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Letter to the Editor

NMR Spectroscopy and X-Ray Crystallography Provide Complementary Information on the Structure and Dynamics of Leucine Zippers

The leucine zipper dimerization motif (Landschulz et al., 1988) has received considerable attention in recent years because of its fundamental role in regulating the activity of the bZIP and bHLH-ZIP families of eukaryotic transcription factors. X-ray diffraction studies (O'Shea et al., 1991; Ellenberger et al., 1992; Ferré-D'Amaré, 1993; König and Richmond, 1993; Glover and Harrison, 1995) and NMR spectroscopic measurements (Oas et al., 1990; Saudek et al., 1991; Junius et al., 1995, 1996) have revealed that the leucine zipper forms a parallel coiled-coil of α-helical strands that wrap around one another with a slight left-handed superhelical twist. The small size of these quaternary protein structures (each leucine zipper comprises only about one-fourth to one-third of a superhelical twist) makes them ideal models for precise examination of the intermolecular forces governing protein-protein interactions (e.g., Zhu et al., 1993) and for testing protein design principles (e.g., Graddis et al., 1993).

Following the standard nomenclature for coiled coils $((abcdefg)_n$; McLachlan and Stewart, 1975), the dimer interface of leucine zippers is comprised of alternating rungs of d-position Leu residues and apolar a-position residues, which generally have β-branched side chains (Val, Ile, and Thr). However, an intriguing feature of many leucine zippers is the presence at the hydrophobic dimer interface of a highly polar a-position Asn residue; this residue is highly conserved in the dystrophin, utrophin, bZIP, and bHLH-ZIP protein families (Hurst, 1994; Blake et al., 1995). It has been demonstrated using both the GCN4 and c-Jun leucine zippers that this Asn residue confers dimerization specificity at the expense of dimer stability (Harbury et al., 1993; Junius et al., 1995).

The x-ray crystal structure of the isolated GCN4 leucine zipper (GCN4-LZ; O'Shea et al., 1991) shows that the side chains of these Asn residues form an intermolecular hydrogen bond at the dimer interface, thus yielding an asymmetric conformation (Fig. 1 *A*). On the other hand, only a single set of ^{1}H NMR resonances are observed for the side chains of this Asn residue in both GCN4 (Saudek et al., 1991) and c-Jun (Junius et al., 1995), suggesting a symmetric arrangement of these side chains in the solution state. This ostensibly implies that a particular conformation has been trapped in the crystal structure. Indeed, in a recent article in this journal that addressed the effects of crystal packing on the structure of leucine zippers, Shen et al. (1996) used our NMR data on the c-Jun leucine zipper (JunLZ) as evidence in favor of this argument.

In fact, our NMR experiments are consistent with exactly the opposite conclusion. The NMR data (Junius et al., 1995) showed that, in the solution state, the Asn side chains of JunLZ are involved in a dynamic hydrogen bond, with each Asn side chain acting alternately as hydrogen bond donor and hydrogen bond acceptor. This can be illustrated using the 1.8-Å resolution crystal structure of GCN4-LZ (O'Shea et al., 1991). Fig. 1 *A* shows a cross-section through this crystal structure illustrating the asymmetric side chain hydrogen bond in which the Asn-O^γ on Helix A acts as a hydrogen bond acceptor and the Asn side chain NH on Helix B acts as a hydrogen bond donor. NMR-derived hydrogen-deuterium exchange rates, temperature titration experiments, and chemical shifts enabled us to propose that this conformation is in fast exchange with an alternate conformation (Fig. 1 *B*) whereby the Asn NH on Helix A now becomes the hydrogen bond donor (by rotation around the C^β-C^γ bond and rolling out of the side chain away from the dimer interface) and the O^γ on Helix B acts as a

