Effects of Hydrostatic Pressure on the Conformational Equilibrium of Tryptophan Synthase

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Introduction

Bacterial Tryptophan (Trp) synthase is an $\alpha_2\beta_2$ tetramer, arranged as individual α-subunits attached to the opposite ends of a β_2-dimer. The activities of the α- and β-subunits are coordinated by complex allosteric interactions. The equilibrium between the low activity (open) and high activity (closed) conformations of Trp synthase is known to be affected by a wide range of variables, including α-subunit ligands, monovalent cations, solvents, pH and temperature. However, the effects of hydrostatic pressure on the conformational behavior of Trp synthase have not been examined previously.

We have now found that hydrostatic pressure exerts large effects on the conformational equilibrium in Trp synthase, as monitored by the absorption spectrum of the PLP-serine complex. Hydrostatic pressure stabilizes the open, rather than the closed, conformation of Trp synthase.

Materials and Methods

Sample Preparation

Trp synthase $\alpha_2\beta_2$ complex from *Salmonella typhimurium* was prepared from cells of *E. coli* containing pEBA, with the *trp*A and *trp*B genes of *S. typhimurium*.

Dilution of the enzyme, to a final concentration of 21.6 µM (αβ dimers), was made in 0.05 M triethanolamine hydrochloride buffer, pH 8.0, with either 0.1 M NaCl or NH_4Cl, and 0.1 M L-Ser, immediately prior to collection of the data.

Equipment

The effects of hydrostatic pressure on the absorption spectra were measured using a Cary 14 UV/Vis spectrophotometer, modified by OLIS, Inc. to contain the (HPCell) High Pressure Cell from ISS, Inc.; the high pressure cell was equipped with a manual pressure pump. The temperature of the cell was maintained at 25°C with an external circulating water bath. The enzyme solutions were contained in 1 mL quartz bottles with a 9 mm pathlength, capped with teflon tubing and immersed in spectroscopic grade ethanol as the pressurizing fluid.

The buffer, triethanolamine hydrochloride, pH 8.0, was chosen since amine buffers have relatively a small pressure dependence for the pKa, so the changing pressure will not significantly change the pH. The enzyme solutions were scanned at 1 bar, and immediately after each incremental increase in pressure.
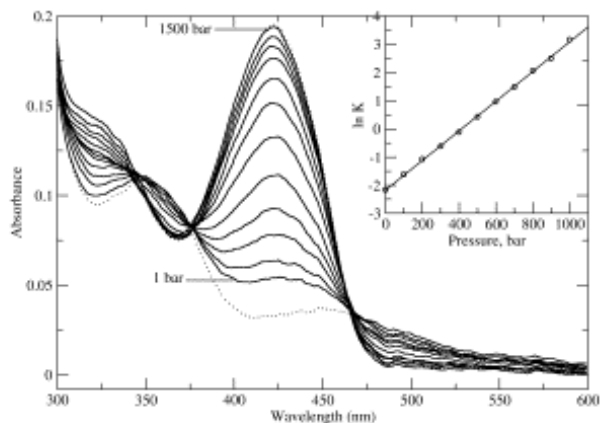
Figure 1. Absorption spectra of the complex of Trp synthase with L-Ser in the presence of 0.1 M NaCl with pressure increasing from 1 to 1500 bar.

TA buffer blank at 1 bar was used to obtain a baseline reading. The total time to measure the spectra through the range of pressures was approximately one hour. The spectra were analyzed by global analysis using the GlobalWorks program obtained from OLIS, Inc.

Results

Figure 1 shows the effect of increasing hydrostatic pressure from 1 bar to 1500 bar on the absorption spectrum of the complex of Trp synthase with L-Ser in the presence of 0.1 M NaCl. There is a dramatic increase in the absorption of the 423 nm species, corresponding to the open conformation, as the pressure is increased from 1 to 1500 bar.

There are isosbestic points at 376 and 467 nm, indicating that the equilibrium involves only two major species. The changes in the spectra in Figure 1 in the 350 to 550 nm region are fully reversible upon release of pressure.

The small increase in absorbance below 350 nm is not reversible and is probably due to slow formation of pyruvate by β-elimination of L-serine during the experiment. The equilibrium constant at each pressure, K_p is logarithmically proportional to the applied pressure (Figure 1, inset).

The spectral data in Figure 1 were fitted by global analysis to Equations 1 and 2, where K_p is the equilibrium constant at pressure p, K_0 is the equilibrium constant at 0 bar, ΔV_0 is the volume change for the reaction, A_0 is the absorbance at 0 bar, A_p is the absorbance at pressure p, and A_∞ is the absorbance at infinite pressure. The global fit gives a K_0 for L-serine aldimine formation of 0.12 ± 0.01 at 25 °C, in excellent agreement with the K_{eq} value of 0.11 at 25 °C calculated from the published thermodynamic parameters obtained from thermal analysis.

Global analysis also provides the ΔV_0 for the reaction, -126 ± 2 mL/mol. The calculated spectra of the components from global analysis are shown as the dotted lines in Figure 1. The effect of increasing hydrostatic pressure on the spectra of the Trp synthase L-Ser complex with 0.1 M NH_4Cl is similar; however, higher pressures of between 1-2 kbar are required to see a significant

change (data not shown). The values of K_0 and ΔV_0 with NH_4Cl, obtained by global fitting of the data, are $(2.34 \pm 0.08) \times 10^{-4}$ and -171 ± 2 mL/mol:

$$K_P = K_0\, e^{-\frac{P \Delta V_0}{RT}} \quad [1]$$

$$K = \frac{(A_P - A_0)}{(A_\infty - A_P)} \quad [2]$$