Stable Isotope Aided Nuclear Magnetic Resonance Study To Investigate the Receptor-Binding Site of Human Interleukin 1β†

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ABSTRACT: Resonance assignments for interleukin 1β at neutral pH were made by using three-dimensional NMR in combination with specific labeling and double-labeling methods with stable isotopes. On the basis of the present assignments, ^{15}N single-quantum coherence spectra of N-terminal truncated and fusion mutants were compared with that of the wild-type. Although these mutants have reduced biological activity, they showed ^{15}N-SQC spectra similar to that of the wild-type. However, small but significant chemical shift changes were observed for amino acid residues within a loop 86–99, in spite of the modification at the N-terminus, supporting the idea that this loop forms a biologically active part of interleukin 1β. Receptor-binding activity was studied for mutants (Asp-93)-, (Leu-93)- and des-(Arg-98)interleukin 1β's. The results show significant loss of the receptor-binding activity. The N-terminus, the C-terminus, and the loop 86–99 form a part of the open end of a β-barrel [Finzel, B. C., Clancy, L. L., Holland, D. R., Muchmore, S. W., Watenpaugh, K. D., & Einspahr, H. M. (1989) *J. Mol. Biol. 209*, 779–791; Clore, G. M., Wingfield, P. T., & Gronenborn, A. M. (1991) *Biochemistry 30*, 2315–2323], which forms the receptor-binding site of IL-1β.

Interleukin 1β (IL-1β),[1] a member of a family of cytokines, modulates a wide variety of immune and inflammatory responses by binding to a receptor on the cell membrane (Kampschmidt, 1984; Oppenheim et al., 1986; Dinarello, 1984; Auron et al., 1984; March et al., 1985). In order to identify the functionally active minimal size of IL-1β, a number of peptide fragments and mutants of IL-1β have been made (Antoni et al., 1986; Palaszynski, 1987; Gronenborn et al., 1986; Huang et al., 1987; Horuk et al., 1987). There are 147 out of 153 residues (numbers 4–150) necessary for its biological activities. In particular, Arg-4 is suggested to have a critical role. In addition, N-terminal truncated or N-terminal fusion mutants also show decreased biological activities, suggesting that the biologically active site resides both on the N-terminal and C-terminal portions of IL-1β (Mosley et al., 1987; Kamogashira et al., 1988a,b). Recently, the three-dimensional structure of IL-1β has been elucidated by X-ray crystallography (Priestle et al., 1989; Finzel et al., 1989) and nuclear magnetic resonance (NMR) spectroscopy (Clore et al., 1990a,b, 1991; Clore & Gronenborn, 1991). Since IL-1β consists of 12 tightly interacting β-strands via hydrogen bonds forming a β-barrel, mutation on the specific site might cause an alteration of the biologically active region of IL-1β. Thus, the results of the mutational study are difficult to interpret without the structural knowledge of the wild-type and mutants. This prompted us to start a comparative structural study of the wild-type and mutants to identify the location of possible biologically active region of IL-1β. For this purpose, we studied truncated and fusion mutants of IL-1β at the N-terminus by using NMR.

IL-1β has 153 amino acid residues, and its molecular mass is 17.4 kDa. For such proteins, conventional 2D NMR techniques are not sufficient due to signal overlap and signal broadening. So we applied stable isotope aided NMR techniques for assigning the backbone signals of IL-1β. Although the resonance assignments of IL-1β have been made by using ^{15}N and/or ^{13}C uniformly labeled IL-1β's at pH 5.4 (Driscoll et al., 1990a,b), we have made the resonance assignments of ^{15}N, ^{15}NH, and $C^{\alpha}H$ of main chains at neutral pH (pH 7.2), independently; we used selective labeling with [^{15}N]amino acids and 1-^{13}C/^{15}N double-labeling (Kainosho & Tsuji, 1982; Torchia et al., 1988a,b, 1989) and ^{15}N uniform-labeling methods in combination with ^{15}N three-dimensional (3D) NMR spectroscopy (Marion et al., 1990). Sequential assignment was accomplished by using 3D NMR as well as conventional 2D NMR starting from resonances assigned type-specifically, sequence-specifically, or site-specifically at physiological pH (7.2). The assignments of the resonances at physiological pH are essential since the biological activity is measured at this pH. On the basis of the assignments of wild-type IL-1β, we analyzed ^{15}N–^{1}H correlation spectra of the truncated and fusion mutants. Analysis of the NMR data

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[1] Abbreviations: IL-1β, interleukin 1β; IL-1α, interleukin 1α; Met-IL-1β, IL-1β mutant containing one methionine residue fused at its N-terminus; IL-1β(4–153), IL-1β mutant in which first three residues are truncated; IL-1β(7–153), IL-1β mutant in which first six residues are truncated; 2D NMR, two-dimensional NMR; 3D NMR, three-dimensional NMR; NOESY, NOE spectroscopy; TOCSY, totally correlated spectroscopy; HOHAHA, homonuclear Hartmann–Hahn spectroscopy; DQF-COSY, double-quantum-filtered correlated spectroscopy; SQC, single-quantum coherence; HMQC, heteronuclear multiple-quantum coherence.

