements.

Papain digestion of reduced and alkylated IgG2a gave Fab and Fc(papain) fragments as described previously (Kato et al., 1991a). Fv, Fab* and Fc fragments were prepared by clostripain digestion of IgG2a(s) that had been reduced and alkylated (Kato et al., 1991a,b; Takahashi et al., 1991a,b).

The bis-(dansyl)cadaverine-IgG2a complex was prepared according to the procedure described by Phillips et al. (1990). Bis-(dansyl)cadaverine and IgG2a were mixed at a molar ratio of 1:1 with an antibody concentration of 25 mg/ml. The reaction mixture was subjected to NMR measurements. It was confirmed that the complex obtained in the above reaction gives a single peak on a Pharmacia Superose 6 column.

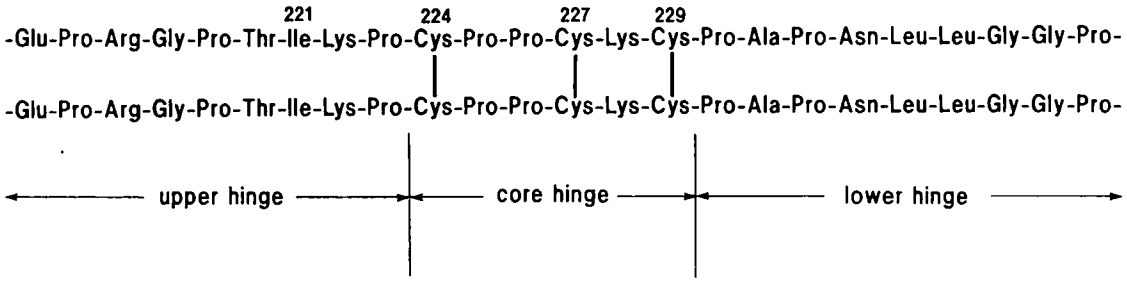

Fig. 1. Amino acid sequences of the hinge region of a mouse IgG2a antibody (Kabat et al., 1987).

NMR measurements

The protein solutions were concentrated by ultrafiltration to a final volume of 2 ml in 5 mM phosphate buffer, pH 7.4, containing 0.2 M NaCl and 3 mM NaN_3 in D_2O unless otherwise stated. A 10-mm NMR sample tube was used with a final protein concentration of 0.1–0.3 mM. NMR measurements were made on a Bruker AM 400 spectrometer. ^{13}C NMR spectra were recorded at 100 MHz using a Waltz-16 composite pulse decoupling sequence. For the selective detection of resonances with longer T_2 relaxation times, a conventional CPMG pulse train was employed without ^1H-decoupling. The free induction decay was recorded with 32 K data points and a spectral width of 24000 Hz, and was multiplied by exponential window function prior to Fourier transformation. Chemical shifts are given in ppm from internal dioxane. The probe temperature was 30°C.

RESULTS

The IgG2a analog selectively labeled with [1-^{13}C]Cys is designated using the one-letter abbreviation for the amino acid as [C]IgG2a. Similar notations will be used for other types of labeled proteins, e.g., [I]Fc and [P]IgG2a.

In IgG2a, Cys-224H, Cys-227H, and Cys-229H form the inter heavy chain disulfide bridges in the core hinge* (see Fig. 1). The IgG2a antibody possesses an additional disulfide bridge formed between Cys-214L and Cys-132H*. All of these four inter chain disulfide bridges can be cleaved under mild reductive conditions. A reduced IgG2a antibody, which is stable under nitrogen, was alkylated by iodoacetic acid or iodoacetate. Figure 2 compares the ^{13}C NMR spectrum of [C]IgG2a (A) with those of three kinds of reduced [C]IgG2a preparations, i.e., (B) reduced and kept under nitrogen, (C) reduced and alkylated with iodoacetic acid, and (D) reduced and alkylated with iodoacetamide. The IgG2a antibody, which is reduced and alkylated with iodoacetic acid, is designated IgG2a-RA and was employed throughout the present work.

The spectral data given in Fig. 2 indicate that, upon reduction (and alkylation) of the inter chain disulfide bridges, five resonances marked by arrows are significantly shifted. In addition,

*The *numbering system* used in the present paper for the constant region is based on human myeloma protein Eu for the heavy chain (Edelman et al., 1969) and mouse myeloma protein MOPC21 for the light chain (Kabat et al., 1987). Amino acid residues in the heavy and light chains are identified by H and L, respectively, e.g., Cys-132H and Cys-214L.

