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Interaction of the tail with the catalytic region of a class II E2 conjugating enzyme

Nadine Merkley & Gary S. Shaw*
Department of Biochemistry & McLaughlin Macromolecular Structure Facility, The University of Western Ontario, London, Ontario, N6A 5C1, Canada

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Abstract

Ubiquitination plays an important role in many biological processes, including DNA repair, cell cycle regulation, and protein degradation. In the latter pathway the ubiquitin-conjugating enzymes or E2 enzymes are important proteins forming a key E2-ubiquitin thiolester prior to substrate labelling. While the structure of the 150-residue catalytic domain has been well characterized, a subset of E2 enzymes (class II) carry a variable length C-terminal 'tail' where structural detail is not available. The presence of this C-terminal extension plays an important role in target recognition, ubiquitin chain assembly and oligomerization. In this work NMR spectroscopy was used to determine the secondary structure of the 215-residue yeast E2 protein Ubc1 and the interactions of its C-terminus with the catalytic domain. The C-terminal tail of Ubc1 was found to contain three α-helices between residues D169-S176, K183-L193 and N203-L213 providing the first evidence for a well-defined secondary structure in this region. Chemical shift mapping indicated that residues in the L2 loop of the catalytic domain were most affected indicating the C-terminus of Ubc1 likely interacts with this region. This site of interaction is distinct from that observed in the E2-ubiquitin thiolester and may act to protect the catalytic C88 residue and direct the interaction of ubiquitin in the thiolester intermediate.

Abbreviations: Ub – ubiquitin; Ubc1Δ – catalytic domain of *S. cerevisiae* Ubc1 (residues 1-150).

Introduction

An important cellular regulatory mechanism in the cell is ubiquitin-dependent proteolysis. This process is responsible for the turnover of damaged or misfolded proteins, therefore providing an integral control mechanism for protein levels in the cell. The ubiquitination process is usually depicted as a cascade of events in which ubiquitin (Ub) is passed from one protein to another until it reaches a protein selected for degradation (Pickart, 2001; Hershko and Ciechanover, 1998). The first step of this process involves ligation of the Ub C-terminal glycine (G76) to an Ub-activating enzyme, E1 in an ATP-dependant process (Hershko et al., 1980). Ubiquitin is then transferred to an Ub-conjugating enzyme, E2 forming a covalent thiolester linkage between the cysteine of the E2 and terminal glycine (G76) of Ub. Labelling the target protein by Ub is achieved by either direct transfer of the Ub to the target by E2 (Pickart and Rose, 1985; Hershko et al., 1983) or through an Ub-ligating protein, E3 (Scheffner et al., 1995). While the basics of this scheme are generally accepted, the details of many of the protein interactions involved and the mechanism whereby ubiquitin is recognized by each enzyme and the targeted protein are poorly understood.

A key enzyme in the ubiquitination pathway is the E2 protein. These proteins are structurally conserved through many species including yeast (Goebl et al., 1988; Jentsch et al., 1987), rabbit reticulocytes (Pickart and Rose, 1985), and wheat (Sullivan and Vierstra, 1989, 1991). In yeast, the E2 pro-

*To whom correspondence should be addressed. E-mail: gshaw1@uwo.ca

teins contain a 150-residue catalytic domain, having > 25% sequence identity between the 13 yeast enzymes identified. The three-dimensional structure of some of these class I E2 proteins have been determined by x-ray crystallography (Worthylake et al., 1998; Cook et al., 1993, 1997) and NMR spectroscopy (Miura et al., 2002). The structures show a conserved arrangement of four α-helices (α1-α4) and a four strand anti-parallel β-sheet (β1-β4) is observed for Ubc1 (Hamilton et al., 2001), Ubc2 (Worthylake et al., 1998), Ubc4 (Cook et al., 1993), Ubc7 (Cook et al., 1997), and Ubc2b (Miura et al., 2002). The active site cysteine residue required for E2-Ub thiolester formation is found on a relatively unstructured region after the last β-strand (β4) and prior to the second helix (α2) in the catalytic domain.

