Comparison of the Three-dimensional Structures of Human, Yeast, and Oat Ubiquitin*

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The crystal structure of human ubiquitin has been solved by x-ray diffraction methods and refined by standard procedures to a conventioanal crystallographic R factor of 0.176 at 1.8-Å resolution (Vijay-Kumar, S., Bugg, C. E., and Cook, W. J. (1987) J. Mol. Biol. 194, 525–538). Crystals of yeast and oat ubiquitin have been grown using human ubiquitin crystals as seeds. Diffraction data for yeast and oat ubiquitin have been collected to a resolution of 1.9 and 1.8 Å, respectively. Difference Fourier electron-density maps reveal that the structures of yeast and oat ubiquitin are quite similar to human ubiquitin. All the amino acid changes are clustered in two small patches on one surface of the molecule. This surface is probably not involved in conjugation with proteins destined for ATP-dependent proteolysis.

Ubiquitin is a small protein that is probably present in all eukaryotic cells (1). It consists of a single 8565-dalton polypeptide chain of 76 amino acids and it appears to be one of the most conserved of all eukaryotic proteins. Ubiquitin has been isolated and sequenced from a variety of sources, and the most conserved of all eukaryotic proteins. Ubiquitin is due to its primary role in intracellular ATP-dependent protein degradation (2). Protein breakdown in this pathway requires the formation of covalent conjugates in which the carboxyl terminus of ubiquitin becomes attached to the target protein via amide linkages to ε-amino groups of their lysine residues (to yield branched ubiquitin-protein conjugates) and/or to the NH₂ terminus. Ubiquitin is also present in the nucleus, where it is conjugated to histone 2A (5), and it has been suggested that this conjugate may be involved in the transcription of active genes (6). Most recently ubiquitin has been identified on the cell surface as part of a lymphocyte homing receptor (7) and as part of the receptor for platelet-derived growth factor (8), but its function in these locations is still unclear.

The structure of human erythrocytic ubiquitin at 2.8-Å resolution has been reported (9), and the model has been refined to 1.8 Å (10). We now report the crystal data for yeast and oat ubiquitin and describe the changes in conformation from human ubiquitin. The relationship of the sites of amino acid substitution to the other portions of the molecule is also described.

EXPERIMENTAL PROCEDURES

Crystallization Procedures—Yeast and oat ubiquitins were purified as described (3, 11). Efforts to crystallize yeast and oat ubiquitin under the same de novo conditions as human (12) were unsuccessful. Therefore, crystals of human ubiquitin were used for seeding. For seeding experiments, the hanging drop vapor-diffusion method was used. Lyophilized yeast or oat ubiquitin was dissolved at a concentration of 20 mg/ml in distilled water. The drop was formed by mixing 3 μl of the ubiquitin solution with 3 μl of a solution of 40% polyethylene glycol 4000 (PEG-4000) (w/v) in 0.05 M cacodylate buffer, pH 5.6. The coverslip was then inverted and set over the reservoir containing 1.0 ml of 24% PEG-4000 (w/v) in 0.05 M cacodylate buffer, pH 5.6. The drops were placed in a 4 °C cold room, and seeding was carried out after 2–4 h. A single crystal of human ubiquitin (0.05 × 0.1 × 0.2 mm) was washed in a stabilizing solution of 20% (w/v) PEG-4000 in 0.05 M cacodylate buffer, pH 5.6, and then transferred with as little liquid as possible to the drop. Introduction of the seed crystal into the drop was accomplished by using a 0.5-mm capillary and a micromanipulator. The coverslip was then reinverted over the reservoir, and the tray was kept in the cold room. After 2–4 days at 4 °C, large crystals were obtained (Fig. 1). Two applications of the seeding technique were required to obtain good crystals. In the first attempt, seed crystals of human ubiquitin were used and these produced large imperfect crystals within which the seed was still clearly visible (Fig. 1a). However, small (0.1 × 0.1 × 0.2 mm) perfect crystals of the yeast or oat ubiquitin were also formed in the same drop. For the second seeding attempt, these new crystals were used as seeds, and large perfect rectangular prisms were obtained (Fig. 1b). The crystals seem to be stable indefinitely at 4 °C, and they can be kept for weeks at room temperature in a stabilizing solution of 32% (w/v) PEG-4000 in 0.05 M cacodylate buffer, pH 5.6.