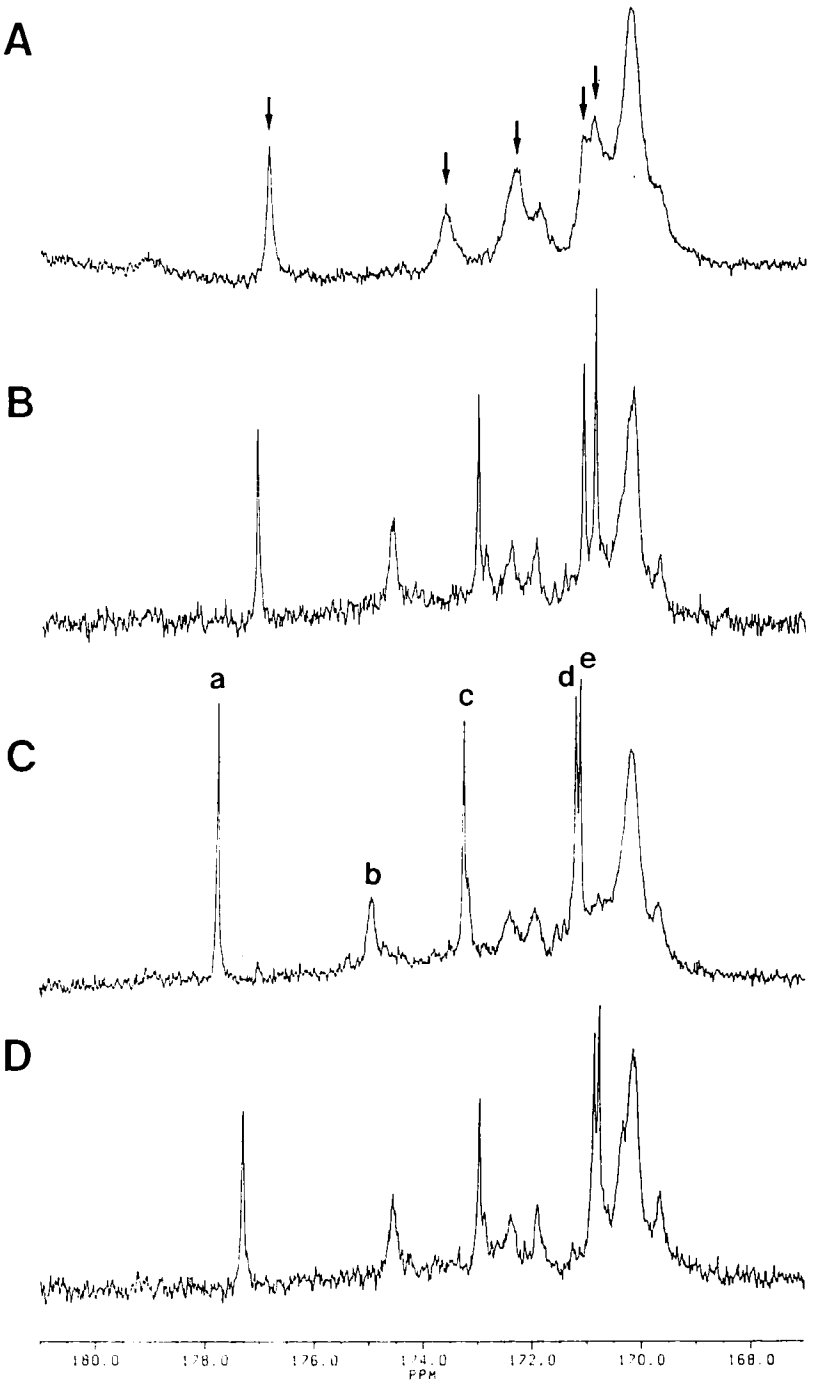

Fig. 2. 100 MHz ^{13}C NMR spectra of (A) [C]IgG2a, (B) reduced [C]IgG2a, (C) [C]IgG2a reduced and alkylated by iodoacetic acid, and (D) [C]IgG2a reduced and alkylated by iodoacetamide. Protein concentrations and the pH of the sample solutions were in the range 0.1–0.3 mM and 7.2–7.4, respectively. Reduced [C]IgG2a was prepared in situ by adding 10 mM DTT to the NMR sample of [C]IgG2a, which was kept under nitrogen throughout the measurement. For each measurement, 32 K data points were used with a spectral width of 24000 Hz and a delay time of 0.3 s. 32000–180000 transients were accumulated for the measurements. The probe temperature was 30°C.

