Solubility of Thaumatin

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ABSTRACT: Thaumatin, an intensely sweet protein, crystallizes rapidly in the presence of tartrate ions. The ease with which crystals form has led to the use of thaumatin over the past decade as a model system for the study of protein crystallization. The available data on the solubility of this protein, however, are inconsistent. We have purified thaumatin and determined its solubility with the L and D enantiomers of the tartrate ion. We find that the crystal habit and solubility are significantly different for the two precipitants: the solubility increases with temperature in L-tartrate, while it decreases with temperature in D-tartrate. Our results suggest that the chirality of precipitants is an important factor that should be controlled when determining the solubility of proteins.

Proteins are crystallized by trial and error.1 Indeed, obtaining high-quality crystals is still a challenge, and it is common to read that “protein crystallization has always been a bit more of an art than a science”.3 As this state of affairs hampers the determination of X-ray structures for proteins, much work continues to be carried out to improve our understanding of protein phase behavior.4,5 In particular, a common approach is the use of model proteins to gain insight into the process of crystallization.6–10

One of these model proteins is thaumatin. The thaumatins are a family of single-domain, highly homologous globular proteins (molecular weight approximately 22 kDa) found in the fruit of Thaumatococcus daniellii.11 The two most abundant components are thaumatin I and thaumatin II, which differ by five amino acids.12

The thaumatins are among the sweetest compounds known, and their physical and chemical properties (in particular, those related to taste) have been studied extensively.13

In 1994, it was shown that the addition of L-tartrate ions leads to the rapid formation of bipyramidal crystals of thaumatin.14 Since then, the thaumatin—tartrate system has been used to examine many aspects of protein crystallization and X-ray structure determination, including crystallization in microgravity,15 in gels,16 and under controlled hydrostatic pressure;17 crystallization mechanisms;18 new crystallization platforms;19,20 radiation damage;21 cryocooling;22 and optimization of X-ray data collection.23

An important quantity in the crystallization of any protein is the solubility—the concentration at which the protein solution is in equilibrium with the crystal phase under a given set of conditions. Several groups have measured the solubility of thaumatin, and the results are contradictory. It has been reported that the solubility of thaumatin increases with temperature,17 decreases with temperature,18 or is essentially zero.18 Here we report our results for the solubility of purified thaumatin with tartrate. We demonstrate that the choice of tartrate stereoisomer strongly influences both the thermodynamics and the kinetics of crystallization.

We obtained industrial preparations of thaumatin from Natex UK Limited and purified the protein by low-pressure size-exclusion chromatography. We characterized the purified protein by quasielastic light scattering, size-exclusion, and cation-exchange high-performance liquid chromatography, and electrospray ionization mass spectrometry (see Supporting Information). Our results show that purified protein is essentially a homogeneous preparation of thaumatin I.

Figure 1 shows the solid phases that formed in our crystallization experiments with Natex thaumatin (for experimental details see Supporting Information). We obtained well-formed protein crystals in solutions with 0.5 M sodium L-tartrate (Figure 1A) and 0.5 M sodium D-tartrate (Figure 1B). The bipyramidal crystals in L-tartrate are the “rapid” crystals which have made thaumatin a popular model system. Crystals around 50 μm in size form within a few hours and sometimes even faster. The prismatic and stubby crystals in D-tartrate also can form rapidly, but they usually take longer to appear and to reach a comparable size. Both types of crystals are birefringent; the bipyramids are more strongly birefringent than the prismatic crystals. The two crystal habits are not chiral in shape, but the underlying crystal structures are different: the unit cell in the bipyramidal crystal is tetragonal, whereas the unit cell in the prismatic and stubby crystals is orthorhombic (N. Asherie, J. Jakoncic, C. Ginsberg, A. Greenbaum, V. Stojanoff, B. Hrnjez, and S. Blass, unpublished data).

Some investigators have used the commercially available racemic mixture of sodium DL-tartrate to produce thaumatin crystals.15,17 We therefore crystallized the protein in 0.5 M sodium DL-tartrate. In the experiments with the racemate, we see bipyramids, prisms, and amorphous precipitate; the proportions of these structures...
Figure 2. The solubility of purified thaumatin. (A) The solubility in 0.5 M sodium l-tartrate (squares) and 0.5 M sodium d-tartrate (triangles). All solutions contained 10 mM sodium phosphate (pH = 7.3) with 0.002% sodium azide. (B) Log-linear plot of the solubility data used to extract the enthalpies and entropies of crystallization. The straight lines are least-squares fits to eq 1. Here \( \nu \) is the volume fraction of protein in solution and \( T_0 \) is a reference temperature taken to be 293 K.

depend on the concentration of protein and the temperature. The variability in the solid structures that form may reflect the heterogeneous nature of the racemic solution.