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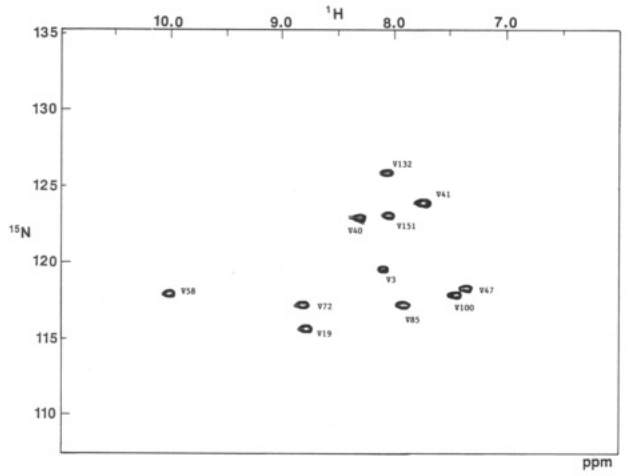

FIGURE 1: ^{15}N-SQC spectra of IL-1β labeled with [^{15}N]Val. The signal assignments are also shown.

(a)

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1           11          21          31
APVRSLNCTL  RDSQQKSLVM  SGPYELKALH  LQGQDMEQQV
41          51          61          71
VFSMSFVQGE  ESNDKIPVAL  GLKEKNLYLS  CVLKDDKPTL
81          91          101         111
QLESVDPKNY  PKKKMEKRFV  FNKIEINNKL  EFESAQFPNW
121         131         141         151
YISTSQAENM  PVFLGGTKGG  QDITDFTMQF  VSS
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(b)

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1           11          21          31
APVRSLNCTL  RDSQQKSLVM  SGPYELKALH  LQGQDMEQQV
41          51          61          71
VFSMSFVQGE  ESNDKIPVAL  GLKEKNLYLS  CVLKDDKPTL
81          91          101         111
QLESVDPKNY  PKKKMEKRFV  FNKIEINNKL  EFESAQFPNW
121         131         141         151
YISTSQAENM  PVFLGGTKGG  QDITDFTMQF  VSS
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FIGURE 2: Amino acid sequence of IL-1β. (a) For hatched amino acid residues, type-specific assignments were made by using ^{15}N-labeled amino acids. In addition, group-specific assignments for (Gly, Ser) and (Asp, Asn, Glu, Gln) were made on the basis of cross labeling from Gly and Asp. (b) For hatched amino acid residues, sequence-specific or site-specific resonance assignments were made by double-labeling experiment.

together with knowledge of the biological activities of the mutants allows a conclusion to be drawn about the receptor-binding site of IL-1β.

Materials and Methods

Purification of IL-1β. Wild-type interleukin 1β was prepared from transformed *Escherichia coli*. The expression plasmid ptrpIL-1β used in our experiments was constructed as described previously (Nishida et al., 1987; Kikumoto et al., 1987). *E. coli*, strain HB 101, was used for preparation of single- and 1-^{13}C/^{15}N double-labeled samples, and strain MC4100 was used for uniformly ^{15}N-labeled samples. Three mutants of IL-1β used in the present NMR study were as follows: IL-1β(4–153) and IL-1β(7–153) in which the first three and six residues were truncated, respectively, and Met-IL-1β, which contained one methionine residue fused at its N-terminus. In addition, (Asp-93)-, (Leu-93)-, and des-(Arg-98)IL-1β mutants were prepared for the receptor-binding assay. All expression plasmids used for preparing the mutants were constructed (Kamogashira et al., 1988a,b) and transformed into *E. coli* strain MC4100. All variants of IL-1β were purified according to the procedures of Kikumoto et al. (1987). The stable isotope labeled samples were obtained by growing the cells in minimal medium supplemented with unlabeled and labeled amino acids, except for tryptophan which is an inhibitor of the expression of IL-1β.

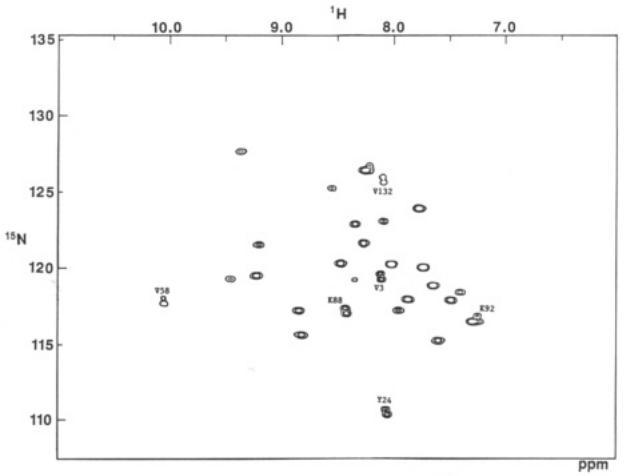

FIGURE 3: ^{15}N-SQC spectrum of IL-1β labeled with [1-^{13}C]Pro/[^{15}N]Val, Lys, and Tyr. The cross peaks with doublet patterns were sequence-specifically assigned as shown.

NMR Samples. The buffer used for all NMR experiments contained 20 mM sodium phosphate buffer (pH 7.2), in 90% H_2O–10% ^2H_2O or in 100% ^2H_2O. The protein concentration was in a range of 0.5–2 mM, and the temperature was 28 °C.