Data Collection—Intensity data were collected with a Picker FACS-1 diffractometer at room temperature using an omega step-scan procedure (scan width, 0.6–0.8°) and nickel-filtered CuKα radiation. The reflections were divided into 20 shells containing 100 reflections each and were collected from high to low resolution. Diffraction data for yeast and oat ubiquitin were collected to a resolution of 1.9 and 1.8 Å, respectively. Two crystals of each protein were used for data collection. Data for one crystal were collected from 1.8 (or 1.9 Å) to

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† The abbreviation used is: PEG-4000, polyethylene glycol 4000.
least squares to an $R$ index of 0.176 at a resolution of 1.8 Å. The details of the structure solution and refinement have been described (9, 10). Changes in the yeast and oat ubiquitin structures were revealed by difference Fourier maps at 3.0-Å resolution. The difference maps were calculated using modified phases of human ubiquitin and the observed amplitudes of yeast or oat ubiquitin minus the modified calculated amplitudes of human ubiquitin. In each case, the phase angles were calculated after replacing the three substituted amino acids with glycine residues. In each case, the surface of the molecule was essentially unchanged.

The most prominent secondary structural features of ubiquitin are shown in Fig. 3. A full description of the secondary structure of ubiquitin has been given (10), but several features should be emphasized here. There is a mixed $\beta$-sheet that contains five strands. Two of the inner strands, composed of residues 1–7 and 64–72, are parallel. The other three strands, which are composed of residues 10–17, 40–45, and 48–50, run in an antiparallel direction. There is an $\alpha$-helix that includes residues 23–34; this helix fits into the concavity formed by the sheet. There is also a short piece of $3_1\alpha$-helix that includes residues 56–59. All of the amino acid changes identified in yeast and oat ubiquitin are clustered in two small patches on one surface of the molecule (Fig. 3). In yeast ubiquitin two of the substitutions, Glu-to-Asp at position 24 and Ala-to-Ser at position 28, are one turn apart in the $\alpha$-helix. Both occur on the surface of the molecule, and one would not expect these changes to have large effects on the structure. Similarly, in oat ubiquitin, two of the substitutions, Glu-to-Asp at position 24 and Ser-to-Ala at position 57, are one turn apart in the $\alpha$-helix. One might have suspected that this substitution would cause changes in the relative orientation of the helix to the $\beta$-sheet, but there appears to be no significant effect. In summary, the secondary structure of ubiquitin is conserved in each case in spite of the three amino acid substitutions.

DISCUSSION

It is noteworthy that the major hydrogen bonding interactions within human ubiquitin are maintained in the yeast and oat structures. One portion of the molecule displays an unusual region that involves a symmetric arrangement of the two helices and two Type I reverse turns. In human ubiquitin the main chain N atoms of the first 2 residues of the $\alpha$-helix (isoleucine 23 and glutamate 24) form hydrogen bonds to the carbonyl oxygen atoms of the 2nd and 4th residues in the turn involving residues 51–54. Similarly, the main chain N atoms of the first 2 residues of the $3_1\alpha$-helix (leucine 56 and serine 57) form hydrogen bonds to the carbonyl oxygen atoms of the 2nd and 4th residues in the turn involving residues 18–21. There is almost 2-fold symmetry in this portion of the
FIG. 2. Stereo drawing of residues 18–21 with the superimposed electron density contour surfaces. Difference maps were calculated by using the modified refined phases of human ubiquitin and the amplitudes of yeast or oat ubiquitin minus the modified calculated amplitudes of human ubiquitin. a, yeast ubiquitin; b, oat ubiquitin.
structure. It is interesting that, even though two of the three amino acid changes in yeast ubiquitin and all three of the amino acid changes in oat ubiquitin occur in this region, the hydrogen bonding pattern and the secondary structural features are preserved. These interactions may make important contributions to the unusual stability of the molecule.

Most studies suggest that ubiquitin functions primarily in intracellular ATP-dependent protein degradation. Protein breakdown in this pathway requires the formation of covalent conjugates in which carboxyl termini of ubiquitin molecules become attached to the target protein via amide linkages.

Comparison of human and yeast ubiquitin demonstrated no difference in the ability of these two proteins to stimulate protein degradation (3). Kinetic analysis of oat ubiquitin demonstrated only 50% of the activity of human ubiquitin (11), but this difference may reflect COOH-terminal heterogeneity, secondary to partial proteolysis or modification by plant polyphenols during extraction.2 The extreme evolutionary stability of ubiquitin suggests that the structural constraints necessary for catalytic activity or recognition of ubiquitin by other proteins are quite strict. Since the only sequence changes identified thus far are confined to two small regions on one surface of the molecule, it seems unlikely that these regions are directly involved in the function of ubiquitin in ATP-dependent proteolysis.

REFERENCE


2 R. D. Vierstra, unpublished data.

FIG. 3. Stereo drawing of the α-carbon backbone of human ubiquitin. The side chains of the residues involved in amino acid substitutions (at positions 19, 24, 28, and 57) are included.