computed by using equal area elements on the sphere. This was done with equal increments in β (typically 0.5°) and variable increments in α proportional to sin β.

Supplementary Material Available: Tables of calculated hydrogen atom coordinates, anisotropic and isotropic thermal parameters, and bond distances and angles for the X-ray structures of **1** to 298 K, **2** at 298 K, and **3** at 128 and 298 K (27 pages); listing of structure factor amplitudes (79 pages). Ordering information is given on any current masthead page.

Protein Structure and Interactions by Combined Use of Sequential NMR Assignments and Isotope Labeling

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Abstract: The 10 leucyl residues in the DNA-binding domain consisting of the 76 N-terminal residues of *Salmonella* phage P22 c2 repressor were labeled with ^{15}N. By use of a novel $^{15}N(\omega_2)$ half-filter technique, simplified two-dimensional nuclear Overhauser enhancement spectra were obtained, which contain exclusively resonance peaks relating to at least one ^{15}N-bound hydrogen atom. Observation of nuclear Overhauser effects in these edited subspectra greatly facilitated sequential resonance assignments leading to sequence-specific assignments for all 10 leucines. Further potentialities of the combined use of residue-selective isotope labeling and sequential assignment procedures for studies of protein conformation in solution and for investigations of intermolecular interactions with proteins are discussed.

Nuclear magnetic resonance (NMR) is presently the only technique besides diffraction methods with single crystals that can be employed for three-dimensional structure determination with biopolymers.[1] With regard to studies of structure–function correlations in proteins and nucleic acids the significance of this new approach lies in the fact that NMR studies can be performed in aqueous solution or in other environments which may closely mimic the physiological milieu. Structure determination by NMR relies on one's ability to obtain sequence-specific ^1H NMR assignments.[1,2] For small proteins these can efficiently be obtained by the sequential assignment technique.[1,3,4] An alternative, straightforward approach for NMR assignments is by site-specific labeling with ^{13}C or ^{15}N,[1,5-8] which has, however, for practical reasons been used only on a limited scope. The present paper illustrates the potentialities of the combined use of sequential assignments and residue-specific isotope labeling with proteins. This approach enables NMR applications with more complex macromolecular systems and opens new avenues for studies of spatial structure and of intermolecular interactions.

Materials and Methods

For the experiments in this paper we used the DNA-binding domain consisting of the 76 N-terminal residues of *Salmonella* phage P22 c2 repressor, which was previously shown to retain the ability of the intact repressor protein for specific binding to DNA.[9,10] c2 repressor 1–76 contains 10 Leu residues, which were all labeled with ^{15}N in the extent of >85%.[11]

NMR spectra were recorded with a Bruker AM-360 spectrometer. For studies of the[2-^{15}N]Leu c2 repressor 1–76 a new $^{15}N(\omega_2)$ *half*-filter experiment was introduced,[12] which is related to a previously described heteronuclear zero-quantum filter procedure.[13] A two-dimensional ^1H nuclear Overhauser enhancement (NOESY) spectrum with a $^{15}N(\omega_2)$ half-filter was obtained by using the pulse sequence $90°(^1H)-t_1-90°(^1H)-\tau_m-90°(^1H)-\tau/2-180°(^1H,^{15}N)-\tau/2-[180°(^{15}N)]$ acquisition. The editing $180°(^{15}N)$ pulse shown in brackets is applied in every second scan, and the difference of the spectra recorded with and without the editing pulse is taken. This experiment selects for the resonances of ^{15}N-bound amide protons along ω_2 but does not discriminate along ω_1, thus providing dramatically simplified ^1H NOESY spectra with a high content of structural information.

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Sequence-specific assignments for the ^{15}N-labeled amino acid residues were obtained with the usual sequential assignment procedures,[1-4] as is described in more detail in the following section.

Results and Discussion

Figure 1 shows different regions of a ^1H NOESY spectrum recorded with a $^{15}N(\omega_2)$ half-filter. On the diagonal from the upper right to the lower left there are exclusively the peaks of the ^{15}N-bound protons, which are split into four components by the one-bond $^{15}N-^1H$ scalar coupling of ca. 90 Hz (Figure 1A). Nuclear Overhauser effects (NOE) between different ^{15}N-bound protons give rise to pairs of cross peaks symmetrically arranged with respect to this diagonal, which are split into four components by one-bond $^{15}N-^1H$ scalar couplings along both ω_1 and ω_2. Figure 1A contains two such cross peaks between different ^{15}N-labeled leucines. Finally, there are all the NOEs between leucine amide protons and protons not bound to ^{15}N. The corresponding NOESY cross peaks consist of two fine structure components separated by the $^{15}N-^1H$ scalar coupling along ω_2, and they are observed