NMR Measurements. ^{1}H 2D spectra, NOESY (Jeener et al., 1979; Macura et al., 1981), TOCSY/HOHAHA (Braunschweiler & Ernst, 1983; Bax & Davis, 1985; Davis & Bax, 1985), and DQF-COSY (Rance et al., 1983), were recorded on a JEOL JNM-GX500 NMR spectrometer operating at 500 MHz for ^{1}H resonance. All inverse detection spectra were measured on a JEOL JNM-GX400 NMR spectrometer working at 400 MHz for ^{1}H resonance. For the NOESY spectra with a 150-ms mixing time and DQF-COSY spectra, 512 t_1 increments were collected, each with 1024 complex points, over a spectral width of 6 kHz in both dimensions. Data were transformed after zero filling to yield real two-dimensional spectra of 1024 × 1024 points. For ^{15}N-SQC spectra, 128 t_1 increments were collected with 128 scans with 1024 complex points in t_2. Spectral widths in the F_1 and F_2 dimensions were 1250 and 6000 Hz, respectively. Data were zero filled to yield the final two-dimensional data of 256 × 1024 real points. For ^{15}N 3D NMR spectra of NOESY-HMQC and HOHAHA-HMQC (Marion et al., 1990), 64 t_1 increments and 32 t_2 increments were collected with 512 complex points in t_3, where 16-scan data were accumulated. The spectral width in both ^{1}H axes and the ^{15}N axis were 6000 and 1250 Hz, respectively. Data were zero filled to yield the final data set of 512 × 64 × 512 points in F_1, F_2, and F_3 dimensions. In all 2D and 3D NMR experiments, quadrature detection was accomplished with the method of States et al. (1982). 2D and 3D NMR spectra were processed on PDP-11 computers connected to JEOL spectrometers with standard built-in Fourier transformation software and simple home-written programs.

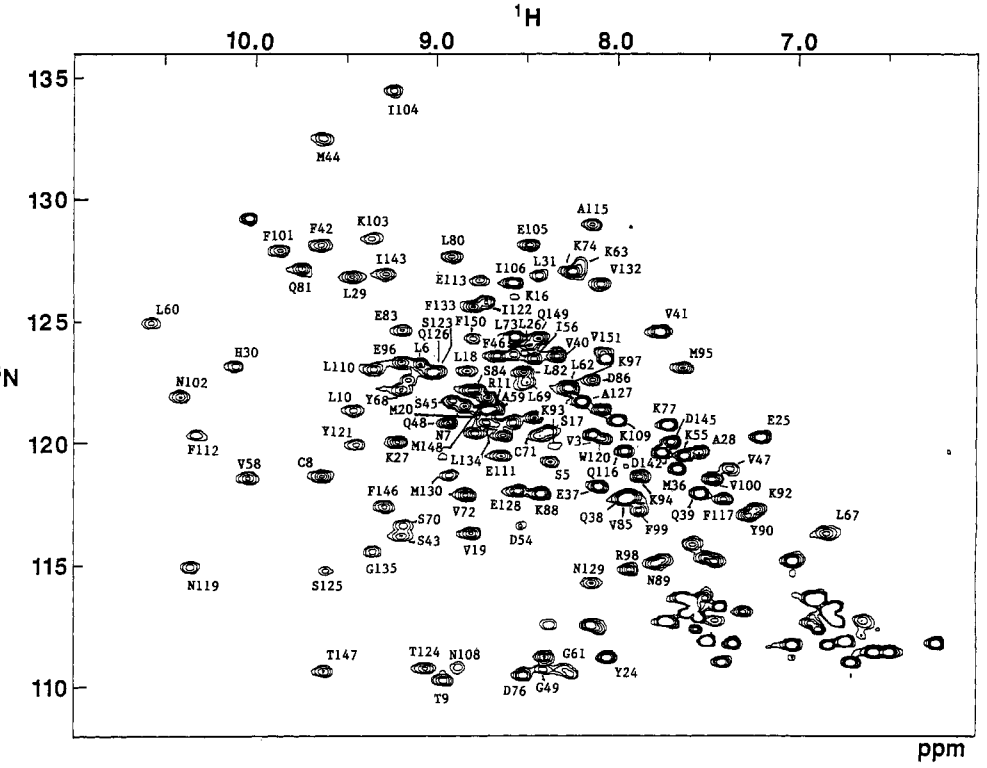

FIGURE 4: ^{15}N-SQC spectrum of IL-1β uniformly labeled with ^{15}N. The assignments of cross peaks are also shown.

Receptor-Binding Assay. Human (Asp-36,Ser-141)IL-1α was labeled with ^{125}I by utilizing Iodogen reagent (Pierce Chemical Co.). The radioactive human (Asp-36,Ser-141)-IL-1α was purified by gel filtration. (Asp-36,Ser-141)IL-1α is more stable than human IL-1α. The membrane fraction of Chinese hamster ovary (CHO) cells transformed with an expression plasmid of the full length of mouse T-cell IL-1 receptor (Sims et al., 1988) was incubated with the ^{125}I-labeled (Asp-36,Ser-141)IL-1α in the presence of various concentrations of unlabeled IL-1β analogues. The membrane fraction was washed twice with phosphate-buffered saline containing 0.1% bovine serum albumin and then counted in a γ counter (Aloka, ARC-300).

Results and Discussion

Assignments of Amide Nitrogen and Protons to Amino Acid Residue Types. Type-specific assignments of amino acid residues are a prerequisite for sequential resonance assignment procedures. For larger proteins with molecular weights much greater than 10000, the efficiency of the polarization transfer among protons through ^{1}H spin–spin coupling interactions is significantly reduced, so that the spin system identification of each amino acid type by conventional TOCSY/HOHAHA and/or COSY is difficult. In addition, the increasing number of resonance peaks in the spectrum leads to greater overlap and chemical shift degeneracy in the 2D spectrum. In order to solve these obstacles, we extensively applied stable isotope aided NMR methods.

