The Stability of *Bacillus Subtilis* Pectate Lyase

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The stability of the beta helix protein pectate lyase to a variety of environmental conditions has been studied. The structure of the protein, as monitored by circular dichroism and fluorescence spectroscopy and by differential scanning calorimetry, is found to be stable to pressure treatment at 800MPa for 30min at neutral pH. Denaturation occurs below pH3, acid conditions do not decrease the stability of the protein to pressure. [stability, pressure, temperature, pH, pectate lyase]

1. Introduction

The alpha-1,4 glycosidic bonds comprising the sugar homogalacturan backbone of pectins is cleaved in a beta elimination reaction which can be catalysed by pectin and pectate lyases. Pectin lyases act on highly methoxylated pectins whereas pectate lyases act on pectins of low degree of esterification and on polygalacturonic acid. The action of pectate lyase is dependent on calcium.

The structure of pectate lyases from *B.subtilis* (BSpel) [1,2] and from *Erwinia chrysanthemi* (PelC and E) [3] has been studied by X-ray crystallography, the structure of the enzyme from *B.subtilis* being solved with calcium bound to the protein. These enzymes are found to contain a large content of a novel secondary structure, the beta helix. The interior of the beta helix region of the BSpel enzyme contains a striking array of aromatic amino acids exhibiting stacking interactions. We are studying the stability of this and other enzymes to pressure and other physical and chemical conditions.

2. Results

Pressure stability of pectate lyase

The effect of pressure treatment on the structure, stability and activity of BSpel was studied. Pressurisation was performed at 800MPa for 30min in 20mM Tris buffer pH7.2 and also in MOPS buffer, the results being independent of the buffer used. Circular dichroism spectroscopy was used to study the secondary structure of the protein. The beta helix structure of BSpel is characterised by a strong band of negative ellipticity centred at 218nm., as shown in fig1 pressurisation causes only a slight change in the far UV circular dichroism spectrum of the protein showing that the secondary structure is little changed by this treatment.

Fig.1. Effect of pressure on the circular dichroism spectrum of pectate lyase.

Fluorescence spectroscopy was also used to probe the environment of aromatic residues before and after pressurisation. Figure 2 shows that there is little change in the fluorescence spectrum following pressurisation. The environment of the aromatic residues of the protein is not changed by pressure treatment indicating that the protein tertiary structure, in addition to the secondary structure, is stable to pressure treatment.

Fig.2. Effect of pressure on pectate lyase fluorescence spectrum.

Table1. The effect of pressure treatment on the activity, spectroscopic properties and thermostability of pectate lyase.

	activity (U/mg)	ellipticity (mdeg)	fluorescence maximum (nm)	T _m
unpressured	504	-5.6	337	63.6°C
pressured	462	-4.9	338	63.5°C

In addition to the spectroscopic studies of the effect of pressure on the BSpel structure differential scanning calorimetry was used to investigate the effect of pressure treatment on the thermal stability of the protein. Figure 3 shows dsc thermograms for pressurised and control samples of BSpel. The denaturation temperature is the same for both samples. It can be concluded from these results that pectate lyase is very stable to pressure at neutral pH. In addition to structural stability enzymatic activity is also stable to pressure, table1.

Fig.3. Differential scanning calorimetry of pressurised and unpressurised pectate lyase.

The pH stability of pectate lyase

In order to further define the stability parameters for pectate lyase the stability of the protein was measured by differential scanning calorimetry as a function of pH (fig4) and in concert with this the structure of the protein at low pH was studied by circular dichroism spectroscopy.

Fig.4. Effect of pH on thermostability.

The temperature at which the protein denatures is fairly constant at 63°C above pH3. The circular dichroism spectrum of the protein in this region also remains constant. Below pH 3 the T_m is decreased as is the enthalpy of the denaturation. At pH2.5 the T_m is 46.5°C while at pH2 only a small transition is observed at 33.5°C. Circular dichroism spectroscopy also shows that the protein becomes unfolded at low pH, the spectrum at pH2 being predominantly that of a random coil. Interestingly the denaturation is maximally reversible (though only ~10%) at pH3, the pH of onset of destabilisation. Thermal denaturation at pH values above 3.5 causes precipitation of the protein.

Pressure did not denature pectate lyase at neutral pH. The effect of pressure was tested at low pH. Again the thermal stability of the protein was not affected by pressure treatment as shown in fig 5, despite the protein being of markedly lower thermal stability at this pH.

Fig.5. Thermostability at pH2.5 of pressurised and unpressurised protein.

3. Conclusion

The beta helix protein pectate lyase is very stable to pressure treatment. It is perhaps to be expected that an extracellular protein may have evolved to be stable to a wide range of conditions and this may also have introduced pressure stability into the structure. BSpel does contain a number of stacked aromatic residues in the interior of the beta helix, such interactions have negative

volumes of reaction [4] and this may enhance the pressure stability of this protein. The protein becomes unstable at low pH, however even when the structure is stressed it retains pressure stability. It is of course possible, if unlikely, that the protein unfolds at high pressure but folds rapidly on depressurisation, studies of protein structure at high pressure will answer this question.

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References

- [1] Pickersgill,R., Jenkins,J., Harris,G., Nasser,W. & Robert-Baudouy,J. *Structural Biology* **1**, 717-723 (1994)
- [2] Henrissat,B., Heffron,S.E. & Yoder,M.D. *Plant Physiol.* **107**, 963-976 (1995)
- [3] Yoder,M.D., Keen,N.T. & Jurnak,F. *Science* **260**, 1503-1507 (1993)
- [4] Mozhaev,V.V., Heremans,K., Frank,J., Masson,P. & Balny,C. *Protein-Struct.Funct.Genet.* **24**, 81-91 (1996)