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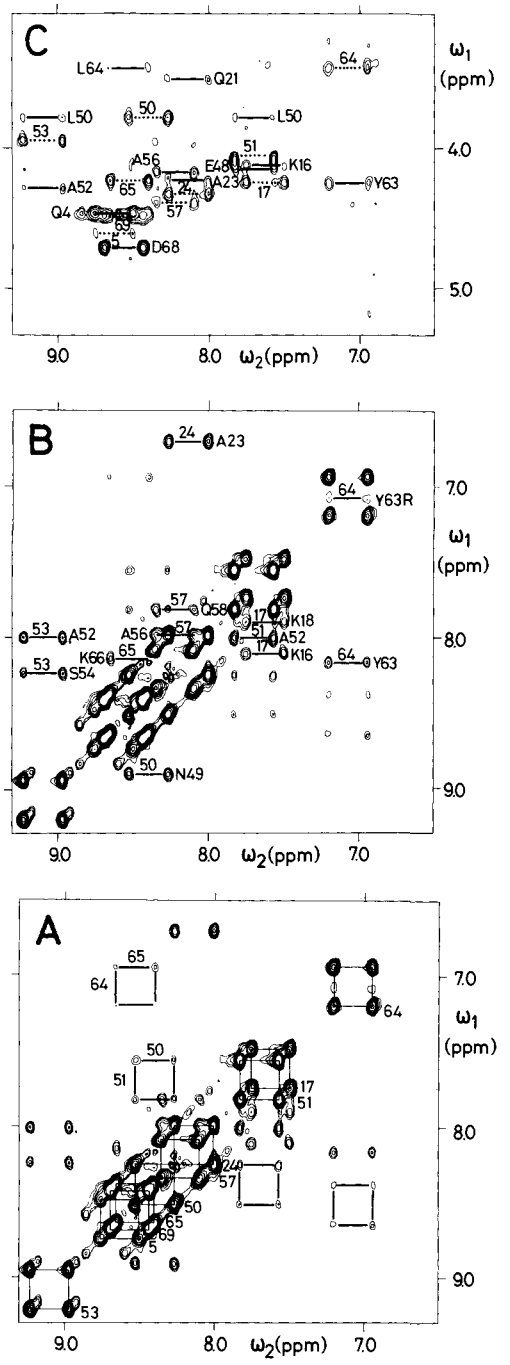

Figure 1. ^1H NOESY spectrum of [2-^{15}N]Leu c2 repressor 1–76 recorded at 360 MHz by using a $^{15}N(\omega_2)$ half-filter (protein concentration 8 mM in H_2O, pH 4.8, 20 °C, mixing time 100 ms, filter delay 5.55 ms). (A) Spectral region (ω_1 = 6.6–9.3, ω_2 = 6.6–9.3 ppm). The fine structure components of 10 diagonal peaks from [2-^{15}N]Leu are connected by thin lines and those of cross peaks between two ^{15}N-bound protons by thicker lines. These peaks are identified with the sequence number of the corresponding Leu residues. (B) Same region as A with identification of ^{15}NH–NH cross peaks. The fine structure components are connected by horizontal lines and identified with the Leu sequence number, the one-letter symbol, and the sequence position of the second residues. R indicates an aromatic proton of Tyr-63. (C) Region (ω_1 = 3.1–5.3, ω_2 = 6.6–9.3 ppm) containing NOEs between C^α protons (ω_1 = 3.1–5.3 ppm) and ^{15}N-labeled amide protons. Cross peak identification as in B. Dotted lines indicate the 10 intraresidue ^{15}NH–$C^\alpha H$ cross peaks of the leucines. The sequence-specific peak assignments indicated in this figure are one of the principal results obtained with the experiments described in this paper.

only once, either above *or* below the diagonal. The cross peaks identified in Figure 1B manifest NOEs between ^{15}NH's of Leu and other amide protons; those identified in Figure 1C are between ^{15}NH's and C^α protons.