^{15}N-SQC spectra of IL-1β selectively labeled with ^{15}N enriched amino acids provide us with residue type assignments of backbone amide nitrogen and proton signals. For the measurement of ^{15}N-SQC spectra, we incorporated spin-lock pulses in the original ^{15}N-SQC pulse sequence (Bodenhausen & Ruben, 1980; Tate et al., 1991). These spin-lock pulses are effective for suppression of a water resonance and make it possible to observe weak signals in ^{15}N-SQC spectra. The ^{15}N-labeled amino acids at the α position, [^{15}N]Phe, [^{15}N]Tyr, [^{15}N]Ile, [^{15}N]Leu, [^{15}N]Met, [^{15}N]Lys, [^{15}N]Ala, [^{15}N]Val, [^{15}N]His, [^{15}N]Gly, and [^{15}N]Asp, were used for preparing the specifically labeled IL-1β. ^{15}N-SQC spectrum of each of these samples was measured. In Figure 1, we showed the ^{15}N-SQC spectrum of IL-1β labeled with [^{15}N]Val as an example. In the spectrum, 11 cross peaks were observed, which is consistent with the number of Val residues involved in IL-1β. In other samples, we also observed an identical number of cross peaks as expected from the amino acid composition. However, for ([^{15}N]Gly)- and ([^{15}N]Asp)IL-1β's, a larger number of cross peaks were observed, showing that the cross labeling to other types of amino acids occurred through metabolic pathways in *E. coli*. In the sequential assignment process, it was found that Gly was cross labeled to Ser and Asp, Asn, Gln, and Glu, respectively. The signals due to cross labeling were helpful in confirming sequence-specific resonance assignments. Thus, type-specific assignments of the cross peaks were accomplished as shown with hatching in Figure 2a.

1-^{13}C/^{15}N Double Labeling. When IL-1β is labeled using two types of amino acids, one specifically labeled with ^{13}C at α-carbonyl position and the other labeled with ^{15}N at α-amino position, we observe doublet cross peaks in the ^{15}N-SQC spectrum only where ^{15}N are involved in ^{13}C–^{15}N peptide bonds. This splitting is due to a spin–spin coupling between ^{13}C and ^{15}N. Thus, the observation of the doublet signals in the ^{15}N-SQC spectrum makes sequence-specific resonance assignment possible from amino acid sequence information alone (Kainosho & Tsuji, 1982). In Figure 3, we showed the ^{15}N-SQC spectra of IL-1β labeled with [1-^{13}C]Pro, [^{15}N]Val, [^{15}N]Lys, and [^{15}N]Tyr. Cross peaks with doublet patterns were obviously observed in the spectrum. Thus, sequence-specific or site-specific resonance assignments of the doublet peaks could be made by reference to type-specific assignments of the amino acid residues. It is to be noted that the observation of the doublet pattern is only possible by using ^{15}N-SQC in the present experiment rather than HMQC, due to intrinsic

