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Proton, carbon, and nitrogen chemical shifts accurately delineate differences and similarities in secondary structure between the homologous proteins IRAP and IL-1β

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SUMMARY

$^{1}H^{\alpha}$, $^{13}C^{\alpha}$, and $^{15}N^{\alpha}$ secondary chemical shifts, defined as the difference between the observed value and the random coil value, have been calculated for interleukin-1 receptor antagonist protein and interleukin-1β. Averaging of the secondary chemical shifts with those of adjacent residues was used to smooth out local effects and to obtain a correlation dependent on secondary structure. Differences and similarities in the placement of secondary structure elements in the primary sequences of these structurally homologous proteins are manifested in the smoothed secondary chemical shifts of all three types of nuclei. The close correlation observed between the secondary chemical shifts and the previously defined locations of secondary structure, as defined by traditional methods, exemplifies the advantage of chemical shifts to delineate regions of secondary structure.

INTRODUCTION

Secondary structure-dependent chemical shifts in proteins have been recognized for some time (Dalgarno et al., 1983; Pardi et al., 1983). As the number of proteins for which sequential assignments are known has increased, several studies regarding the generalization of secondary structure-dependent chemical shifts have appeared (Pastore and Saudek, 1990; Williamson, 1990). Recently, a large database of protein chemical shifts has been correlated with secondary structure (Wishart et al., 1991), and has led to the proposal of a chemical shift index to define regions of

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Abbreviations: IL-1, interleukin-1; IL-1β, interleukin-1β; IRAP, interleukin-1 receptor antagonist protein.

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protein secondary structure (Wishart et al., 1992). A major conclusion from these investigations is that secondary chemical shifts are nearly as reliable as traditional NOE-based methods for delineating regions of secondary structure (Wishart et al., 1992). In order to determine if secondary chemical shifts can accurately reflect subtle differences in secondary structure between structurally homologous proteins, we have compared secondary $^1H^\alpha$, $^{13}C^\alpha$, and $^{15}N^\alpha$ chemical shifts for two 153-residue proteins: interleukin-1 receptor antagonist protein and interleukin-1β.

IRAP and IL-1β are naturally occurring cytokines that bind to the IL-1 receptor. Based on structural homologies, the two proteins share 25% sequence identity. However, although the binding of IL-1β elicits changes in neurologic, metabolic, hematologic, and endocrinologic systems (Dinarello et al., 1991), the binding of IRAP does not (Carter et al., 1990; Eisenberg et al., 1990). Our research is focused on understanding the structural differences and similarities between these two predominantly β-sheet proteins that underlie these physiological properties.

We previously reported the secondary structure and topology of IRAP, as defined by hydrogen-exchange and NOE-based methods (Stockman et al., 1992). In comparison to the solution topology of IL-1β (Driscoll et al., 1990b), different regions of the primary sequences contribute to structurally homologous parts of the secondary structure, as shown in Fig. 1. Some of the differences are large, up to five residue shifts, while others are subtle shifts of a single residue. In addition, several identical areas of the primary sequences were found to be structurally homologous. With the availability of nearly complete $^1H^\alpha$, $^{13}C^\alpha$, and $^{15}N^\alpha$ resonance assignments, we have ana-

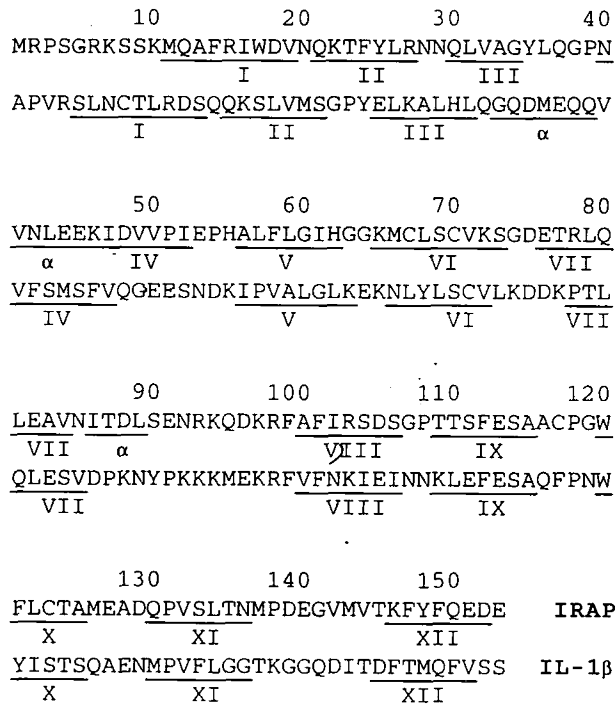

Fig. 1. Comparison of the primary sequences of IRAP (top) and IL-1β (bottom). The locations of secondary structure elements are underlined in each sequence. Structurally homologous β-strands are identified by Roman numerals; helical regions are identified by α's.

lyzed secondary chemical shifts for both proteins to determine how well they define regions of secondary structure.