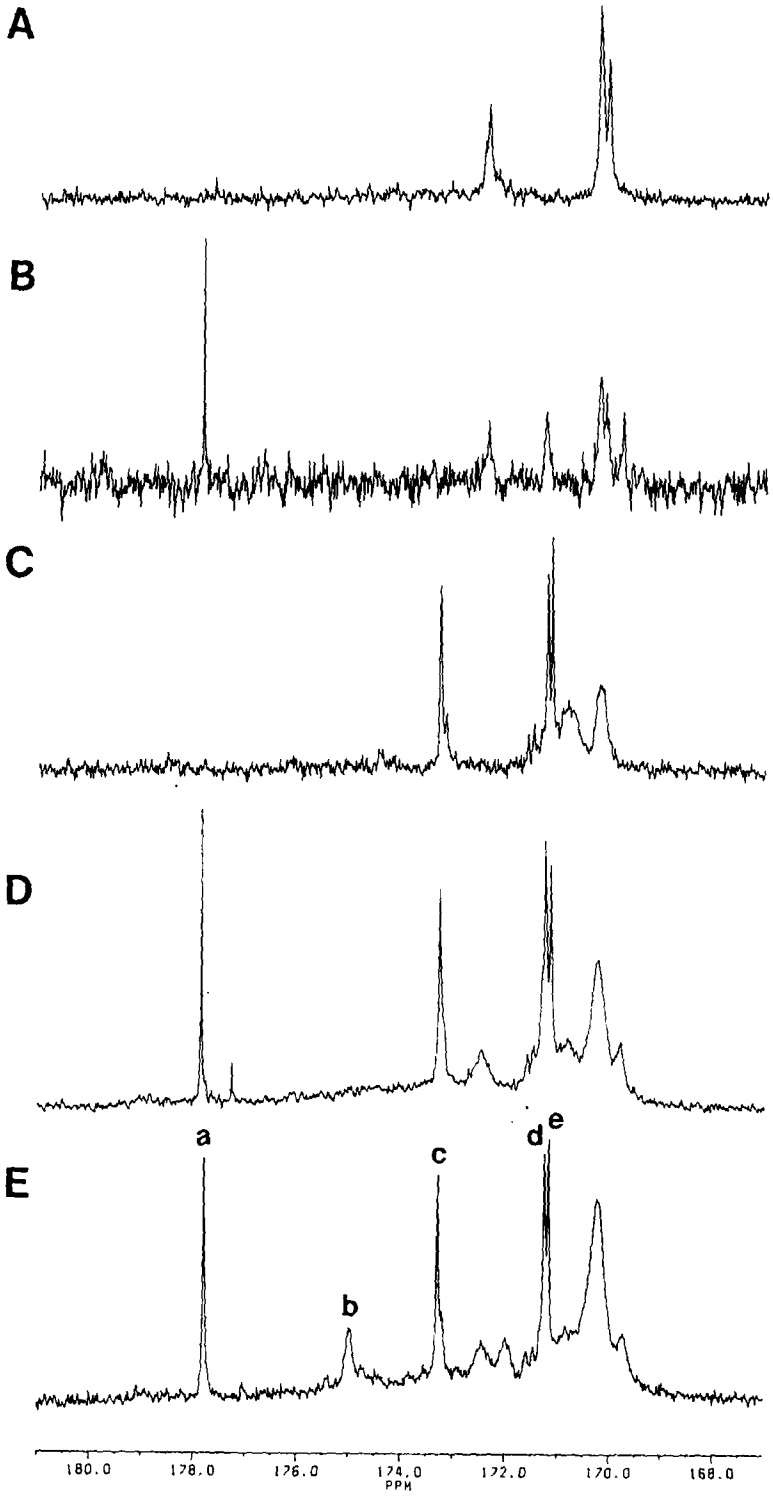

Fig. 3. 100 MHz ^{13}C NMR spectra of (A) [C]Fv, (B) [C]Fab*, (C) [C]Fc, (D) IgG2a(s)-RA, (E) [C]IgG2a-RA. Experimental conditions are as in Fig. 2.

four of the five resonances showed dramatic line narrowing. No significant changes in chemical shift and line width were observed in other parts of the observed spectra (vide infra). The spectral changes observed are essentially the same for the three kinds of reduced preparations of the IgG2a antibody. These results indicate that the five resonances a–e originate from the Cys residues which participate in the formation of inter chain disulfide bridges, i.e., Cys-132H, Cys-224H, Cys-227H, Cys-229H, and Cys-214L (see Fig. 1).