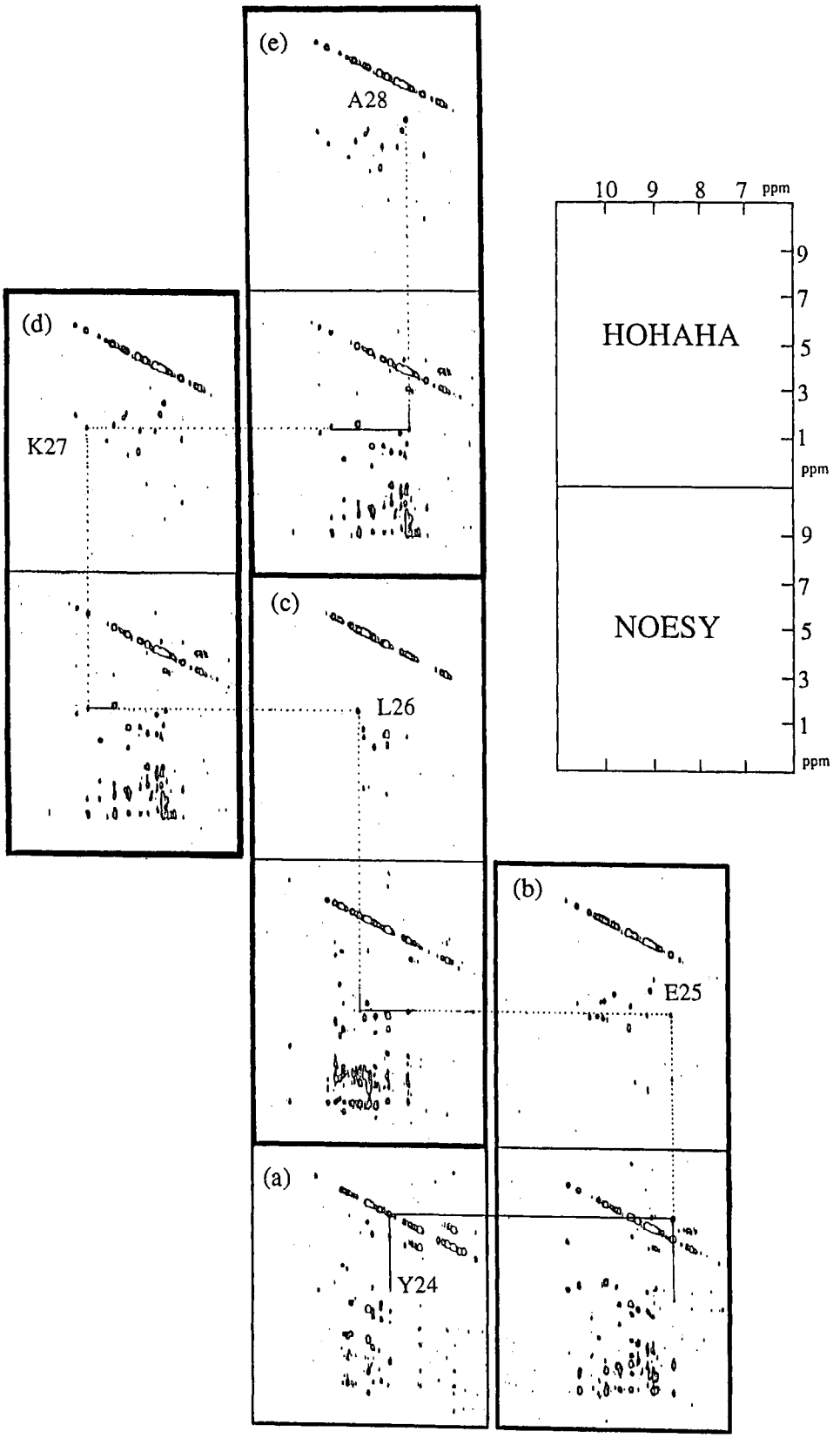

FIGURE 5: Sequential connectivities from Tyr-24 to Ala-28 in the 3D NMR spectra of IL-1β. NOESY and HOHAHA planes with the same ^{15}N chemical shifts are boxed by bold lines. ^{15}N chemical shift for each plane is (a) 110.3 ppm, (b) 119.4 ppm, (c) 122.7 ppm, (d) 119.2 ppm, and (e) 118.7 ppm.

higher resolution along t_1 axis for ^{15}N-SQC (Norwood et al., 1989, 1990; Bax et al., 1990; Tate et al., 1991). On the primary sequence, we also show sequence-specifically or site-specifically assigned amino acid residues with hatching in Figure 2b. Thus, in the present study, we made type-specific assignments for 70 amino acid residues (Phe, 9; Tyr, 4; Ile, 5; Leu, 15; Met, 6; Lys, 15; Ala, 4; Val, 11; His, 1), sequence-specific assignments for 16 amino acid residues (Pro–Val, 3; Pro–Lys, 2; Val–Phe, 3; Lys–Ile, 2; Leu–Lys, 3; Phe–Val, 3), and site-specific assignment for 11 amino acid

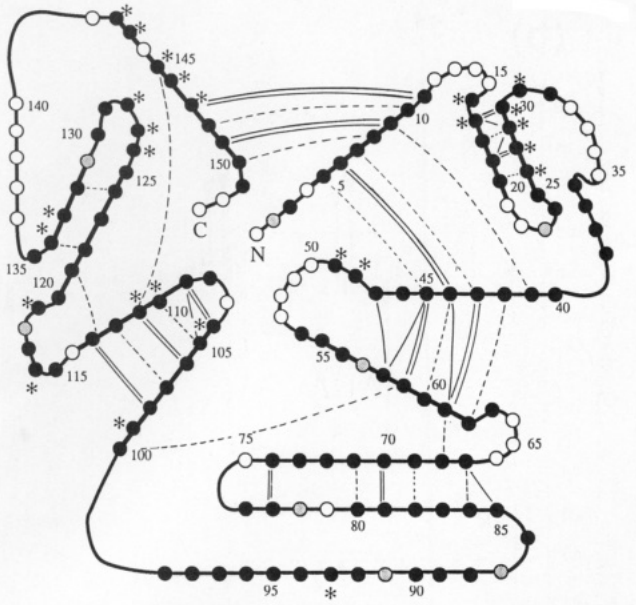

FIGURE 6: Long-range NOE connectivities characteristic to the antiparallel pleated β-sheet structure found in the present study were plotted on the schematic secondary structure (Finzel et al., 1989); double lines denote d_{NH-NH}, single lines denote $d_{NH-C^{\alpha}H}$, and dotted lines denote $d_{C^{\alpha}H-C^{\alpha}H}$. Closed circles show assigned residues in the present study, and shaded circles represent Pro residues. Open circles show unassigned residues due to the absence of the cross peaks or the failure to find the connectivities. The residues with asterisks show a small but appreciable change of chemical shifts between pH 5.4 (Driscoll et al., 1990a) and 7.2. Those residues are mainly located on the closed end of the β-barrel.

residues (Met-20, Tyr-24, Ala-28, His-30, Ala-59, Tyr-68, Leu-73, Met-95, Leu-110, Val-119, Leu-134).

Sequential Resonance Assignment. Sequential resonance assignment of IL-1β was made using ^{15}N 3D NOESY-HMQC and 3D HOHAHA-HMQC spectra (Marion et al., 1990), together with conventional 2D NOESY, DQF-COSY, and TOCSY/HOHAHA spectra. It should be noted that the use of the ^{15}N 3D NMR spectra resolves the chemical shift degeneracy of amide protons, but the chemical shift degeneracy of α-protons still remains. In the present study, this ambiguity was removed by using a large number of resonances assigned by the type-specific, sequence-specific, and site-specific labeling methods.

The assignments of the observed resonances are shown in the ^{15}N SQC spectrum of ^{15}N uniformly labeled IL-1β (Figure 4). Under our experimental conditions (pH 7.2), some of the amide protons disappeared or broadened due to exchange with solvent protons, so that sequential resonance assignment was interrupted at several sites. However, it was possible to assign almost all observed signals of the backbone amide nitrogens and amide protons by extensive use of the isotope labeling methods together with the 3D NMR. In the following, we show the sequential assignment process for residues 24–31 as an example.