Several E2 proteins are more complex than the simple class I members and have either N-terminal or C-terminal extensions. Class II E2 proteins have a C-terminal extension or a 'tail', whereas class III E2 proteins have an additional N-terminal sequence (Scheffner et al., 1998). The C-terminal tail of E2 proteins ranges from 45-100 residues with very little sequence similarity and having a variety of mechanistically distinct functions (Silver et al., 1992). An important function of the E2 C-terminal tail is substrate specificity (Silver et al., 1992; Jentsch et al., 1990; Hadleman et al., 1998). E2 enzymes with appended C-terminal tails enhanced the removal of selected proteins. Appendage of a 15-amino acid S-peptide binding sequence to the C-terminus of *Arabidopsis thaliana* Ubc1 specifically ubiquitinated free S-protein. Ubiquitin conjugates with S-protein were not detected with unmodified Ubc1 (Gosink and Vierstra, 1995). In addition the tail region of this protein has been suggested to promote its dimerization (Leggat and Candido, 1997; Ptak et al., 1994). For example, UBC-1, an E2 from *C. elegans* has a 40-residue C-terminal tail and self-associates to form dimers and tetramers. Deletion of residues from the C-terminal tail significantly decreased the self-association (Leggat and Candido, 1997). The pattern of ubiquitination is also altered by the presence of a C-terminal tail on E2 (Leggat and Candido, 1997; Hodgins et al., 1996). Ubc1 from yeast produces a multi-Ub chain anchored at K93 in the absence of E3. Removal of the C-terminal tail in Ubc1 causes the number of Ub molecules and the linkage pattern to be modified (Hodgins et al., 1996). The C-terminal tail of an E2 has also been shown to be important for anchoring an E2 to the cystolic side of the endoplasmic reticulum (Sommer and Jentsch, 1993).

Despite the important functions of the C-terminal tail in class II E2 proteins there has not been any three-dimensional structure showing the conformation of the C-terminal tail or its interaction with the catalytic domain. Yeast Ubc1 is a 215-residue E2 protein with a 65-residue tail. The C-terminus of this protein has been shown to be essential for the G0-G1 transition accompanying spore generation (Seufert et al., 1990) and the site of ubiquitination. In this work, we have used NMR spectroscopy to determine the secondary structure of Ubc1. Chemical shift mapping was used, in combination with the three-dimensional structure of the Ubc1 catalytic domain (Ubc1Δ), to determine the residues of the catalytic domain important for interactions with the C-terminal tail region.

Experimental procedures

Materials

$^{15}NH_4Cl$, [$^{13}C_6$]glucose and deuterium oxide were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA).

Uniformly labelled recombinant yeast Ubc1(K93R) was over-expressed and purified in *Escherichia coli* BL21(DE3)pLysS strain as previously described (Hodgins et al., 1996). Isotopic labelling was achieved using M9 minimal media containing 1.0 g/l 99% $^{15}NH_4Cl$ and 2.0 g/l 99% [$^{13}C_6$]glucose for double labelling and 1.0 g/l of 99% $^{15}NH_4Cl$ for uniform ^{15}N labelling. Electrospray mass spectrometry confirmed almost 100% isotopic enrichment of the uniformly labelled samples. Fractions containing Ubc1 from gel filtration (Sephadex-75) in 25 mM Tris, 1 mM DTT, 1 mM EDTA, 150 mM NaCl were pooled and concentrated. NMR samples were prepared at pH 7.5 in 90% H_2O/10% D_2O to final concentrations of either 0.34 or 0.4 mm.

NMR spectroscopy

NMR experiments were acquired at 35 °C on a Varian INOVA 600 MHz spectrometer equipped with a pulse field gradient triple resonance probe. Carrier frequencies were centered at 117.91 (^{15}N), 4.67 (^{1}H), 55.96 ($^{13}C^{\alpha}$), 45.96 ($^{13}C^{\beta}$) and 173.96 ($^{13}C'$). Sequential assignment of the backbone resonances were made from HNCA, HNCACB, CBCA(CO)NH, ^{15}N-edited TOCSY and ^{1}H-^{15}N HSQC experiments (Bax and

Grzesiek, 1993; Muhandiram and Kay, 1994). NOE $d_{NN}(i,i+1)$ cross peaks were identified from a ^{15}N-edited NOESY-HSQC spectrum (Zhang et al., 1994) with a mixing time of 150 ms. The $d_{\alpha N}(i,i+3)$ NOE cross peaks were obtained from either a ^{13}C-edited NOESY-HSQC spectrum (Muhandiram et al., 1993) collected on a Varian INOVA 800 MHz spectrometer with a 100 ms mixing time or from the ^{15}N-edited NOESY-HSQC spectrum. Data was processed and analyzed using NMRDraw, NMRPipe (Delaglio et al., 1995), Pipp and Stapp (Garrett et al., 1991) programs on a Sun Sparc5 workstation.