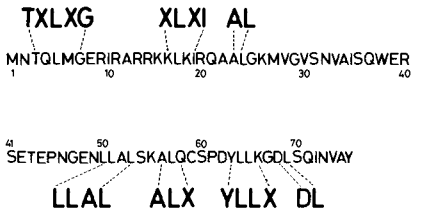

Figure 2. Amino acid sequence of *Salmonella* phage P22 c2 repressor 1–76. The 10 Leu residues were labeled with ^{15}N in the extent of >85%. The bigger size letters above and below the sequence identify segments of neighboring amino acid spin systems in the primary structure, which were identified by sequential assignments with ^1H–^1H NOEs and led to sequence-specific assignments of all 10 leucines. X stands for a residue from the group Glu, Gln, Met, Arg, Lys, and Pro for which the spin systems were not individually identified (see text).

The primary advantage gained with the ^{15}N labels is that the resonances of the 10 leucyl amide protons and the NOEs of the leucyl amide protons with other protons can be observed without interference from the bulk of the protein (Figure 1 contains only approximately 10% of the total number of peaks that would be present in the same region of a normal ^1H NOESY spectrum of the c2 repressor). Full use of the facilitated observation of these NMR lines for studies of protein structure and function can, however, only be made after *sequence-specific assignments* were obtained with the *method of sequential assignments*.[1,3,4]

Assignment of a protein ^1H NMR spectrum starts with the identification of the ^1H *spin systems* of the individual amino acid residues, using through-bond, scalar spin–spin couplings (a spin system comprises all protons attached to one residue). These are characteristic for the different amino acid *types*, but since a protein usually contains multiple copies of any given amino acid (Figure 2), the sequence positions cannot be determined from this information alone. Therefore, by use of *through-space*, dipolar couplings observed as ^1H–^1H NOEs, two or several sequentially neighboring amino acid spin systems are identified (sequential assignments).[1,3,4] The peptide segments thus determined are then matched against the primary structure, and as a result the sequence-specific assignments are obtained.[1] For the c2 repressor the identification of the complex ^1H spin systems of Leu, Glu, Gln, Met, Arg, Lys, and Pro was difficult because of spectral overlap (in the following, X stands for a spin system of this group). In this situation, which can generally be anticipated for larger proteins or for small proteins with unfavorable spectral properties, sequence-specific assignments were greatly facilitated by the identification of the Leu spin systems through ^{15}N labeling. This is readily apparent from the following example: A tripeptide segment XXX identified by sequential assignments in the nonlabeled protein matches the sequence positions 4–6, 13–15, 14–16, 15–17, 16–18, and 64–66 in the c2 repressor sequence (Figure 2). With ^{15}N labeling of the leucines the ambiguities of the sequence-specific assignments are greatly reduced, since XXX then matches only with the locations 13–15 and 14–16 whereas 4–6 and 16–18 are recognized as XLX and 64–66 as LLX.

Sequence-specific assignments for all 10 leucines and subsequently for the entire polypeptide chain of the c2 repressor 1–76 (Otting, G.; Senn, H.; Wüthrich, K., unpublished results) resulted from combination of Figure 1 with sequential assignments for the nonlabeled residues obtained from a normal ^1H NOESY spectrum. Cross peaks in NOESY manifest close spatial approach between two protons. The sequential assignment strategy relies on the fact that short distances between different amide protons or between α-protons and amide protons in proteins prevail predominantly between residues that are neighbors in the sequence.[1,3] It was, therefore, likely that the two ^{15}NH–^{15}NH cross peaks in Figure 1A corresponded to the residues 50–51 and 64–65. Starting from one of these two cross peaks, subsequent NOEs ^{15}NH–Ala and Ala–^{15}NH were found (Figure 1B), identifying a peptide segment LLAL, which matches with the residues 50–53 in the c2 repressor sequence (Figure 2). Similarly, the peptide segments YLLX, ALX, AL, and DL were identified from Figure 1 and assigned to the positions 63–66, 56–58, 23–24, and 68–69 (Figure 2). To

distinguish between Leu-5 and Leu-17, information from NOEs between different nonlabeled residues was needed in addition to that contained in Figure 1, as is indicated in Figure 2.

On the basis of the sequence-specific assignments, Figure 1 provides direct information on the secondary polypeptide structure near several [2-^{15}N]Leu residues. Thus both Leu–Leu dipeptide segments are located in α-helices, as evidenced by the strong sequential NOEs d_{NN} throughout the polypeptide segments 49–54 and 63–66 (Figure 1A,B) and by the short distances $d_{\alpha N}$ (48, 51) and $d_{\alpha N}$ (50, 53).[1,14] Similarly, there is evidence that Leu-24 is located in a helical segment of the polypeptide chain.

