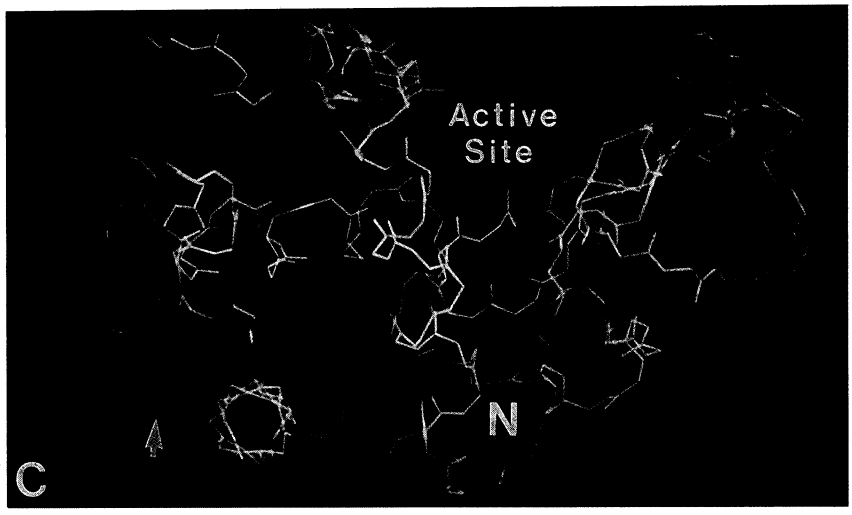

FIG. 2. Comparisons of 6,127-rcm-lysozyme and native chicken egg white lysozyme crystal structures. The main-chain tracing from the 1.75-Å native lysozyme structure (22) is shown in green superimposed upon the two 6,127-rcm-lysozyme conformers (blue and red) determined to 1.9 Å. Where the structures coincide, they appear white; disulfide bonds are shown only for the native structure, and are in green. The C termini, N termini, and active site are indicated. The Cys-6 Cys-127 disulfide, broken in the 6,127-rcm derivative, is identified by the arrow. The Lys-13 ubiquitination site (not shown here; see text) extends from the prominent helix (residues 5–15) that is linked via the Cys-6 Cys-127 disulfide to the C terminus. All three structures are nearly identical except for several residues at the C termini.

of native lysozyme that had been determined from crystals belonging to space group $P4_32_12$ (22). The resultant 1.9-Å resolution atomic model for 6,127-rcm-lysozyme includes two molecules per asymmetric unit and has been refined to an *R* factor of 0.185 (Table 1).

Overall, the conformations of 6,127-rcm-lysozyme and native lysozyme are extremely similar, and significant deviations (i.e., displacements > 1.0 Å) in the polypeptide backbones are limited to only a few regions. Fig. 2 shows the main-chain polypeptide tracings for both of the 6,127-rcm-lysozyme molecules in the asymmetric unit, rcmA and rcmB, superimposed upon the 1.75-Å resolution native lysozyme structure obtained from tetragonal crystals (22). Differences are slight except at the C terminus and, to a lesser extent, for a short peptide segment centered about residue 71 (regions II and IV in Fig. 3 *Upper Left*). Comparisons with the 1.5-Å native lysozyme structure obtained from triclinic crystals (29, 30)[§] revealed deviations for two additional regions centered about residues 47 and 101 (Fig. 3 *Upper Right*). For regions I–III, similar main-chain deviations are observed between the native lysozyme structures (Fig. 3 *Lower Left*, tetragonal vs. triclinic), whereas only regions I and IV differ between the two 6,127-rcm-lysozyme molecules (Fig. 3 *Lower Right*, rcmA vs. rcmB). Positional differences within the pairs of native or 6,127-rcm-lysozyme structures are unequivocal evidence of conformational mobility. Note, however, that region IV of the native lysozyme main chain appears fixed (Fig. 3 *Lower Left*). Thus, upon Cys-6 Cys-127 reduction and carboxymethylation, only the C terminus is seen to deviate from the conformations accessible to lysozyme in the native state.

Although Cys-6 Cys-127 disulfide cleavage frees lysozyme's C terminus from its interaction with the body of the protein, the position of the N-terminal peptide that encompasses Cys-6 is essentially unchanged. An inventory of the contacts in native lysozyme made by residues near the N and C termini shows that this might be expected. Other than the Cys-6 Cys-127 disulfide itself, few interactions stabilize the native conformation of lysozyme's five C-terminal residues, which contribute to only 4 hydrogen bonds as compared to 11 for the first five (N-terminal) residues.

[§]Brookhaven Protein Data Bank reference 2LZT.

Destabilization of the native conformation at the C terminus of 6,127-rcm-lysozyme also must be due in part to electrostatic repulsion of the side chain carboxylates of carboxymethylcysteines 6 and 127. In rcmA and rcmB, residue 127 is oriented to position its side chain into solvent and away from the buried hydrophobic environment of Cys-127 in native lysozyme. These new conformations entail a redirection of the polypeptide backbone, as is evident in Fig. 2. For Cys-6, however, positions of the main-chain and C_β atoms are nearly identical for native lysozyme and 6,127-rcm lysozyme. Placement of the Cys-6 carboxymethyl moieties within the model was precluded by weak electron density, however, which indicates that this side chain is quite mobile.

Conformational Flexibility of the C-Terminal Residues. The conformations of the C-terminal portions of rcmA and rcmB differ significantly from each other and from the native structure. These residues therefore are expected to be mobile in solution, and inspection of lattice contacts suggests that these motions may be much larger when free of the crystalline environment. As illustrated in Fig. 4, the last five residues of 6,127-rcm-lysozyme have contacts to symmetry-related molecules. These contacts are similar for rcmA and rcmB and, in each case, are as extensive as the noncovalent intramolecular interactions. Thus, the deviations of the 6,127-rcm-lysozyme C-terminal residues seen from the crystal structures may severely underestimate the true range of motion in solution.

In contrast, and despite the absence of restrictive lattice contacts (see Fig. 4), the conformations of the N-terminal residues are essentially identical. Two-dimensional NMR measurements by Radford *et al.* (18) support our conclusion that the N-terminal portion of 6,127-rcm-lysozyme is virtually the same as that of the native protein. From an immunochemical study, however, it appeared that the 6,127-rcm-lysozyme N-terminal region may be distorted relative to the native conformation (19). The immunochemical and structural data can be reconciled if the N-terminal epitopes that were probed also included Cys-6 or distal portions of the antigen such as its C terminus.

Recognition by Ub–Protein Ligase. Although movement of the Leu-129 side chain was apparent from the NMR study of 6,127-rcm-lysozyme, whether there was a more extensive rearrangement of the C terminus could not be determined