Data analysis

Changes in the chemical shifts between full length Ubc1 and the catalytic domain (Ubc1Δ) were normalized using the following formula: $\Sigma\Delta\delta = \Delta\delta|(^1H)| + 0.2^*\Delta\delta|(^{15}N)|$ (Shuker et al., 1996). The normalized chemical shift differences were grouped into four broad ranges ($0 < \Sigma\Delta\delta \leq 0.02$ ppm, $0.02 < \Sigma\Delta\delta \leq 0.08$ ppm, $0.08 < \Sigma\Delta\delta \leq 0.10$ ppm and $\Sigma\Delta\delta > 0.10$ ppm) and assigned relative shift values of 0 (smallest effect), 1, 2, 3 (largest effect). Prolines and unassigned residues were assigned values of zero.

Results

Ubc1 has a 65-residue C-terminal tail that directs the pattern of ubiquitination and may alter substrate binding. One possibility is that the Ubc1 tail interacts with the catalytic domain of Ubc1 in order to modify its interaction with ubiquitin during thiolester formation. In order to probe this interaction, assignment of the ^{1}H and ^{15}N resonances of Ubc1 were completed and compared with those for the isolated catalytic domain (Ubc1Δ).

Sequential assignment of Ubc1

All experiments utilized the K93R mutant of Ubc1 and Ubc1Δ. Although not a factor in the current work, this mutant inhibits ubiquitination of the E2 enzyme at K93 in the presence of ubiquitin The backbone assignment of Ubc1 was completed using standard triple resonance experiments including HNCA (Kay et al., 1990; Grzesiek and Bax, 1992a), HNCACB (Wittekind and Mueller, 1993), and CBCA(CO)NH (Grzesiek and Bax, 1992b). Excluding the proline residues 195 out of the 201 expected backbone amide residues were identified in the ^{15}N-HSQC spectrum (Figure 1). Many of the peaks were well resolved except for the region between 116.6 and 127.2 ppm. Residues R93 and R192, R132 and Q70, A20 and H34, I11 and I186, and I7 and Q156 had degenerate amide proton and amide nitrogen resonances. Extent of backbone ^{15}N, ^{13}C and ^{1}H resonance assignments excluding proline residues, are as follows: 96% of $^{13}C^{\alpha}$, 95% of $^{13}C^{\beta}$, 89% of $^{13}C'$ and 84% of $^1H^{\alpha}$.

Secondary structure determination

In general the pattern of the ^{15}N-HSQC bears many similarities for residues within the catalytic domain Ubc1Δ which lacks the 65-residue C-terminal tail. For example the ^{1}H-^{15}N correlation for N79 is found well downfield in the ^{1}H dimension while that of S97 is shifted significantly upfield. In the three-dimensional structure of Ubc1Δ N79 is partially buried and hydrogen bonded to the backbone carbonyl of D120 between sheet β5 and helix α3 and S97 is on the surface of the protein and hydrogen bonded to the carbonyl of F64. The uniqueness of these chemical shifts likely indicates that the environments of these residues are maintained to the level of hydrogen bonding. Examination of many of the outlying resonances indicates that many residues in helices such as I100, L102, L106 and S108 (α2), N119, D120 and Q122 (α3) and S136 and N138 (α4) have very similar chemical shifts to those observed in the catalytic domain, Ubc1Δ. Further residues in extended regions such H33 and L40 (β2), D55 and E57 in (β3) and G44, T45, Y47 (linker between β2 and β3) have near identical chemical shifts in both the ^{15}N-HSQC of Ubc1 and that of the catalytic domain. Qualitatively this indicates the fold of the catalytic domain of Ubc1 is similar to that of the isolated domain. The secondary structure of Ubc1 was identified by NOE connectivities (Wüthrich, 1985) from ^{15}N or ^{13}C-edited NOESY-HSQC spectra, TALOS (Cornilescu et al., 1999) and CSI (Wishart and Sykes, 1994) (Figure 2). The results indicate that Ubc1 is comprised of six α-helices and six β-strands. In the catalytic domain three α-helices (α1, A4-D16; α2, L102-L112; α3/4, A124-L147)* and five β-strands (β1, I22-E25; β2, H34-L40; β3, V53-V58; β4, K68-Q70; β5, A86-I88) were observed. The tail region of Ubc1 contains three additional α-helices (α5, D169-S176; α6, K183-L193; α7, N203-L213) and a β-strand (β6, S197-D199). The α-helices were assigned by the presence $d_{NN}(i,i+1)$ and $d_{\alpha N}(i,i+3)$

*Ubc1Δ has two α-helices between 125-147 (α3, α4), from herein α3 and α4 are residues 125-132 and 136-147.

