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SOME COMPARISONS BETWEEN SOLUTION AND CRYSTAL PROPERTIES OF THIOSULFATE SULFURTRANSFERASE

Paul M. Horowitz and Kuldeep Patel

Department of Biochemistry
The University of Texas Health Science Center
San Antonio, Texas 78284

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SUMMARY: The activity and crystal stability of the enzyme thiosulfate sulfurtransferase were studied as a function of ionic strength. At 2 M ammonium sulfate, where the x-ray structural studies of this protein were done soluble enzyme has low activity (<16% of the activity of the enzyme at an ionic strength of 0.1) and crystals of the enzyme are stable when substrates are added. However, at 1.4 M ammonium sulfate, crystals of TST rapidly dissolve in 1 mM CN^- but are relatively stable in 1 mM $S_2O_3^=$. These results are consistent with a conformational change on converting the sulfur substituted form of the enzyme (ES) to the sulfur-free form (E) and helps to explain why this change was not observed in the crystallographic studies.

INTRODUCTION

A number of solution studies of the enzyme thiosulfate sulfurtransferase (TST; EC2.8.1.1; rhodanese) have been interpreted as indicating that the protein has structural flexibility and that a reversible conformation change accompanies catalysis (1,2,3). However, comparisons between the recently available 2.5 Å x-ray structure of TST and these solution studies reveal a number of discrepencies. TST cycles between sulfur free (E) and sulfur substituted forms (ES) during catalysis. Addition of CN^- to crystals of ES gives E but crystallographic analysis does not show the large conformational changes that would be expected from kinetic and spectral studies of TST in solution (4,5). Solution studies further show that the active site sulfhydryl (SH) group (residue 247) can form an intramolecular disulfide bond with another SH group in the protein (either cys-254 or cys-263) (6,7,8). However, the crystallographic studies show that these sulfhydryl groups are located in such a way that disulfide bond formation is very unlikely (4,5). These differences are of interest because they involve features that are directly related to the

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catalytic event, and to the extent they can be supported they make TST an interesting model for studying functional protein flexibility.

In this paper we have studied the activity and crystal stability of TST as a function of the concentration of ammonium sulfate (AS) to try to understand the discrepencies between the solution and x-ray studies.

MATERIALS AND METHODS

Bovine liver TST was prepared and assayed as previously described (9) and the crystalline enzyme was stored at -70°C as an ammonium sulfate (AS) suspension.

TST was prepared in the E form by the inclusion of the sulfur acceptor substrate KCN at 1 mM in the buffers. The enzyme was prepared in the ES form by inclusion of the sulfur donor substrate $Na_2S_2O_3$ at 1 mM in the buffers.

For studies of the effect of ammonium sulfate on enzyme activity the TST assay mixture was supplemented with the salt at the required levels. Control assays with the added product SCN^- showed that the color development in the assay was unaffected. The enzyme concentrations in the assays were kept below 1 µg/ml and there was no precipitation at any ammonium sulfate concentration used.

The crystal solubility was measured by treatment of TST crystals with successively lower concentrations of ammonium sulfate under controlled conditions until all the crystals dissolved. A stored microcrystalline suspension of TST was washed 3 times with 2 M AS (pH 7.9) and 800 µg of protein were resuspended in 50 µl of 0.03 M sodium phosphate pH 7.9 which was 2 M in AS. This sample was incubated for 2 minutes on ice and centrifuged for 1 minute in an Eppendorf 5412 centrifuge operated at 4°C. Fifty microliters of the supernatant was removed and assayed for TST activity and a 50 microliter aliquot of a lower concentration of AS was used to resuspend the crystals. This procedure was repeated until all the crystals had dissolved. The AS concentration in the assays never exceeded 2 mM.

The time dependence of the solubility of TST crystals was measured by adding crystals (198 µg) to 50 µl of 1.4 M AS in 30 mM sodium phosphate pH 8.0 containing either 0.97 mM $Na_2S_2O_3$ or 0.97 mM NaCN. The samples were incubated at 0° for various times, centrifuged and the supernatants were assayed for TST activity. The samples were remixed to give a uniform suspension and the incubation was continued. At the end of the experiment any remaining crystals were dissolved in 50 µl of sodium phosphate buffer and assayed.

RESULTS AND DISCUSSION

It is known that catalysis by TST depends on ionic interactions involving charged groups on both the anionic substrate, thiosulfate, and the enzyme. The cationic groups on the enzyme that were deduced to be important from the solution studies can be seen in the x-ray structure (4,5). These groups, in fact, appear to be involved in stabilizing the transferred sulfur atom which is held in ES as a persulfide (4,5). Figure 1 shows the activity of soluble TST as a function of the concentration of AS. At 2 M AS where the x-ray studies were done, the enzyme has less than 16% of the activity observed in buffer. This asymptotic