The ^{13}C spectra of [C]Fv, [C]Fab*, [C]Fc, [C]IgG2a(s)-RA, and [C]IgG2a-RA are compiled in Fig. 3. A comparison of the spectrum of [C]IgG2a-RA with that of [C]Fc clearly indicates that resonances c, d, and e are conserved in both spectra, but resonances a and b are missing in the spectrum of [C]Fc. In view of the fact that the hinge region is retained in Fc, we conclude that resonances c, d, and e are due to the three Cys residues that exist in the core hinge (see Fig. 1). This conclusion is consistent with the observation that resonances c, d, and e are missing in the spectrum of Fc(papain) (data not shown). These results indicate that resonances a and b originate from Cys-132H and Cys-214L, respectively, or vice versa.

In order to proceed further, we have taken advantage of the fact that in IgG2a(s) the entire C_H1 domain is deleted, but the rest of the molecule is identical with IgG2a in the amino acid sequences (Igarashi et al., 1990). The Cys-132H-Cys-214L disulfide bridge does not exist in IgG2a(s), since IgG2a(s) lacks Cys-132H that is the C_H1 domain residue. A comparison of the spectra of [C]IgG2a-RA and [C]IgG2a(s)-RA clearly indicates that resonance b is missing in the case of [C]IgG2a(s)-RA, whereas resonances a, c, d, and e are conserved in both spectra. We therefore assign resonances a and b to Cys-214L and Cys-132H, respectively. This conclusion has been confirmed by a double-labeling experiment.

The three Cys residues in the core hinge possess the following dipeptide sequences: Cys-224H-Pro-225H, Cys-227H-Lys-228H, and Cys-229H-Pro-230H. The ^{13}C spectrum of [C, K]IgG2a-RA (Fig. 4A) clearly indicates that resonance c is split into a doublet due to $^1J_{CN}$ coupling. Resonance c can therefore be assigned unambiguously to Cys-227H. At the present stage, Cys-224H and Cys-229H resonances cannot be differentiated, since both of these Cys residues are followed by Pro in the amino acid sequence.

In addition to resonances a–e, [C]IgG2a-RA gives a number of broad peaks, most of which are severely overlapping with each other. In contrast to what has been observed for resonances a–e, the chemical shifts and line widths of these broad peaks do not significantly change before and after the reduction and alkylation (see Fig. 2). This result is again consistent with the above conclusion about the assignments for the Cys residues participating in the formation of the inter chain disulfide bridges.

Each of the immunoglobulin domains contains a pair of Cys residues, which form the disulfide bridge in the *pin region* (Lesk and Chothia, 1982). Presumably all of these Cys residues possess little freedom of internal flexibility, giving broad Cys resonances. It should be noted that the spectrum of [C]IgG2a(s)-RA is simply a superposition of those of [C]Fab* and [C]Fc (see Fig. 3). Comparison of the spectra of [C]Fv, [C]Fab*, [C]Fc, [C]IgG2a(s)-RA, and [C]IgG2a-RA has led to *fragment-specific* (and in the cases of C_H1 and C_L *domain-specific*) assignments of all the Cys resonances to Fv, C_H1, C_L, and Fc. Site-specific assignment for [C]Fv has already been accomplished using the double labeling method (Kato et al., 1991b).

In Fig. 5 the ^{13}C NMR spectrum of [P]IgG2a is compared with that of [P]IgG2a-RA. It is observed that [P]IgG2a gives two sharp resonances. After reduction and alkylation, line narrowing