In conclusion, the experiments described here illustrate that isotope labeling of a *single*, strategically selected amino acid type in a protein (Figure 2) can dramatically enhance the power of ^{1}H NMR for studies of such systems, provided that labeling is used in combination with sequential assignment procedures.[1,3,4] The latter can be greatly facilitated by the ease with which distinct resonance lines are observed in the labeled protein (Figure 1). Once sequence-specific assignments are obtained, site-directed information on the spatial polypeptide structure or on interactions with other molecules can be collected in systems which might otherwise be too complex for detailed investigations. The present experiments are part of the first phase of an investigation aimed at studies of protein–DNA interactions in the P22 c2 operator system. We plan to use protein analogues with ^{15}N labels on amino acids that are likely to be in direct contact with the DNA. The resulting NOEs can then be measured in the simplified ^{1}H NOESY spectra obtained with the ^{15}N half-filter technique, where intermolecular ^{1}H–^{1}H NOEs between ^{15}NH's and nonlabeled protons would appear as doublets along ω_2 (Figure 1B,C). Compared to the recently described X-filter technique,[15] which selects for peaks connecting protons which are *both* bound to the *same* X-spin, the half-filter experiment[12] used here can thus be combined with suitable residue-selective isotope labeling for novel investigations of structural and functional features in proteins.

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Effect of Ligand on Ring Contraction of Six-Membered Nickel-Containing Cyclic Esters, $L_nNiCH_2CH_2CH_2COO$, to Their Five-Membered-Ring Isomers, $L_nNiCH(CH_3)CH_2COO$. Kinetic and Thermodynamic Control of Asymmetric Induction by Chiral Diphosphines in the Ring Contraction

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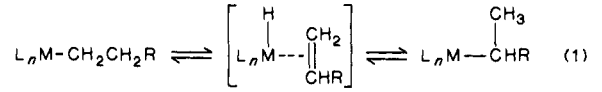
Abstract: Ring contraction of $L_nNiCH_2CH_2CH_2COO$ (**1**) to $L_nNiCH(CH_3)CH_2COO$ (**2**) is accelerated by coordination of bulky ligands: coordination of a bulky diphosphine like 1,2-bis(diphenylphosphino)ethane (dpe) causes an extensive ring contraction to afford (dpe)$NiCH(CH_3)CH_2COO$. Use of a chiral diphosphine affords a mixture of unequal amounts of diastereomers (chiral diphosphine)-(*R*)-$NiCH(CH_3)CH_2COO$ ((*R*)-**2**) and (chiral diphosphine)-(*S*)-$NiCH(CH_3)CH_2COO$ ((*S*)-**2**). When (*S*,*S*)-chiraphos is used, (*R*)-**2** is kinetically favored, but it isomerizes to thermodynamically favored (*S*)-**2** obeying the first-order kinetics. The equilibrated reaction mixture after the isomerization contains ((*S*,*S*)-chiraphos)$NiCH(CH_3)CH_2COO$ in 54% diastereomer excess at 24 °C, and the kinetic and thermodynamic parameters for the *R* to *S* isomerization are as follows: $\Delta H^\ddagger = 93 \pm 2$ kJ/mol, $\Delta S^\ddagger = -8 \pm 6$ J/(K mol), $\Delta G^\ddagger = 95$ kJ/mol, $\Delta H° = 13 \pm 2$ kJ/mol, $\Delta S° = 54 \pm 6$ J/(K mol), and $\Delta G° = -3.0$ kJ/mol at 24 °C. Use of (*R*,*R*)-dipamp gives a result opposite to that of (*S*,*S*)-chiraphos concerning the kinetically and thermodynamically favored species. Data obtained by use of (*R*)-prophos, *trans*-cypenphos, and *trans*-renorphos are also given. The kinetic and thermodynamic control of the asymmetry can be explained by considering the effects of arrangement of two phenyl groups bonded to each phosphorus atom of the diphosphine ligands on the metallacycle entity.

Isomerization processes involving β-hydrogen elimination and reinsertion mechanism[1-7] have been considered responsible for skeletal isomerization in metal-catalyzed homogeneous catalytic reactions as well as in isolated transition-metal alkyls.



In the skeletal isomerization of transition-metal alkyls isomerization from *sec*-alkyl to *n*-alkyl has been often observed, a process favored by the steric bulkiness of the coordinated ligand. Such isomerization processes are important in catalytic processes such as hydroformylation for producing a linear chain product. For metallacycles, however, such isomerizations have been reported in very limited cases. Whitesides[8] and Schrock[9] suggested oc-

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