Assignments of Residues 24–31. Figure 5 shows the sequential connectivities through residues Tyr-24–Ala-28 in the 3D spectra: 3D HOHAHA-HMQC and 3D NOESY-HMQC (Marion et al., 1990). From the double-labeling experiment, site-specific assignment of Tyr-24 was made, which was a solid starting point for the following sequential resonance assignment process. Furthermore, in this sequence, site-specific assignment of Ala-28, sequence-specific assignment of Leu–Lys, and type-specific assignment of Leu were established in the ^{15}N-SQC spectrum, which made the analysis of NOESY walk in the 3D spectra in an unambiguous manner. In the NOESY plane taken at the chemical shift of [^{15}N]-Tyr-24 (110.3 ppm), a NH–NH cross peak corresponding to a d_{NN} connectivity was observed. The mirror image of this cross peak was found in the NOESY plane at the chemical shift of ^{15}N = 119.4 ppm. The ^{15}N–^{1}H correlation peak corresponding to both ^{15}N and ^{15}NH chemical shifts of this mirror image was found in the ^{15}N-SQC spectrum of [^{15}N]-Asp-labeled IL-1β, which was due to cross labeling from Asp to Asn, Gln, or Glu and could be assigned to Glu-25. The Glu-25 NH–C$^{\alpha}$H correlation peak was subsequently searched in the corresponding HOHAHA plane (^{15}N = 119.4 ppm). At the chemical shift of Glu-25 C$^{\alpha}$H, there were five NOE cross peaks on the 3D NOESY-HMQC plane. Since Glu-25 should be connected to Leu-26, NOESY planes were searched at the ^{15}N chemical shifts of Leu residues. It was found that only one of these five NOE signals was consistent with the NOESY cross peaks with Leu NH (^{15}N = 122.7 ppm). Thus, this Leu NH is assigned to Leu-26 NH, and subsequently Leu-26 C$^{\alpha}$H was identified from the HOHAHA plane (^{15}N = 122.7 ppm). Two NOE cross peaks were observed degenerately at the Leu-26 C$^{\alpha}$H chemical shift. However, when we compared the NOESY plane at the cross peaks of three lysine residues with a Leu–Lys sequence in the 3D NOESY-HMQC spectrum, the cross peak between Leu-26 C$^{\alpha}$H and Lys-27 NH was identified (^{15}N = 119.2 ppm), resulting in the assignment of Lys-27 C$^{\alpha}$H in the HOHAHA plane. Since Ala-28 was site-specifically assigned, the identification of the cross peak between Lys-27 C$^{\alpha}$H and Ala-28 NH was easily made, and the assignment of Ala-28 C$^{\alpha}$H was established in a similar manner. Thus, we can identify the connectivity from the α-protons to the amide protons unambiguously by using specific isotope labeling method together with 3D NMR. In a similar manner, the NOESY walk was extended from Ala-28 to Leu-31 through Leu-29 and His-30, utilizing type-specific assignment of Leu and site-specific assignment of His-30 in the ^{15}N-SQC spectrum.

Secondary Structure of IL-1β in Aqueous Solution at pH 7.2. The detailed structural analyses by X-ray (Finzel et al., 1989; Priestle et al., 1989) and NMR (Driscoll et al., 1990b; Clore et al., 1991a,b) were reported, and similar secondary and tertiary structures were established in both states. Here, we compared the assignments of ^{15}N and ^{15}NH resonances at pH 7.2 and 28 °C with those reported by Driscoll et al. (pH 5.4, 36 °C), although their assignments were made using the mixture of IL-1β and des-(Ala-1)IL-1β. In Figure 6, we show the residues assigned in the present study in filled circles on the schematic drawing of IL-1β secondary structure proposed by Finzel et al. (1989). Long-range NOEs between NH–NH, NH–C$^{\alpha}$H, and C$^{\alpha}$H–C$^{\alpha}$H proton resonances obtained from NOESY spectra (pH 7.2, 28 °C) are also shown. Although under the present experimental conditions (pH 7.2), some of the amide proton resonances broadened due to chemical exchange with bulk water, resulting in attenuation of the NOESY cross peaks, we observed a large number of the NOESY cross peaks characteristic to the antiparallel pleated β-sheet structure. These NOEs are consistent with those observed at pH 5.4, supporting the view that the secondary structure in solution at pH 7.2 is similar to those in the crystalline state and in solution at pH 5.4. Open circles represent unassigned residues in our experiment due to the failure to observe the signals and/or NOE connectivities. Most of these residues are located on the exposed loops. Figure 6 also shows the amino acid residues which have small but appreciable chemical shift differences between pH 5.4 and 7.2 with asterisks (more than 0.5 and 0.05 ppm for ^{15}N and ^{15}NH, respectively). These are mainly localized on the β-hairpins