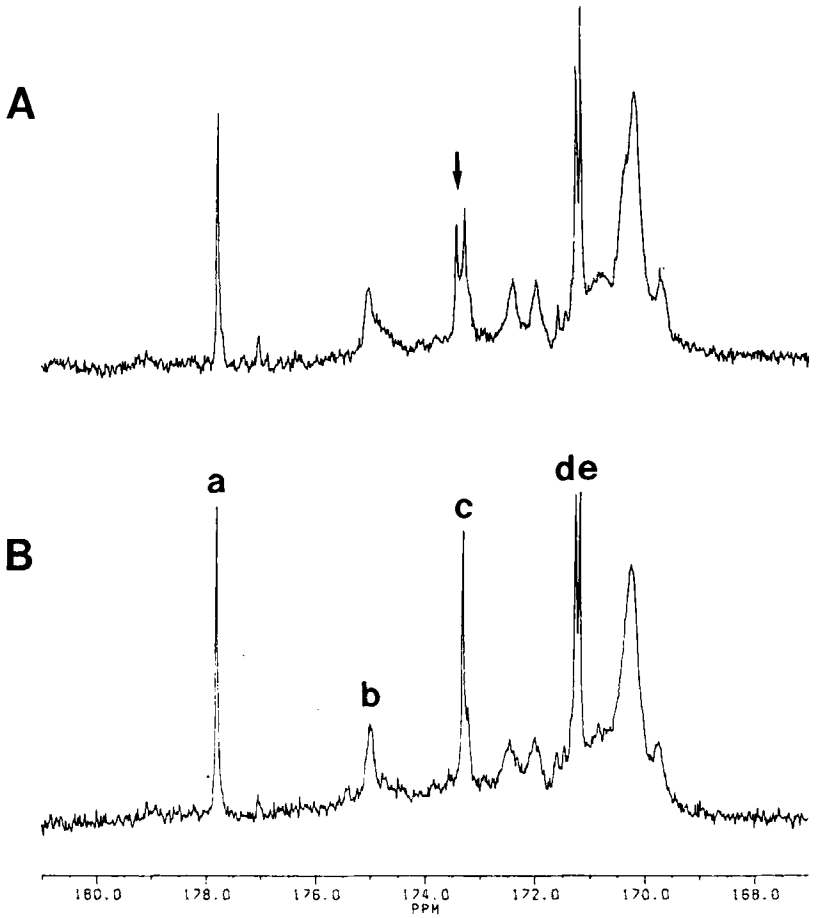

Fig. 4. 100 MHz ^{13}C NMR spectra of (A) [C, K]IgG2a-RA and (B) [C]IgG2a-RA. Experimental conditions are as in Fig. 2. The Cys-227H resonance is indicated by the arrow. See text.

was observed for four additional resonances. In view of the results described above for [C]IgG2a, we suggest that all of these six resonances originate from the hinge region.

Figure 6A shows the ^{13}C NMR spectrum of [I]IgG2a. The CPMG spectrum of [I]IgG2a (Fig. 6B) indicates that the peak marked by the arrow is the only one which survived under the conditions used for the measurement. Figure 6C shows that this peak is conserved in the spectrum of [I]Fc where the hinge region is retained. It was confirmed that this peak does not exist in [I]Fc-(papain) where the hinge region had been cleaved off (data not shown). As Fig. 1 shows, there is one Ile in the hinge region, i.e., Ile-221H in the upper hinge. These results indicate that the peak under consideration originates from Ile-221H. We have also confirmed this assignment by a double labeling experiment. In Fig. 6, the ^{13}C spectrum of [I]IgG2a is compared with that of a hapten-linked dimeric [I]IgG2a. Upon formation of the dimer, a number of resonances observed in the spectrum of the monomeric [I]IgG2a were broadened out. It is of interest that most of the survived resonances have their counterparts in the spectrum of [I]Fc and therefore originate from Fc and the hinge region. Similar results were obtained using [C]IgG2a (data not shown).