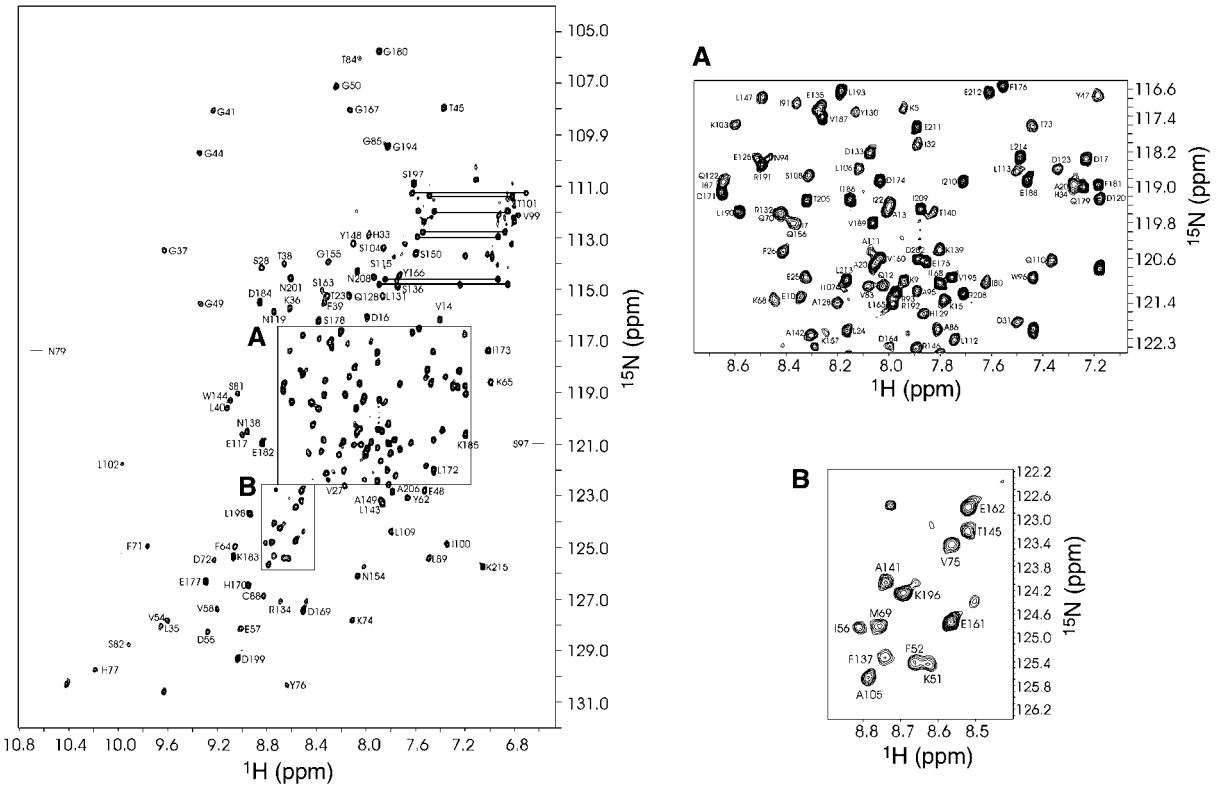

Figure 1. ^{1}H-^{15}N HSQC spectrum of 0.4 mm uniformly ^{15}N-labelled Ubc1 in 25 mM Tris, 1 mM DTT, 1 mM EDTA, 150 mM NaCl and pH 7.5 acquired at 35 °C on a Varian INOVA 600 MHz spectrometer. Cross peaks for the backbone amides are labelled with their one-letter amino acid code and number. The insets (A, B) are an expanded view of crowded regions. Sidechain amide cross peaks have lines connecting the pairs of resonances.

NOE cross peaks and were in agreement with CSI and the Φ and Ψ angles from TALOS. Absence of any strong $d_{NN}(i,i+1)$ NOE cross peaks confirmed the position of the β-strands (Wüthrich, 1985).

Interaction of the tail with the catalytic domain in Ubc1

Despite many similarities in chemical shifts between Ubc1 and the isolated catalytic domain, several significant differences were noted (Figure 3). To quantify these, the amide proton and amide nitrogen chemical shifts were measured in Ubc1 and were compared to those for Ubc1Δ (Hamilton et al., 2000a). The only difference between the two proteins is the 65-residue tail region found in Ubc1, but lacking in Ubc1Δ. Further, NMR experiments for the proteins were both done at pH 7.5 decreasing the chances of small pH dependent chemical shift differences. Therefore it was reasoned that any significant chemical shift changes between the two proteins must result from either a direct interaction of the tail region with the catalytic domain or a conformational difference in the catalytic domain as a result of the additional tail residues.