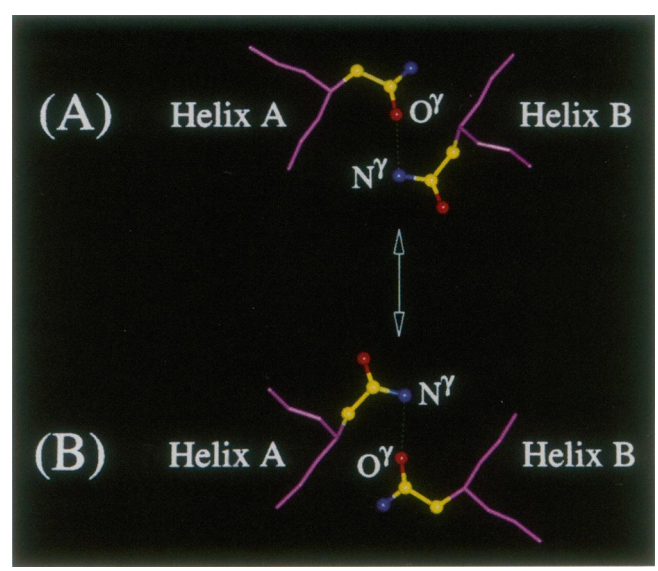

FIGURE 1 (*A*) A cross-sectional view of the x-ray crystal structure of GCN4-LZ (O'Shea et al., 1991) illustrating the hydrogen bond between the side chains of the interfacial Asn residues. (*B*) The alternate conformation of GCN4-LZ as proposed on the basis of solution-state NMR data (Junius et al., 1995). It is postulated that the two conformations are in rapid exchange, as indicated by the double-headed arrow. Note, however, that the two conformations are, from a purely chemical point of view, formally equivalent as they are related by a 180° rotation around an axis normal to the plane of the page. In both figures, the helix backbone is shown in magenta, and the Asn side chains are drawn in yellow with the exception of the O^γ and N^γ atoms, which are colored red and blue, respectively.

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hydrogen bond acceptor (by a similar rotation around the C^{β}-C^{γ} bond in concert with movement of the side chain toward the dimer interface).

A rapid equilibrium between these alternate conformations would explain why, on average, the Asn side chains appear to be symmetric based solely on NMR chemical shift information. However, the crucial point to be noted is that the conformations shown in Fig. 1, *A* and *B*, are actually chemically equivalent—the conformations are related by a simple 180° rotation about an axis normal to the page. Thus, we do not believe a specific conformer has been trapped in the crystal form because the crystal structure is equivalent to each of the two interconverting, but chemically equivalent, conformations shown in Fig. 1.

This situation represents an excellent example of the often mooted, but seldom demonstrated, complementarity of NMR and x-ray structural data. The exact nature of the hydrogen bonding pattern could not be ascertained from the NMR data alone but neither could the exchange phenomenon outlined in Fig. 1 be visualized using x-ray crystallography. The driving force for the exchange phenomenon remains to be determined; we have proposed that it might provide some entropic compensation for the enthalpically unfavorable desolvation that occurs when the Asn residues are buried at the hydrophobic dimer interface (Mackay et al., 1996).

Glenn F. King

Department of Biochemistry
The University of Sydney
Sydney, Australia

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Response to G. F. King

In his Letter to the Editor, ("NMR Spectroscopy and X-Ray Crystallography Provide Complementary Information on the Structure and Dynamics of Leucine Zippers"), Glenn F. King commented on our recent paper published in *Biophysical Journal* (Shen et al., 1996). We welcome his contribution and the opportunity to enter into a deeper discussion of conformational variability and dynamics of peptide structures in the crystal and in solution.

As King reminds us, NMR spectroscopy in solution shows single resonances for the intrahelical GCN4 Asn 16 residues (Saudek et al., 1991) and their Jun equivalents (Junius et al., 1995). This observation implies a symmetrical arrangement of side chain protons in the Asn-Asn contact atoms. King suggests a structurally plausible "flip-flop" rotation of the mutually hydrogen-bonded Asn amide groups, by 180°, around the Cβ—Cγ bond. The Asn-Asn side chain CO-NH_2 hydrogen bond is asymmetrical, but a rapid Cβ—Cγ flip-flopping would establish, on the average, a symmetrical condition with the Asn 16 NH_2 protons from the two α-helices of the GCN4 dimer equally engaged

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