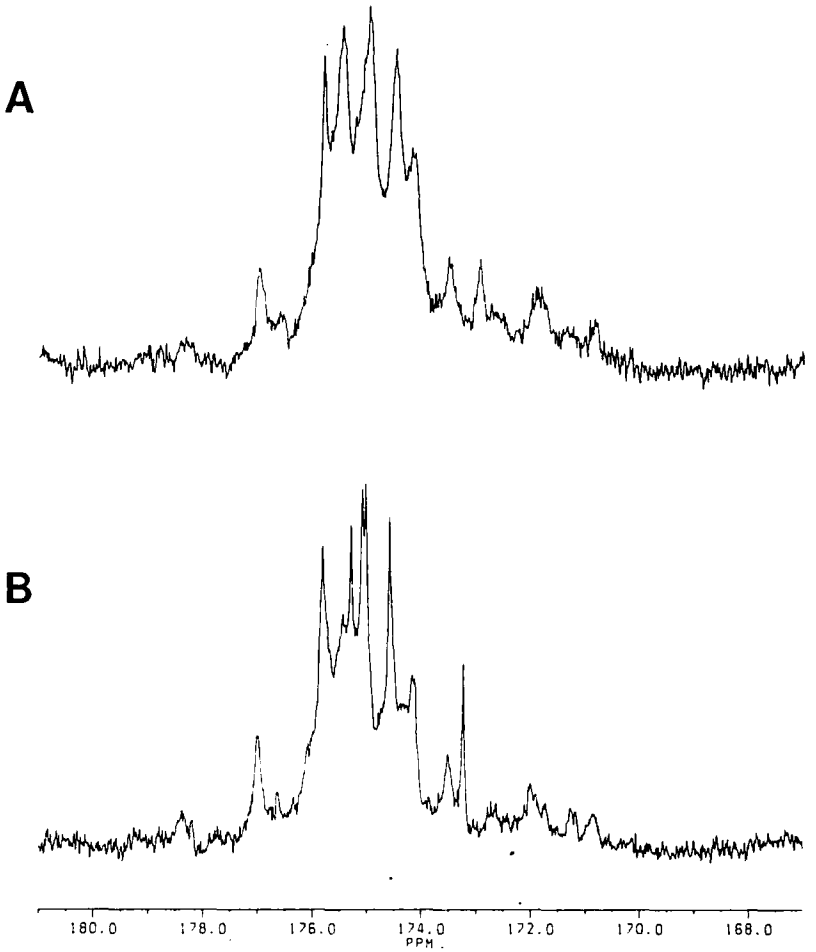

Fig. 5. 100 MHz ^{13}C NMR spectra of (A) [P]IgG2a and (B) [P]IgG2a-RA. Experimental conditions are as in Fig. 2.

DISCUSSION

Line widths of the carbonyl carbon resonances

As shown in the previous papers (Kato et al., 1989a,1991a,b), the carbonyl carbon resonances can be *built-in* spectroscopic probes for the structural analyses of the IgG molecule with a molecular weight of 150 K. This result has been confirmed in the present work. It should particularly be noted that ^{13}C NMR can be a direct means of obtaining information concerning the dynamic structure of the hinge region of the intact antibody molecule (see below for further discussion).

The carbonyl carbon does not have in its neighborhood proton(s) which can be a strong source of spin relaxation. Thus the line widths of the carbonyl carbon resonances are generally determined by the chemical shift anisotropy (Abragam, 1961). Except for the resonances originating from the hinge region, the ^{13}C line widths observed for the intact IgGs are generally of the order of 20 Hz at the observation frequency of 100 MHz (Kato et al., 1989a,1991a,b). It has been re-

