2005.11.23-25

NMR study of the unusually slow refolding mechanism of a protein from a hyperthermophile : NMR peak assignment and H/D exchange reaction

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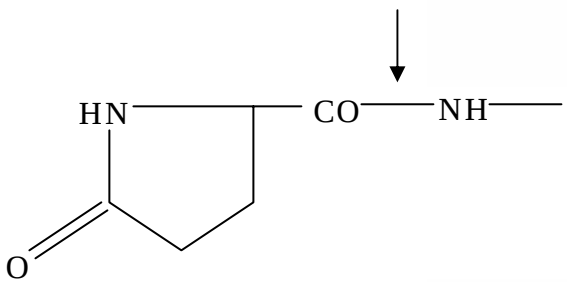
pyrrolidone carboxyl peptidase (PCP) from a hyperthermophile, *pyrococcus furiosus*

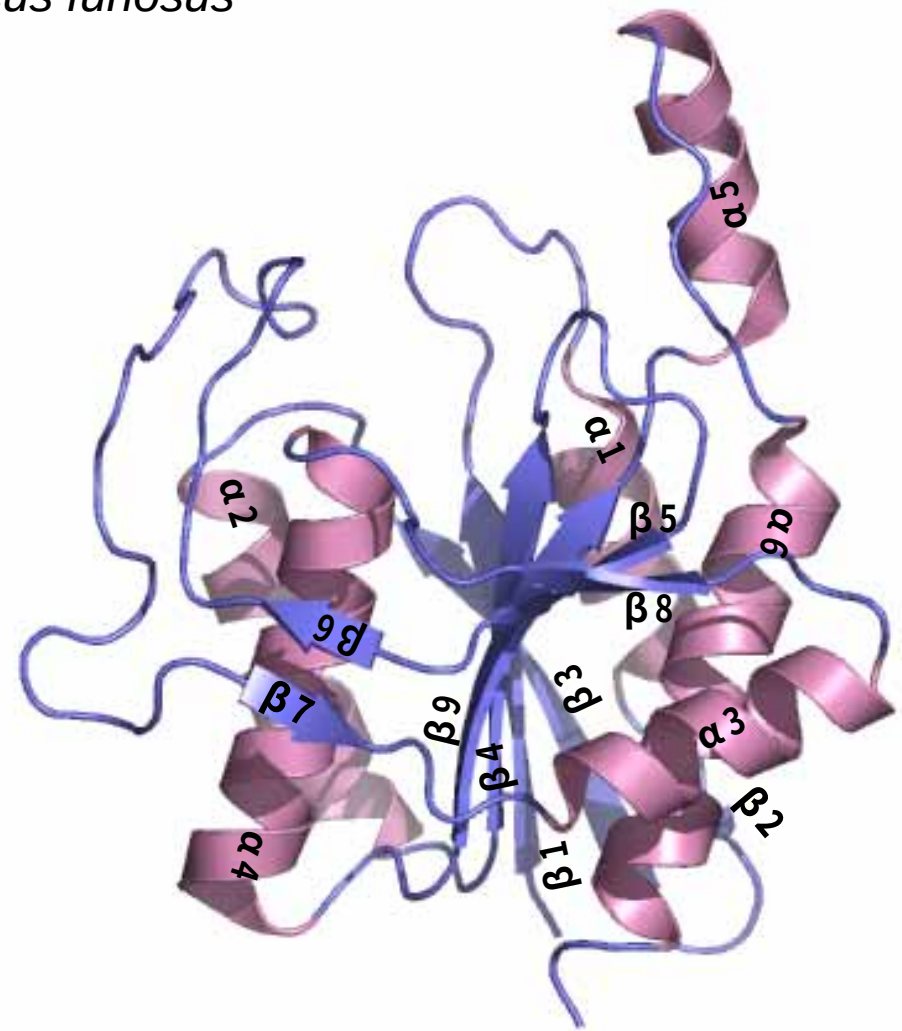
Number of Residues
:208(2Cys at 142and188,Tyr8,Trp0)

Molecular Weight
:22800

Molecular Form
:Homo tetramer around neutral pH
:Monomer below pH 2.5

Catalytic Function
:Removal of N-terminal pyroglutamic acid
active site residue: Cys 142





In all experiment Cys142/188Ser mutant(PCP-0SH) was used to avoid difficulties in the analysis of refolding reaction. By this mutation no change in 3D structure was occurred.

abstract

The refolding and unfolding rates of pyrrolidone carboxyl peptidase (PCP) from *P.furiosus* are unusually slow at low temperatures and low pH[1]. In the refolding process of PCP-0SH (SH mutant of PCP, Cys142/188Ser), there was a burst phase from completely disordered state, D_2 state, in the concentrated denaturant, to non-random denatured state, D_1 state, under physiological conditions. In the transition from the D_1 to the N states[2], the unusually slow relaxation kinetics of PCP-0SH was apparently two state transitions and there was no stable intermediate state between them.

In order to elucidate folding mechanism of PCP-0SH at amino acid sequence level we observed H/D exchange reactions and measured 2 D ^{1}H-^{15}N HSQC spectra in the present study. NMR peak assignments of ^{1}H-^{15}N HSQC spectra were performed by HNCACB, HN(CO)CACB, HNCO and HN(CO)CA techniques using ^{13}C/^{15}N double labeled and selectively [^{15}N]-Leu, -Lys, -Val-labeled PCP-0SH. Out of theoretically assignable 192 residues (16 Pro residues are included in 208 residues of PCP-0SH), 144 residues could be assigned on a ^{1}H-^{15}N HSQC spectrum.

From real-time H/D exchange experiments in the native state, residues with elevated protection factors were found in the secondary structure region. However in presence of 2.0 M urea α5-helix in the C-terminal protrusion was less stable than other secondary structure region.

Many NMR resonance peaks in the D_1 state were not observed on HSQC spectra because of the molten-globule like structure. Therefore, the progress of the H/D exchange reactions in the D_1 state was presumed from ^{1}H-^{15}N HSQC spectra after refolding. The results showed that, in the D_1 state, C-terminal α6-helix already formed a native-like rigid structure, and α4-helix and the region around the V44 formed a week hydrophobic core, whereas the central β-sheet regions were not folded.

1) Kaushik et al., *J. Mol. Biol*., (2002) **316**, 991
2) Iimura et al., *Biochemistry* (2004) **43**, 11906

Residual HN proton of PCP-0SH in the D_1 state on the 3D structure

0 min

10 min

1 hour

2.2 hours

12.0 hours

24.0 hours

Residual HN proton of PCP-0SH in the D_1 state on the 3D structure at pH* 3 and 4 ^{o}C.

Black; rapidly H/D exchangeable region in native structure & not assigned region

White; H/D unexchangeable region in the native structure

Red; protected region in D_1 state for limited time