A new polypeptide backbone fold for serine proteases has been identified based on the crystal structure of human cytomegalovirus protease. The structure was determined at 2.5 Å resolution by the multiple-wavelength anomalous diffraction technique using the selenium-methionyl protein and refined at 2.5 Å resolution. It reveals a seven-stranded mostly-antiparallel β-barrel, which is surrounded by seven helices. The active site residues (Ser-132 and His-63) are situated on the outside of the β-barrel and in a groove on the surface of the protein. The structure suggests that the third member of the catalytic triad is probably His-157. The protease of herpesviruses plays an essential role in the production of infectious virions and is an attractive target for the development of antiviral agents. The crystal structure information will help in the design and optimization of inhibitors against herpes virus protease.


Human cytomegalovirus (HCMV) is a beta herpes virus. HCMV, like all other members of the Herpes virus family, encodes a protease that is essential for capsid maturation and production of infectious virus. The catalytic domain of the HCMV protease was produced in E.coli as a single-chain protein and was crystallized in space group C2221 with two dimers per asymmetric unit. The crystal structure was determined at 2.5 Å resolution using the ISAS method and noncrystallographic symmetry averaging. Our current model has been refined against 2.3 Å data collected on an image plate at -170°C. The HCMV protease structure has a new fold different from that of any other known protease. There is a central core comprising two orthogonal 4-stranded beta sheets surrounded by eight alpha-helices. Residues in three flexible surface loops, including two associated with internal cleavage sites at amino acids 143 and 209, have not been modeled into the current structure. Dimerization of HCMV protease is mediated primarily by burying four turns of alpha-helices (218-233) from one monomer into a pronounced depression in the surface of the other monomer. Only two of three residues previously implicated by amino acid sequence alignment and mutagenesis as participating directly in catalysis (Ser-132 and His-63) but not Glu-122 are actually located in the active site. This novel structure is being used to further understand the catalytic mechanism, and to design inhibitors as potential anti-viral agents.