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Adaptation to Low-temperature of a Psychrotrophic Bacterium

Kazufumi TAKANO

Department of Material and Life Science, Osaka University

Research aims

We have previously shown that the cellular content of FKBP22 (SIB1 FKBP22) from a psychrotrophic bacterium *Shewanella* sp. SIB1 increases at 4°C, as compared to that at 20°C.[1] SIB1 FKBP22 exists as a homodimer and exhibits the PPIase (Peptidyl Prolyl *cis-trans* Isomerase) activity like other MIP-like FKBP subfamily proteins. However, the optimum temperature of this protein for activity (10°C) is much lower than that of *E. coli* FKBP22 (>25°C). We proposed that this activity facilitates efficient folding of proteins containing *cis* prolines in psychrotrophic bacteria at low temperatures.

In this work, the N- and C-domains of SIB1 FKBP22 were overproduced in *E. coli* and purified in an amount sufficient for physicochemical studies. By comparing their activities and stabilities with those of the intact protein, we showed that the unfolding temperature of SIB1 FKBP22 is much higher than the optimum temperature for activity. Based on these results, we discuss a role of each domain of SIB1 FKBP22 and a cold-adaptation mechanism of this protein.[2]

Results

PPIase Activity. When the PPIase activity was determined at 10°C by protease coupling assay using ALPF (*N*-succinyl-Ala-Leu-Pro-Phe-p-nitroanilide) as a substrate, C-domain exhibited the activity. The catalytic efficiency (k_{cat}/K_m) of C-domain was estimated to be 1.43 $\mu M^{-1} s^{-1}$, which was 1.6 times higher than that of SIB1 FKBP22. The temperature dependence of the PPIase activity of C-domain was nearly identical to that of SIB1 FKBP22. In contrast, when the PPIase activity was determined by RNase T_1 refolding assay, C-domain exhibited much less activity as compared to that of SIB1 FKBP22.

Thermal stability. Heat induced unfolding of N-domain, C-domain, and SIB1 FKBP22 were analyzed by DSC (differential scanning calorimetry). The DSC curves of N-domain and C-domain showed single peaks at 44.7°C and 35.6°C, respectively. In contrast, the DSC curve of SIB1 FKBP22 showed two distinct peaks at 32.5°C and 46.4°C. These results indicate that the thermal unfolding transitions of SIB1 FKBP22 at lower and higher temperatures represent those of its C-domain and N-domain, respectively.

Discussion

Thermal unfolding of C-domain is also initiated at >25°C. Nevertheless, SIB1 FKBP22 and C-domain both exhibit the maximal PPIase activity at 10°C and their activities are greatly reduced at 20°C. These results suggest that subtle conformational change around the active-site causes great reduction of the enzymatic activity. The large difference in the temperatures for enzymatic inactivation and structural unfolding has been observed for cold-adapted α-amylase and family 8 xylanase from an Antarctic bacterium. The apparent optimal temperatures of these proteins for enzymatic activities are much lower than the temperatures at which any significant conformational event occurs. In contrast, the optimal temperatures for the activities of their mesophilic and thermophilic counterparts closely correlate with the temperatures for their structural transitions. Thus, the large difference in the temperatures for enzymatic inactivation and structural unfolding seems to be a characteristic feature of cold-adapted enzymes. It has been proposed that this difference is caused by a cold-adaptation strategy termed "localized flexibility". Although an increase in flexibility around the active site increases k_{cat} by reducing the energy cost of conformational change during the catalytic reaction, it should increase K_m concomitantly. By restricting the increase of flexibility within small areas, cold-adapted enzymes prevent unfavorable increase in K_m.

References

[1] Suzuki Y, Haruki M, Takano K, Morikawa M, Kanaya S. (2004) Possible involvement of an FKBP family member protein from a psychrotrophic bacterium *Shewanella* sp. SIB1 in cold-adaptation, *Eur. J. Biochem.*, **271**, 1372–1381.

[2] Suzuki Y, Takano K, Kanaya S. (2005) Stabilities and activities of the N- and C-domains of FKBP22 from a psychrotrophic bacterium overproduced in *Escherichia coli*, *FEBS J.*, **272**, 632–642.