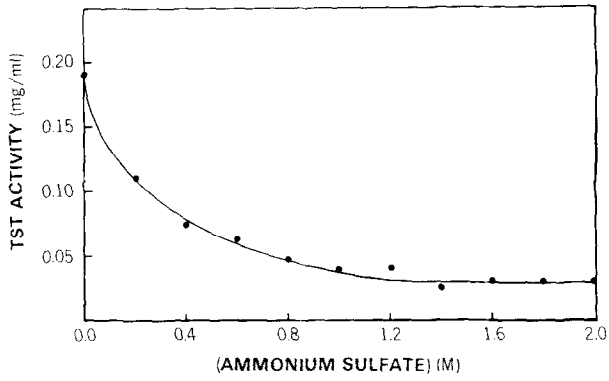
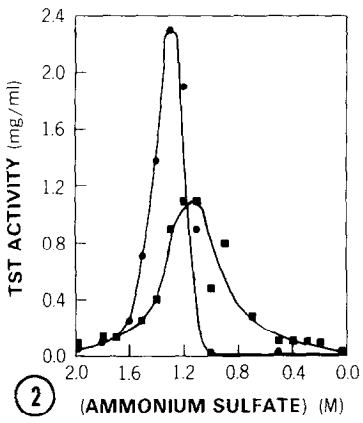


Figure 1. Soluble TST activity as a function of the ammonium sulfate concentration. Conditions are described in Materials and Methods.

value is relatively constant beyond 1 M AS. It therefore seems that under the conditions of the crystallographic experiments it is difficult to draw firm conclusions about solution behavior involving presumably intraprotein ionic interactions.

Figure 2 shows the result when microcrystalline suspensions of TST are treated under controlled conditions with successively lower concentrations of AS. Such a curve should rise when a sufficiently low AS concentration is reached and then fall as the point of optimal solubility under the conditions of the experiment is passed. The conditions of the experiment are such that both the rate of dissolution and the solubility of the crystals affects the measurement. The results show that crystals in the presence of 1 mM CN^- (E form) dissolve at a lower AS concentration than the ES form of the enzyme and give a sharper peak. The area under each peak is proportional to total activity recovered and in Figure 1 the ratio of the area under the E curve to that under the ES curve is 0.93, therefore indicating there is no large change in activity during the measurement. These results are consistent with the solution result showing a conformational change on going from E to ES (seen here as a change in the crystal stability of these two forms) and is also consistent with the report that it has not been possible to crystallize the enzyme in the E form. It is interesting that at 2 M AS where the x-ray



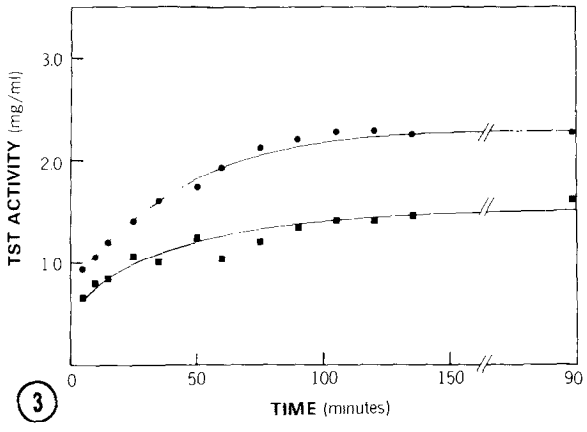


Figure 2. TST crystal solubility under conditions of controlled dissolution as a function of decreasing ammonium sulfate concentrations. • indicates the presence of 1 mM CN^-, ▪ indicates the presence of 1 mM S_2O_3; other conditions are described in Materials and Methods.

Figure 3. Time course of dissolution of TST crystals. • indicates the presence of 1 mM CN^-; ▪ indicates the presence of 1 mM S_2O_3. Other conditions are described in Materials and Methods.

studies were done there is no solubility difference between crystals of E and ES. This is consistent with the idea at high ionic strengths, the crystals of TST are so stable relative to the solution forms that the conformational change expected on the ES to E conversion cannot occur.

Figure 3 shows the dissolution of crystals of TST in the E (upper curve) and ES (lower curve) forms as a function of time after transferring an aliquot of crystals to 1.4 M AS chosen to maximize the difference between E and ES. After approximately 2 hrs the difference between E and ES is stabilized and does not change over the next 18 hours. When the remaining crystals are dissolved in buffer the total activity recoverable from the (E) sample was 94% of that from ES. This shows that total enzyme is largely conserved and that the equilibrium solubility is higher for the E form, again indicating that crystals of ES are destabilized by the presence of CN^-.

The above results are compatible with a model of a stable ES form of the enzyme which is relatively rigid and crystallizable. Addition of CN^- to the crystals produces the E form of the enzyme which at high ionic strengths is

stabilized by the crystal lattice forces in a conformation similar to ES. In fact, the solution studies indicate that conformational relaxation of the ES form to the E form occurs not when the persulfide is cleaved but when the product SCN^- is released. It is of interest therefore, that the x-ray studies show an electron density in the active site region after the addition of CN^- which was not identified but could be bound SCN^-. At lower ionic strengths, entropic considerations would indicate that the dissolved state of the more flexible E form should be favored and therefore crystals of E readily dissolve and once in solution do not readily crystallize. The observed discrepencies are possible because if a conformational change in the enzyme is catalytically linked, it is likely to involve a small amount of energy and the crystal lattice forces may be sufficient to constrain the conformational transitions. Therefore, it is just in those cases in which substrates induce conformational changes that the conformational transition energies would be expected to be small and that discrepencies between the crystal structure and solution behavior may be observed.

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