Interaction of the 65-residue tail of Ubc1 with its catalytic domain was analyzed using the chemical shift perturbation mapping method. This analysis measures the changes in chemical shift of the amide proton and nitrogen resonances in the ^{15}N-HSQC spectrum as a function of added ligand or residues. These changes are classified based on their magnitudes and identification of possible binding sites can be achieved since chemical shifts are very sensitive to chemical environment. This method has been used to monitor binding of small molecules (Shuker et al., 1996), protein-peptide interactions (Barber et al., 1999) or protein-protein interactions (Hamilton et al., 2000b). To determine the possible interaction of the tail region of Ubc1 with its catalytic domain the ^{15}N-HSQC spectra were superimposed and differences in the chemical shifts examined (Figure 3). The resulting spectra indicate that residues A13, T23, L24, V27, S28, I32, T38, F39, G41, G50, K74, V75, Y76, I87, L89, K103,

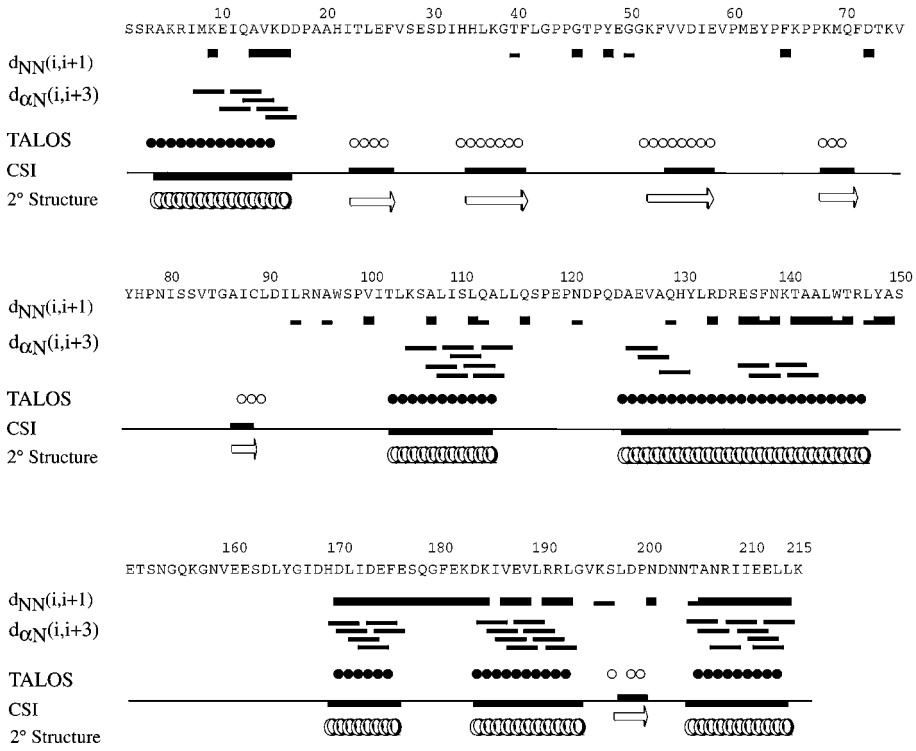

Figure 2. Summary of the sequential and medium range NOE's involving H^N and H^α protons, TALOS and CSI for Ubc1. The rows labelled $d_{\alpha N}(i,i+3)$ and $d_{NN}(i,i+1)$ summarize the sequential NOE cross peaks from ^{15}N and ^{13}C-edited NOESY-HSQC spectra. For $d_{NN}(i,i+1)$ the bar thickness (high, medium and low) represents strong, medium and weak NOE cross peaks. Solid lines represent the presence of $d_{\alpha N}(i,i+3)$ NOE cross peaks. TALOS (Cornilescu et al., 1999) predicted the Φ and Ψ angles based on C^α, C^β, C', H^α and N chemical shift information in Ubc1 where 9 out of 10 predictions fell in the same region of the Ramachandran plot. Closed circles represent α-helices in which Φ and Ψ were in the range -60 +/− 20 and -40 +/− 20, the open circles symbolize β-strands in which Φ and Ψ were in the range -120 +/− 35 and 120 +/− 35. The consensus CSI ($CSI^{C\alpha} + CSI^{C} - CSI^{H\alpha}$) is indicated as positive and negative bars except for residues where there was insufficient chemical shift information. The derived secondary structure of Ubc1 is based on the predictions from NOE connectivity, TALOS and CSI and is represented by spirals for the α-helices and arrows for β-strands.