MATERIALS AND METHODS

Proton, carbon, and nitrogen chemical shift values for IRAP were taken from Stockman et al. (1992). Proton and nitrogen chemical shift values for IL-1β were taken from Driscoll et al. (1990a). Carbon chemical shift values for IL-1β were taken from Clore et al. (1990). A correction factor of 0.4 ppm (Spera and Bax, 1991) was added to the carbon chemical shifts of IL-1β. No corrections were introduced to account for differences in pH, temperature, or other solution conditions. For glycine residues, the average of the two $^{1}H^{\alpha}$ resonances was used. Values for random coil $^{1}H^{\alpha}$ chemical shifts were those used by Wishart et al. (1992). Random coil $^{13}C^{\alpha}$ chemical shifts were from Richarz and Wüthrich (1978). Since random coil nitrogen chemical shifts are not available, average values for the $^{15}N^{\alpha}$ chemical shifts, based on a database compiled by Wishart et al. (1991), were used instead.

For each residue of IRAP and IL-1β, the secondary chemical shift was calculated by subtracting the value of the random coil chemical shift from that of the observed value. To average out local effects such as ring-current shifts (IRAP has 14 aromatic amino acids, IL-1β 15), the secondary chemical shifts were then smoothed over plus and minus two residues (Pastore and Saudek, 1990). In order not to introduce any bias, the smoothing range was not extended to compensate for missing chemical shift assignments. Secondary chemical shift and smoothing calculations and plotting were carried out using a Microsoft® Excel spreadsheet on a MacIIci personal computer. The data file, which can easily be modified to any primary sequence, is available on request.

For the $^{1}H^{\alpha}$ resonances, similar results were obtained using the chemical shift index of Wishart et al. (1992). However, since chemical shift indices for $^{13}C^{\alpha}$ and $^{15}N^{\alpha}$ resonances have not been defined, smoothing of the $\Delta\delta$ values was carried out for each nuclei type in order to facilitate comparisons.

RESULTS AND DISCUSSION

Smoothed secondary chemical shifts for IRAP and IL-1β $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, and $^{15}N^{\alpha}$ resonances, as a function of residue number, are shown in Fig. 2A–C. Gaps in the plot are present when the chemical shift was not assigned. The locations of secondary structure elements, as defined previously by hydrogen-exchange and NOE-based methods (see Figure 1), are indicated for IRAP and IL-1β on the lower portion of each plot. As expected for predominantly β-sheet proteins, large positive deviations from random coil chemical shifts are observed for the $^{1}H^{\alpha}$ and $^{15}N^{\alpha}$ resonances (Pardi et al., 1983; Wishart et al., 1991), and large negative deviations from random coil chemical shifts are observed for the $^{13}C^{\alpha}$ resonances (Spera and Bax, 1991; Wishart et al., 1991).

The out-of-phase appearance of the comparison plots over the first 55 residues corresponds exactly with the relative locations of the structurally homologous β-sheet strands indicated at the bottom of each figure. The β-sheet framework begins at residue 5 in IL-1β and at residue 11 in IRAP. The unassigned N-terminus of IRAP has no structural counterpart in IL-1β. The comparison plots nearly come back in phase near residue 55 because of a five-residue loop in IL-1β that has no structural counterpart in IRAP. The correspondence between the secondary shifts for resi-