While thaumatin was being concentrated, a precipitate always formed once the concentration reached about 40 mg/mL (Figure 1C). Though most of the precipitate was amorphous, crystalline objects could be seen. Furthermore, the precipitate is weakly birefringent, suggesting that it is somewhat ordered. We measured the solubility of this precipitate and found it to be 20 ± 4 mg/mL from 2.5 to 28 °C with an erratic temperature dependence. Therefore, we conclude that this precipitate is not a true equilibrium phase, but a predominantly amorphous solid whose solubility depends on both thermodynamic and kinetic factors.

We also attempted to produce crystals with the third tartrate stereoisomer, sodium meso-tartrate. Under the same conditions used for l- and d-tartrate, we observe mainly precipitates with an occasional crystalline structure (Figure 1D). The meso-tartrate is colored (unlike the solutions of l- and d-tartrate, which are colorless), and this colored contaminant may be interfering with the crystallization of the protein.

Figure 2 shows the solubility of thaumatin. At each temperature we plot the protein concentration \( C \), which is in equilibrium with the crystal phase.\(^{25}\) The solubilities in 0.5 M l-tartrate and 0.5 M d-tartrate are very different. With l-tartrate (where bipyramidal crystals form), the solubility increases with temperature, as is the case for most globular proteins.\(^{26,27}\) In d-tartrate (where prismatic and stubby crystals form), the solubility is retrograde; that is, it decreases as the temperature increases. Although many examples of retrograde solubility in proteins are known,\(^ {26,27}\) it is unusual for a given protein to display both types of solubility. Generally, if a protein crystallizes in more than one habit, the solubilities are of the same type.\(^ {28}\) The reason is that the underlying crystallization mechanisms are different: direct solubility is dominated by energetic effects, while retrograde solubility is driven by entropic interactions.\(^ {27,29}\)

It is known that a single point mutation can alter the solubility of human yD crystallin from direct to retrograde,\(^ {30}\) but the results we report are one of the few examples of an inversion in protein solubility due to a change in precipitant.\(^ {7}\) We do not yet understand why such a dramatic shift in the phase behavior occurs. It is curious that the change depends on the chirality of the precipitant. We are currently conducting X-ray diffraction experiments to examine this phenomenon more closely.

To extract the enthalpies \( \Delta H \) and entropies \( \Delta S \) of crystallization, we fit our solubility data using the van’t Hoff equation:\(^ {31}\)

\[
\ln \nu = \frac{\Delta H}{RT_0} - \frac{\Delta S}{R}
\]

Here, \( \nu \) is the volume fraction of protein in solution; it is related to the concentration by \( \nu = \nu C \), where \( \nu \) is the specific volume of the protein, taken to be 7.2 × 10\(^{-4}\) mL/mg.\(^1\) Also, \( R \) is the universal gas constant and \( T \) is the absolute temperature.

Figure 2B shows the solubilities in l-tartrate and d-tartrate with fits to eq 1 (\( T_0 \) is a reference temperature taken to be 293 K). The enthalpies and entropies of crystallization are listed in Table 1. As expected, the energetic contribution to the free energy dominates in l-tartrate, whereas the entropic contribution dominates in d-tartrate. Additional information on the protein–tartrate interactions can be obtained by studying how the solubility varies with the concentration of tartrate at a fixed temperature; such measurements are in progress.

Our solubility data differs from those of other investigators, and there are several reasons for this. First, previous solubility measurements were made with a less pure formulation of the protein (a mixture of thaumatin I and II) from a different commercial source.\(^ {16-18,24}\) Second, while most studies were conducted with l-tartrate, some used racemic dL-tartrate;\(^ {17}\) the results of the studies with dL-tartrate could be affected by the presence of two populations of crystals with different solubilities. Finally, in earlier investigations the solubility was measured upon crystal growth. It has been shown that it is more difficult to establish equilibrium by this method because, as the crystals grow, their surfaces often become poisoned.\(^ {35}\) As a result, further growth stops, leading to an apparent solubility due to a change in precipitant.\(^ {7}\) We do not yet understand why such a dramatic shift in the phase behavior occurs. It is curious that the change depends on the chirality of the precipitant. We are currently conducting X-ray diffraction experiments to examine this phenomenon more closely.

Table 1. Thermodynamic Parameters for the Crystallization of Thaumatin

<table>
<thead>
<tr>
<th>precipitant</th>
<th>( \Delta H ) (kJ/mol)</th>
<th>( T_0 \Delta S ) (kJ/mol)</th>
</tr>
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<tbody>
<tr>
<td>0.5 M Na l-tartrate</td>
<td>−43 ± 4</td>
<td>−24 ± 1</td>
</tr>
<tr>
<td>0.5 M Na d-tartrate</td>
<td>19 ± 1</td>
<td>33 ± 2</td>
</tr>
</tbody>
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\(^{a}\) The reference temperature \( T_0 \) is 293 K.

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Supporting Information Available: Experimental procedures. This information is available free of charge via the Internet at http://pubs.acs.org.

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