V126, A142, W144, A149 and S150 experienced the largest change in environment in the presence of the C-terminal tail region. Since the tail region begins at position 151 it is not surprising that residues A149 and S150 are affected. Several other residues were affected to a lesser extent while others (described earlier) had little or no change. The changes in chemical shift were categorized into four categories based on the magnitude of the change and mapped to the surface of the protein using the structure of the catalytic domain of Ubc1 (Hamilton et al., 2001). Using this approach it was clear that a majority of the affected residues in Ubc1 were located in the upper half of the protein towards the C-terminal residue S150 (Figure 4). For example residues T23, L24 (β1), V27, S28 and I32 (loop) and T38, F39 (β2) lie opposite S150 where the tail region for Ubc1 commences. Chemical shift mapping also revealed a large surface on the catalytic domain is formed comprising residues from that underwent chemical shift changes in the L2 loop between β4 and α2 (K74, S82, T84, Y76, A86, I87 and L89) and proximal residues in helix α3 (V126) and α4 (W144). The surface presented by these residues is on the same face as the catalytic C88 residue. While the majority of the residues that had chemical shift changes were on the surface of the catalytic domain, several were buried or partially buried including T38, F39, G41 and G50.

Discussion

The C-terminal extensions of E2 conjugating proteins in *Saccharomyces cerevisiae* can be extremely variable ranging from only 23 residues in Ubc2 to more than 100 residues in Ubc3 and Ubc8 (Scheffner et al., 1998; Jentsch, 1992). In addition, these tail regions