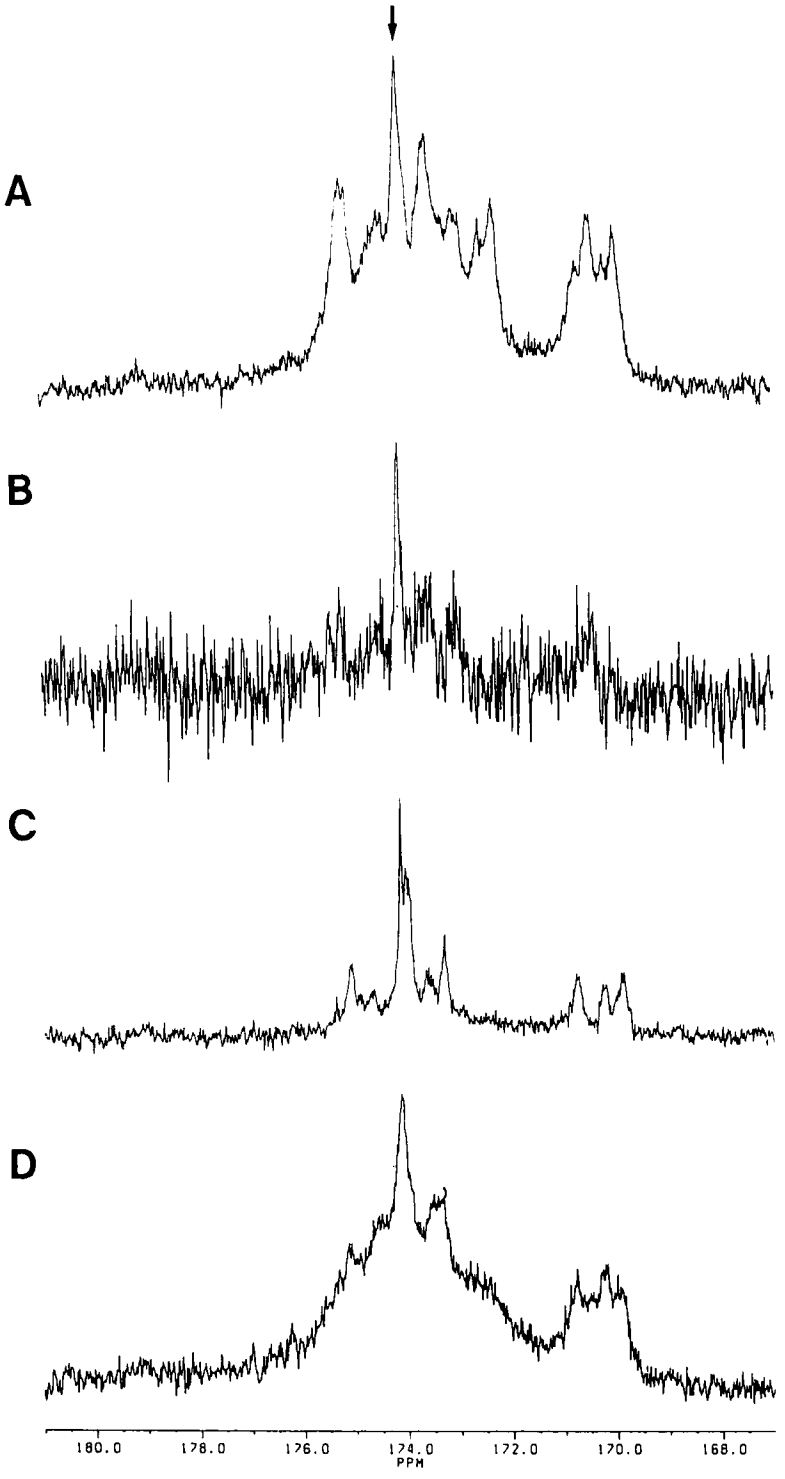

Fig. 6. 100 MHz ^{13}C NMR spectra of (A) and (B) [I]IgG2a, (C) [I]Fc, and (D) bis-(dansyl)cadaverine-[I]IgG2a complex. The spectrum (B) was obtained by using a CPMG pulse sequence $\pi/2\text{-}(\tau\text{-}\pi\text{-}\tau)_n$, with $\tau = 2$ ms and n = 10. Other experimental conditions are as in Fig. 2. The Ile-221H resonance is indicated by the arrow. See text.

ported that the magnitude of chemical shift anisotropy for the carbonyl carbon in peptides is of the order of 150 ppm [Sarkar et al., 1983; see, e.g., Saito and Ando (1989) for a review]. This would lead to a correlation time of the order of 10^{-8} s, which is typically observed for rotational tumbling of a molecule of the size of the intact IgGs. A decrease in the observation frequency to 75 MHz led to a decrease in the line width at a sacrifice of the sensitivity of signal detection. It appears that 100 MHz is a good compromise between the line width and sensitivity. The number of resonances and the size of the molecule in question would certainly have to be taken into consideration for the choice of optimum experimental conditions.

^{13}C line widths and the dynamical structure of the hinge region

As Fig. 6 shows, the resonance originating from Ile-221H in the upper hinge of the intact IgG2a antibody exhibits an extremely narrow line width of less than 10 Hz. We conclude that the upper hinge possesses a significant degree of freedom of internal flexibility. This result is consistent with our previous conclusion, based on the observation of spin diffusion, that the upper hinge is the source of internal flexibility of IgG (Endo and Arata, 1985).

The ^{13}C data obtained in the present work show that the core hinge is comparable in flexibility to other parts of the IgG molecule. It is of great interest that reduction (and alkylation) of the inter chain disulfide bridges resulted in a dramatic decrease in the line width for the core hinge. The line widths thus obtained became comparable to that observed for the Ile-221H resonance for the upper hinge. These results indicate that cleavage of the inter heavy chain disulfide bridges leads to *homogeneous* line narrowing of the hinge region. It should be noted that reduction and alkylation of the hinge region impair the effector functions of antibody molecules (Metzger, 1978). We therefore conclude that a *mosaic* structure of the hinge region with a heterogeneous nature of flexibility as evidenced by the present ^{13}C study is essential for the IgG antibody to mediate effector functions. This conclusion is quite consistent with what we have suggested previously on the basis of the ^{1}H NMR measurements of the hinge peptides obtained by proteolytic cleavage of a human IgG1 protein (Ito and Arata, 1985).

We have shown that Pro resonances originating from the hinge region can be observed separately. Pro characterizes along with Cys a unique structural feature of the hinge region. Although we have to wait until the completion of spectral assignments for any further discussion, this result is encouraging in that we will eventually be able to use Pro as the spectroscopic probe for the elucidation of the dynamical structure of the hinge region.