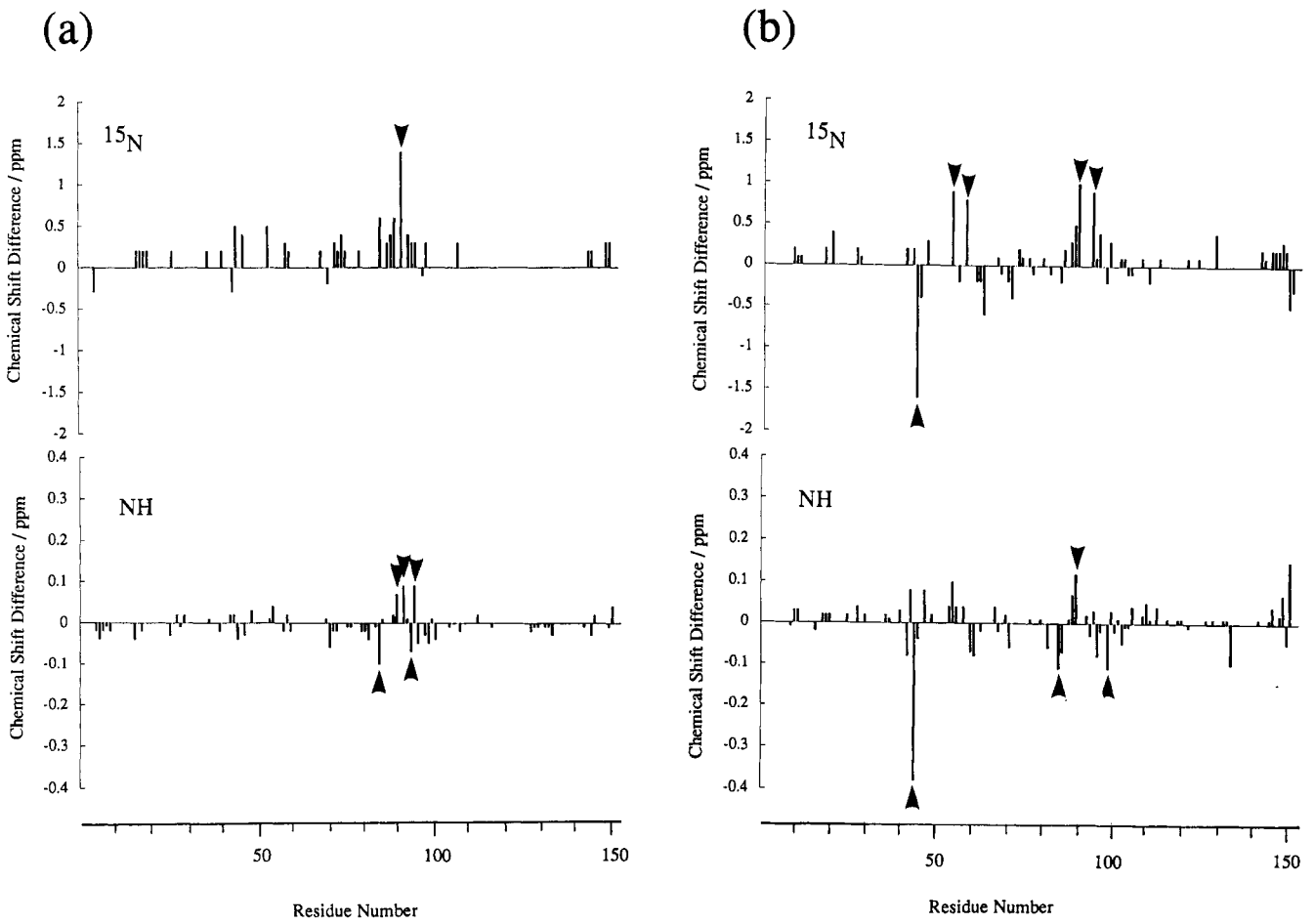

FIGURE 7: Chemical shift differences in the ^{15}N-SQC spectra of (a) IL-1β(4–153) and (b) IL-1β(7–153), compared with that of the wild-type. In each panel, the upper and the lower portions show ^{15}N and ^{15}NH chemical shift differences, respectively. The amino acid residues with large chemical shift difference, Lys-92 (upper) and Val-85, Tyr-90, Lys-92, Lys-94, and Met-95 (lower) in panel a and Met-44, Asp-54, Val-58, Tyr-90, Lys-94 (upper) and Met-44, Val-85, Tyr-90, and Glu-96 (lower) in panel b are shown with arrows. The large chemical shift changes of Met-44, Asp-54, and Val-58 in panel b are due to hydrogen-bond breakage by the deletion of N-terminal six residues.

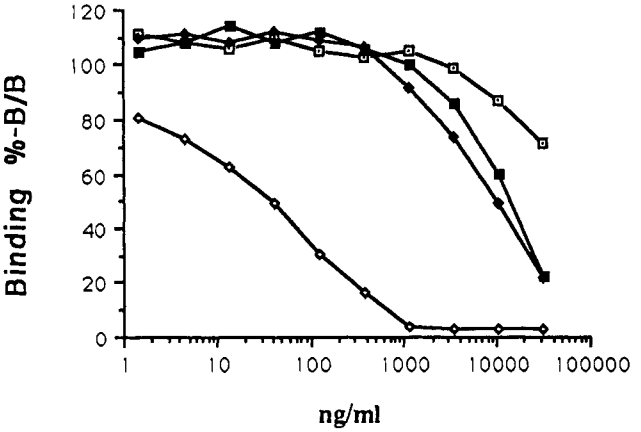

FIGURE 8: Receptor binding assay of IL-1α (◇) and (Asp-93)- (⊡), (Leu-93)- (◆), and des-(Arg-98)IL-1β (■). The vertical axis shows the percent inhibition of [^{125}I]IL-1α binding, and the horizontal axis shows the concentration of proteins. IL-1α and IL-1β show similar binding affinity to the receptor.

