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Evolutionally Conserved Intermediates Between Ubiquitin and NEDD8

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The investigation of common structural motifs provides additional information on why proteins conserve similar topologies yet may have non-conserved amino acid sequences. Proteins containing the ubiquitin superfold have similar topologies, although the sequence conservation is rather poor. Here, we present novel similarities and differences between the proteins ubiquitin and NEDD8. They have 57% identical sequence, almost identical backbone topology and similar functional strategy, although their physiological functions are mutually different. Using variable pressure NMR spectroscopy, we found that the two proteins have similar conformational fluctuation in the evolutionary conserved enzyme-binding region and contain a structurally similar locally disordered conformer (I) in equilibrium with the basic folded conformer (N). A notable difference between the two proteins is that the equilibrium population of I is far greater for NEDD8 ($\Delta G^0_{NI} < 5$ kJ/mol) than for ubiquitin ($\Delta G^0_{NI} = 15.2$ (±1.0) kJ/mol), and that the tendency for overall unfolding (U) is also far higher for NEDD8 ($\Delta G^0_{NU} = 11.0(\pm 1.5)$ kJ/mol) than for ubiquitin ($\Delta G^0_{NU} = 31.3(\pm 4.7)$ kJ/mol). These results suggest that the marked differences in thermodynamic stabilities of the locally disordered conformer (I) and the overall unfolding species (U) are a key to determine the functional differences of the two structurally similar proteins in physiology.

Keywords: ubiquitin; NEDD8; NMR; pressure; high energy conformer

Abbreviations: NOE, nuclear Overhauser effect; HSQC, heteronuclear single quantum coherence; UBL, ubiquitin-like.

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Introduction

The ubiquitin-fold[1] is common for ubiquitin-like proteins and for many domains of larger proteins, although sequence conservation for the proteins is rather poor and their physiological functions are different. This goes against the classical view that a protein folds into a single specific structure to perform a specific function. Recently, many experiments have shown that a protein exists in an ensemble of different structures[2,3] and it is considered that, to function, a protein exploits conformational transitions in a wide conformational space beyond the lowest energy folded structure.[4–10]

Ubiquitin as a post-translational modifier targets many proteins for digestion in the 26 S proteosome as well as for altering function and location or for trafficking of its binding partners. The protein NEDD8[11–14] is a ubiquitin-like protein (UBL), consisting of 81 amino acid residues and sharing 57% amino acid sequence identity with ubiquitin. NEDD8 is also a post-translational modifier and is essential for cellular regulatory mechanisms including cell division and signal transduction.[15,16] NEDD8 is conjugated to the target proteins, in which so far cullin family members of ubiquitin ligases are known.[15,17] The reaction chemistry is quite similar in both proteins such that the C-terminal carboxylate forms an isopeptide bond with a lysyl residue of the target protein aided by a series of enzymes.[18] Although the two modifier proteins have much similarity in their chemistry and functional strategy, they play different roles in cellular physiology.

Despite their different roles in the cell, the basic folded structures of the two proteins are almost identical (R.M.S.D. of the main chain atoms between the two proteins: ~0.6 Å). The structure of the complex of NEDD8 with the E1 enzyme, as determined by X-ray crystallography,[19,20] depicts significant conformational changes in both NEDD8 and the E1 enzyme upon the complex formation; the C-terminal tail (beyond Leu69) of NEDD8 is reoriented by about 30°, while the E1 also undergoes a conformational change for NEDD8 binding. Although no detailed structure is available for ubiquitin–E1 complex, NMR chemical shift perturbation experiments suggest that ubiquitin binds to E1 in a similar manner.[21] Furthermore, our previous high pressure NMR studies indicate that ubiquitin fluctuates to cause local unfolding in the enzyme-binding region.[8,22,23] These results appear to indicate that the enzyme-binding regions of NEDD8 and ubiquitin are made intrinsically labile in order to facilitate their binding to respective E1s.

Here, we examine the dynamics of NEDD8 in detail using spin relaxation and variable-pressure NMR spectroscopy, and compare the results with those previously published for ubiquitin. While spin relaxation is useful for delineating rapid and small-amplitude fluctuations, e.g. within the folded protein, variable-pressure NMR spectroscopy is useful for exploring protein dynamics in a wide conformational space and detecting slow and large-amplitude fluctuations.[24–27] Furthermore, the method allows one to examine directly the structures of high-energy alternative conformers resulting from such fluctuations. Here we focus on the dynamics and high-energy alternative states of NEDD8 and ubiquitin to find the molecular origin for the functional similarity and differences between the two similarly folded proteins.

Results and Discussion

Large-amplitude fluctuations are found within the folded manifold of both NEDD8 and ubiquitin

First, we investigated the backbone dynamics of ^{15}N-uniformly labeled NEDD8 at 1 bar and 30 °C using ^{15}N spin relaxation for individual amide groups. ^{15}N/^{1}H two-dimensional NMR measurements were performed at the ^{1}H frequency of 800.16 MHz. The relaxation parameters, i.e. the longitudinal relaxation rate R_1, the transverse relaxation rate R_2 and nuclear Overhauser effect (NOE), are shown in Figure 1(a)–(c). The model-free analysis of Lipari & Szabo[28,29] gives the order parameters S^2 representing the degree of spatial restriction of individual N-H vectors against motions in the picoseconds to nanoseconds range (Figure 1(d)) and the R_{ex} representing the exchange contribution to R_2 (Figure 1(e)). The S^2 values for residues from 1 to 72 are mostly in the range 0.8–1.0, showing that the basic structure of NEDD8 is well folded. A decrease in the S^2 values for residues beyond 72 shows increased motions of picoseconds to nanoseconds range toward the C-terminal end. The smaller S^2 values for residues 73–76 are found commonly in the NEDD8 and ubiquitin, indicating that both proteins have similar fluctuations in the C-terminal segment, which carries a reactive C-terminal glycine. The R_2 values (10–20 Hz) larger than average (7 Hz) were found for residues 33, 34, 35, 36, 39, 40, 41, 42, 68, 70 and 72, and the R_2 values smaller than average were found for residues 73–76. The larger R_2 values resulting in larger R_{ex} values are considered to arise from slow fluctuations between (or among) different local conformations on the microsecond to millisecond timescale.

In order to compare the slow conformational fluctuations between NEDD8 and ubiquitin, their R_2 values are plotted in Figure 2(a) and (b). While the larger R_2 values are obtained for all residues between residues 33 and 42 except the two proline residues at 37 and 38 and residues 68–72 in NEDD8 at 1 bar (Figure 2(a)), the corresponding R_2 values for ubiquitin at 30 bar (filled circle in Figure 2(b)) show no such large R_2 values, though smaller R_2 values are depicted commonly for residues 73–76 in both proteins. When pressure is increased to 3 kbar, larger R_2 values (10–18 Hz) are found for several