Prospects

It has become possible to use the carbonyl carbon resonances as spectroscopic probes for structural study of IgGs with the molecular weight of 150 K. This is primarily due to the fact that the carbonyl carbon is well *shielded* from the strong source of relaxation which would generally become increasingly efficient for larger proteins such as antibodies. It has also been demonstrated that even the dimeric complex of IgG with a molecular weight of 300 K can be a target of NMR structural analyses at atomic resolution. It appears that the existence of internal flexibility in the hinge region has made it possible to observe resonances originating from Fc which follows the hinge region.

We have also shown that two-dimensional HOHAHA spectroscopy is applicable to antibodies that had been selectively deuterated in the aromatic region, yielding information concerning the

flexible part of the molecule (Kato et al., 1989b). In addition, ^{1}H-^{15}N shift-correlation spectroscopy has been used in our laboratory for the structural analysis of the antibody combining site (Takahashi et al., 1991a,b). On the basis of these experimental findings we are now extending stable-isotope-aided NMR to a variety of larger systems. Progress along this line would certainly open up a new possibility of the use of NMR for structural studies of large proteins.

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REFERENCES

Abragam, A. (1961) *The Principles of Nuclear Magnetism*, Oxford University Press, London, p. 315.

Dangl, J.L. and Herzenberg, L.A. (1982) *J. Immunol. Methods*, **52**, 1-14.

Dangl, J.L., Parks, D.R., Oi, V.T. and Herzenberg, L.A. (1982) *Cytometry*, **2**, 395-401.

Edelman, G.M., Cunningham, B.A., Gall, W.E., Gottlieb, P.D., Rutishauser, U. and Waxdal, M.J. (1969) *Proc. Natl. Acad. Sci. USA*, **63**, 78-85.

Endo, S. and Arata, Y. (1985) *Biochemistry*, **24**, 1561-1568.

Igarashi, T., Sato, M., Katsube, Y., Takio, K., Tanaka, T., Nakanishi, M. and Arata, Y. (1990) *Biochemistry*, **29**, 5727-5733.

Ito, W. and Arata, Y. (1985) *Biochemistry*, **24**, 6467-6474.

Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M. and Gottesman, K.S. (1987) *Sequences of Proteins of Immunological Interest*, 4th ed., U.S. Department of Health and Human Services, National Institutes of Health, Washington, DC.

Kainosho, M. and Tsuji, T. (1982) *Biochemistry*, **21**, 6273-6279.

Kato, K., Matsunaga, C., Nishimura, Y., Waelchli, M., Kainosho, M. and Arata, Y. (1989a) *J. Biochem. (Tokyo)*, **105**, 867-869.

Kato, K., Nishimura, Y., Waelchli, M. and Arata, Y. (1989b) *J. Biochem. (Tokyo)*, **106**, 361-364.

Kato, K., Matsunaga, C., Igarashi, T., Kim, H., Odaka, A., Shimada, I. and Arata, Y. (1991a) *Biochemistry*, **30**, 270-278.

Kato, K., Matsunaga, C., Odaka, A., Yamato, S., Takaha, W., Shimada, I. and Arata, Y. (1991b) *Biochemistry*, **30**, 6604-6610.

Klein, M., Haeffner-Cavaillon, N., Isenman, D.E., Rivat, C., Navia, M.A., Davies, D.R. and Dorrington, K.J. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 524-528.

Lesk, A.M. and Chothia, C. (1982) *J. Mol. Biol.*, **160**, 325-342.

Metzger, H. (1978) *Contemp. Top. Mol. Immunol.*, **7**, 119-152.

Nezlin, R. (1990) *Adv. Immunol.*, **48**, 1-40.

Phillips, M.L., Oi, V.T. and Schumaker, V.N. (1990) *Mol. Immunol.*, **27**, 181-190.

Saito, H. and Ando, I. (1989) *Annu. Rep. NMR Spectrosc.*, **21**, 209-290.

Sarkar, S.K., Sullivan, C.E. and Torchia, D.A. (1983) *J. Biol. Chem.*, **258**, 9762-9767.

Takahashi, H., Igarashi, T., Shimada, I. and Arata, Y. (1991a) *Biochemistry*, **30**, 2840-2847.

Takahashi, H., Odaka, A., Kawaminami, S., Matsunaga, C., Kato, K., Shimada, I. and Arata, Y. (1991b) *Biochemistry*, **30**, 6611-6619.