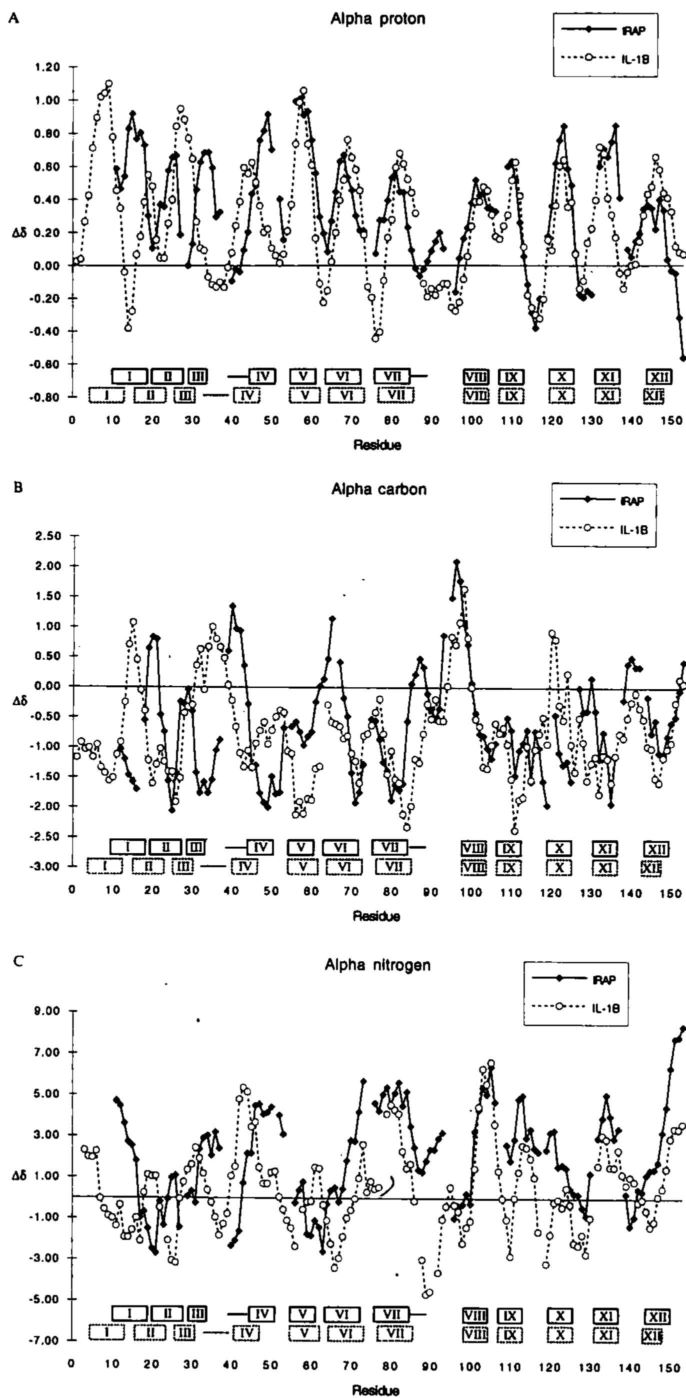

Fig. 2. Comparison of $^{1}H^{\alpha}$ (A), $^{13}C^{\alpha}$ (B), and $^{15}N^{\alpha}$ (C) shifts from random coil (Δδ) for IRAP and IL-1β. Breaks in the plots are located at unassigned resonances. NOE-defined locations of secondary structure for each protein are indicated in the lower portion of each figure. Boxes indicate β-sheet strands, and are labeled with the strand number. Lines indicate helical conformations.

dues 60–153 agrees quite well with the relative locations of β-sheet strands in the two proteins. Furthermore, it is interesting that even the one-residue shifts in the locations of strands VI and VII are manifested in the secondary chemical shift plots, as evidenced by the fact that the maxima in the IRAP plots occur earlier than in the IL-1β plots for these strands.

Although predominantly β-sheet, IRAP and IL-1β have some helical regions as well. One helix, residues 40–45 in IRAP and residues 33–39 in IL-1β, is structurally conserved. These two helices are delineated in the $^{1}H^{\alpha}$ and $^{15}N^{\alpha}$ plots as offset negative secondary shifts, and in the $^{13}C^{\alpha}$ plot as offset positive secondary shifts. As with the β-strands, the secondary chemical shifts accurately delineate the different regions of the primary sequences that adopt helical conformations.

IRAP contains a second short helical segment at residues 86–89 that was not observed in IL-1β. The secondary chemical shift plots of the $^{13}C^{\alpha}$ nuclei show opposite deviations from random coil for this stretch of structurally corresponding residues, with the shifts observed for IRAP being to low field as expected for a helical conformation. In contrast, the $^{1}H^{\alpha}$ and $^{15}N^{\alpha}$ secondary chemical shift plots are similar for these residues in both proteins. This may be due in part to tyrosine^{90} in IL-1β, which has no structural counterpart in IRAP. Ring-current effects may be shifting the IL-1β $^{1}H^{\alpha}$ and $^{15}N^{\alpha}$ resonances to high field, similar to what would be expected for a helical conformation, and thus may obscure the effect of backbone conformation on these chemical shifts.

Overall, the $^{1}H^{\alpha}$ and $^{13}C^{\alpha}$ secondary chemical shifts correlate quite well with the locations of secondary structure elements in both proteins. The weaker correlation observed between $^{15}N^{\alpha}$ secondary chemical shifts and backbone conformation may be attributable to the small database of proteins used to generate the random coil chemical shift values. Alternatively, the jagged appearance of the smoothed $^{15}N^{\alpha}$ plots may be caused by hydrogen-bonding or solvent effects that do not effect $^{1}H^{\alpha}$ or $^{13}C^{\alpha}$ resonances to the same degree. The $^{15}N^{\alpha}$ resonances may be more sensitive to subtle tertiary structural differences than to gross secondary structural differences. A detailed analysis of a larger number of proteins with assigned $^{15}N^{\alpha}$ resonances will be required to explain this observation.

CONCLUSIONS

The excellent correlations observed between the $^{1}H^{\alpha}$ and $^{13}C^{\alpha}$ chemical shifts, and to a lesser extent the $^{15}N^{\alpha}$ chemical shifts, and the actual locations of secondary structure elements indicates the advantage and precision with which chemical shifts can be used to define locations of secondary structure. With the increasing use of non-NOE-based methods of sequential assignments (Ikura et al., 1990), and the concomitant lack of identified short-range NOEs, chemical shifts may become increasingly important in protein structure determination (Wishart et al., 1992).

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