15–31 and 120–136, β-strands 100–106 and 109–115, and the C-terminal tail 141–148 which form the closed end of the β-barrel. Deprotonation of His-30 (pK_a 7.5; Gronenborn et al., 1986) and ionization of carboxylate groups may induce such small but appreciable chemical shift changes of the amino acid residues within the closed end of the β-barrel. However, considering the similarity of the secondary structure as well as the similar ^{15}N SQC spectral patterns between pH 5.4 and 7.2, we can conclude that the tertiary structure of IL-1β at pH 7.2 is essentially the same as that at pH 5.4. Thus, we can discuss the effect of mutation on the basis of the reported tertiary structure of IL-1β.

Spectral Changes Due to Mutation at the N-Terminus. IL-1β(4–153) shows about 20% receptor-binding activity of the wild-type and IL-1β(7–153) almost completely (<0.1%) loses its activity (Mosley et al., 1987). Met-IL-1β has also about 5% biological activity of the wild-type (Kikumoto et al., 1987). Moreover, the replacement of Arg-4 by other kinds of amino acid, especially by Asp, leads to a significant reduction in biological activity and des-(Arg-4)IL-1β completely loses its biological activity (Kamogashira et al., 1988). These mutation studies obviously demonstrate the importance of N-terminal residues for biological activities. In order to investigate the effects of the N-terminal mutation on the structure of IL-1β, we compared the ^{15}N-SQC spectra of the wild-type with those of Met-IL-1β, IL-1β(4–153), and IL-1β(7–153). The chemical shift difference of ^{15}N and ^{15}NH resonances between the wild-type and the mutants was investigated. In the case of Met-IL-1β, small spectral changes were observed for the residues near the mutation site and residue 46 on the opposite β-strand, 41–47. Notably, in addition to these residues, the chemical shift change was observed for residues 94 and 95, which are located within the loop

86–99. For IL-1β(4–153) and IL-1β(7–153), appreciable chemical shift changes were observed mainly for amino acid residues within the loop 86–99 and the β-strand 41–47 (Figure 7a,b). However, for other residues, chemical shift changes were small, supporting the view that the overall structure of these mutants was essentially the same as that of the wild-type. Thus, we can conclude that the significant loss of biological activity is not due to the overall structural change induced by the mutation but the localized structural change of the receptor-binding site comprising the N-terminus, C-terminus, and the loop 86–99. It is to be noted that these regions reside on the open end of the β-barrel. The present study highlighted the importance of the loop 86–99 for the biological activity of IL-1β.

The Receptor-Binding Activity of IL-1β Mutants. The receptor-binding activity of IL-1β mutants (Leu-93)-, (Asp-93)-, and des-[Arg-98]IL-1β's was studied. The results are summarized in Figure 8. A significant decrease in receptor-binding activity was observed for these mutants, showing that these basic residues on the loop 86–99 are important, which is in line with the report by Kamogashira et al. (1988) that a monoclonal antibody to a peptide in the region of residues 82–102 inhibits the binding of IL-1β to the receptor. In addition, IL-1β with chemical modification at either Lys-93 or Lys-94 (Yem et al., 1989) sharply decreases the biological activity. These results confirm the view that the loop 86–99 is involved in the receptor-binding site. According to the NMR structure, the loop is exposed from the core but takes a specific conformation with two type I β-turns. This conformation is stabilized by the hydrophobic interaction and hydrogen-bond formation with the core (Driscoll et al., 1990; Clore et al., 1991; Clore & Gronenborn, 1991). It is to be noted that all the amide protons on the loop can be observed even at pH 7.2, showing that these amide protons are shielded from the solvent. Actually, *B* factor and RMSD values for this loop are lower than others (Finzel et al., 1989; Clore et al., 1991). Driscoll reports that the chemical shifts of the Lys-93 and Lys-94 mutants are altered only for those residues that are located close either in the amino acid sequence or in the tertiary structure (Driscoll et al., 1990), excluding the possibility of the overall structural change by the mutation at position 93.

The Proposed Receptor-Binding Site of IL-1β. On the basis of the biological activity of the mutants combined with the three-dimensional structure of IL-1β in a crystal and in solution, Clore proposed three distinct biologically active regions, which interact with each of three immunoglobulin domains of the IL-1 receptor (Clore et al., 1991). However, it should be noted that the interpretation of the biological activity and/or the receptor-binding activity of mutants is difficult without knowledge of their structures. Although we discussed the structures of Met-IL-1β, IL-1β(4–153), and IL-1β(7–153) in qualitative manner based on the comparison of the chemical shifts of ^{15}N and ^{15}NH resonances of the wild-type and the mutants, the chemical shifts are more sensitive to the structural change than NOE (Folkers et al., 1989). On the basis of the present results, we may conclude that the fusion and deletion mutants of IL-1β take structures similar to that of the wild-type so that significant loss of the biological activity is ascribed to the local conformational change around the receptor-binding site rather than the overall conformational change. The receptor-binding site of IL-1β resides on the N-terminus, the C-terminus, and the loop 86–99. The proper orientation among the loop 86–99, the N-terminus, and the C-terminus is required for the receptor binding of IL-1β, and basic residues Arg-4, Lys-92, Lys-93, Lys-94, and Arg-98 in this region are responsible for the receptor binding.

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Supplementary Material Available

Two figures showing ^{15}N-SQC spectra of single- and double-labeled IL-1β and a table containing chemical shifts of IL-1β at pH 7.2 and 28 °C (13 pages). Ordering information is given on any current masthead page.

Registry No. IL-1β, 97599-21-8.

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