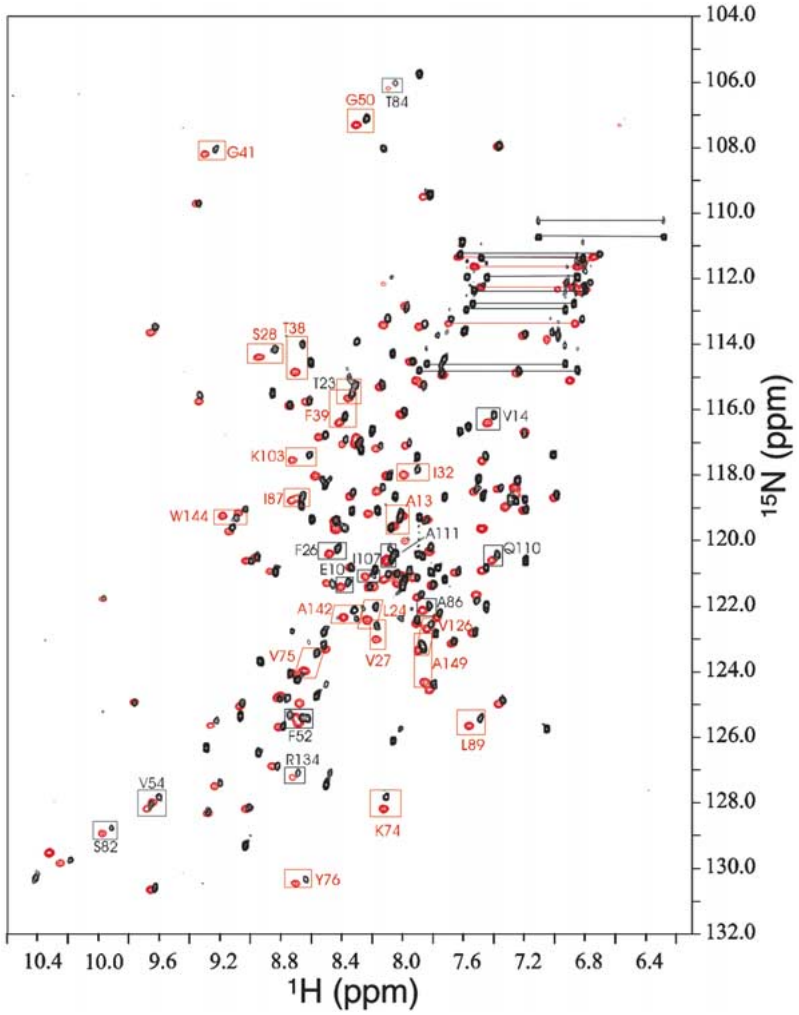

Figure 3. Superimposed ^{1}H-^{15}N HSQC spectra of 0.8 mM Ubc1Δ (red contours) in 40 mM HEPES, 450 mM NaCl and 1 mM EDTA at 30 °C and 0.4 mM of full length Ubc1 (black contours) in 25 mM Tris, 150 mm NaCl, 1 mM EDTA and 1 mM DTT at 35 °C. Both spectra were acquired at pH 7.5. Resonances for the catalytic domain of Ubc1 were calculated according to $\Sigma\Delta\delta = \Delta\delta|(^{1}H)| + 0.2^{*}\Delta\delta|(^{15}N)|$ (Shuker et al., 1996) where Δδ reflects the differences in chemical shift between Ubc1 and Ubc1Δ for each residue. Resonances that are shifted >0.10 ppm are boxed in red and those residues shifted between 0.08 and 0.10 ppm are boxed in black.

have also been noted in mammalian E2 proteins such as E2-25K (Mastrandrea et al., 1998; Hadleman et al., 1997) and the *C. elegans* protein UBC-1 (Leggat and Candido, 1997). The C-terminus in these proteins has been implicated in interactions with specific E3 proteins, modification of ubiquitination patterns and substrate specificity (Pickart, 2001; Scheffner et al., 1998). Previous structural work has indicated that the C-terminus in Ubc2 is likely flexible (Morrison et al., 1988) while the E2-25K tail is predicted to contain two or more α-helices (Mastrandrea et al., 1998). In other cases, such as UBC-1 (Leggat and Candido, 1997) and Ubc3 (Ptak et al., 1994), the C-terminus has been implicated in dimer formation of the E2 proteins.

In solution, Ubc1 behaves as a monomeric species at concentrations of 0.3–0.4 mm and below. Analytical ultracentrifugation experiments under identical buffer conditions as NMR experiments show that Ubc1 is monodisperse having an apparent molecular weight of 21.9 ± 2.3 kDa (data not shown). It is possible that at the higher concentrations used for NMR studies (0.3–0.4 mm), some degree of oligomerization of Ubc1 occurs as has been observed for the Ubc3 and UBC-1 E2 proteins. However, the NMR line widths for Ubc1 are consistent with that of a 24 kDa protein

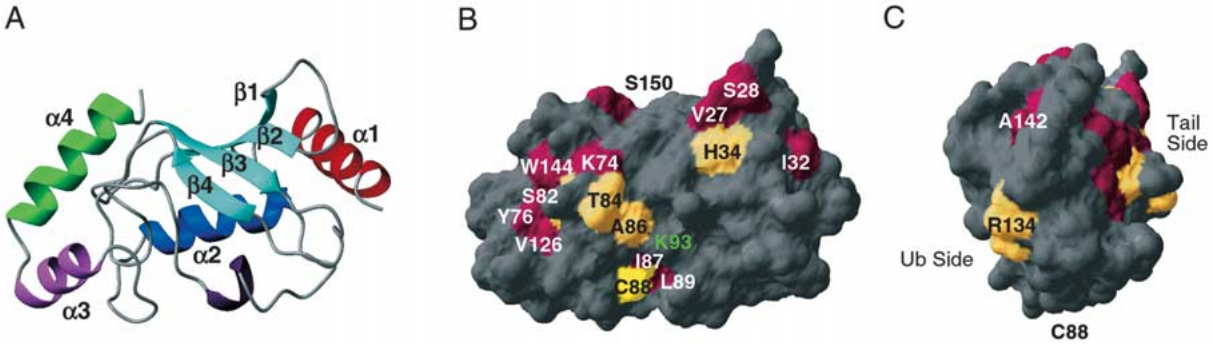

Figure 4. Surface map for the interaction of the C-terminal tail region of Ubc1 with its catalytic domain. (A) Ribbon diagram showing the secondary structure of Ubc1Δ based on the x-ray crystal structure (Hamilton et al., 2001). (B,C) Accessible surfaces in Ubc1Δ showing regions of interaction. Residues affected by the presence of a C-terminal tail were coloured according to their chemical shift change using the accessible surface of Ubc1 generated based on its x-ray crystal structure. Residues were coloured maroon ($\Sigma\Delta\delta \geq 0.10$ ppm) or pale yellow ($0.10 > \Sigma\Delta\delta \geq 0.08$ ppm). The catalytic cysteine involved in thiolester formation, C88, is coloured yellow and residue K93, where polyubiquitin chain synthesis is anchored is green. Diagrams A, B show the same orientation of Ubc1Δ while C is rotated approximately 90°.

and there is no evidence for chemical exchange that might be associated with a monomer-dimer equilibrium. The current NMR work shows that the tail of Ubc1 is comprised of three clearly defined α-helices between residues D169-S176, K183-L193 and N203-L213 providing the first direct evidence for α-helical structure in the C-terminus of an E2 protein. This result is consistent with predictions for E2-25K although the two proteins bear virtually no similarity in their C-terminal regions (Hadleman et al., 1997). It has been suggested that interactions between the catalytic domain, where Ubc1 and E2-25K are highly homologous, and the C-terminus govern the tail function (Hadleman et al., 1997). These interactions may also facilitate the α-helical structure of the tail.

Implications of catalytic domain-tail interactions

Mapping the residues with the largest chemical shift changes onto the catalytic domain of Ubc1 reveals a potential surface for the interaction between the tail region and the catalytic domain (Figure 4). This region comprises many residues from the L2 loop between β4 and α2 (K74, S82, T84, Y76, A86, I87 and L89) prior to or near the catalytic C88 residue. This places the surface on the 'β-sheet side' of the E2 protein opposite helix α2. Some of these residues (K74, S82, I87, L89) are affected by ubiquitin binding in the E2-Ub thiolester intermediate (Hamilton et al., 2001). However in the E2-Ub thiolester there are a larger subset of residues at the protein-protein interface that reside on helix α2 (A105, S108, Q114), the subsequent linker (S115, N119) and the L2 loop region following C88 (L91, L92, W96, S97). Similar interactions between Ubc2b (human) and Ub have also been proposed (Miura et al., 1999). These differences in interactions indicate that the tail region in Ubc1 must be interacting with a surface on the opposite side of the protein from that where a ubiquitin molecule interacts in the E2-Ub thiolester (Figure 4C). The catalytic residue C88 is positioned near the intersection of these two regions.

The C-terminal tail region in Ubc1 could potentially block or direct Ub to its binding site in the E2-Ub thiolester by occupying one side of the catalytic domain. Blockage of the Ub would account for the change in the pattern of ubiquitination that was observed by Hodgins et al. (1996). In this study the multi-ubiquitin chains were linked through K48 (Ub) by the truncated Ubc1, anchored at K93. Full length Ubc1, on the other hand, had a modified ubiquitination pattern suggesting that the E2 tail region interfered with the assembly of the ubiquitin chain. Figure 4B shows that residue K93 in Ubc1 resides on the same side where the C-terminus of Ubc1 likely interacts. It is conceivable that the tail region in Ubc1 may interact with Ub to alter chain assembly.

The proposed surface of interaction between the C-terminus of Ubc1 and its catalytic domain indicates that C88 may be protected to some extent by the tail. For example residues near the active site including A86, I87 and L89 are amongst those most affected by the tail region in the full-length protein. This finding is consistent with observations for the mammalian E2 protein, E2-25K (Hadleman et al., 1997). This protein exhibits poor accessibility to alkylation of its catalytic cysteine (C92) in the presence of its C-terminal

residues. The alkylation rate is markedly increased in the truncated protein lacking the C-terminus indicating the tail region may be folding over or blocking access to the cysteine. The interacting sites for the tail of Ubc1 also comprise residues from the N-terminus of β1 (T23, L24) and helices α3 (V126) and α4 (W144) analogous to regions proposed for E2-25K for tail interaction.

The interaction of the Ubc1 tail does not appear to involve residues from the L1 (V57-P65) or the C-terminal region of the L2 (W96-T107) loop regions. These residues fell into the two lowest categories of shift changes and in many cases had negligible differences between Ubc1 and Ubc1Δ. These regions have been identified as the key areas of interactions with an E3 protein based on the x-ray crystal structures of the HECT E3 (E6AP) bound to HsUbc7 (Haung et al., 1999) and HsUbc7 with the ring finger E3, c-Cbl (Zheng et al., 2000). The E2-E3 binding interface for Ubc1 has been predicted to involve R6 and K9 of the first α-helix (α1), M60, E61, P63, F64 and K65 in L1 and S97, P98, V99 and T101 in L2 (Hamilton et al., 2001). The lack of changes in chemical shifts between Ubc1 and the truncated Ubc1Δ in these regions suggests the C-terminal tail of Ubc1 does not interact with these residues and likely does not directly interfere with binding of E3 to Ubc1. However, it is possible that the tail region may directly interact with an E3 protein as has been observed for Ubc2 (Madura et al., 1993; Xie and Varshavsky, 1999) and Ubc3 (Mathias et al., 1998).

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