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BIOCHEMISTRY OF DISSIMILATORY SULFUR OXIDATION

PROGRESS REPORT

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DISCLAIMER

A. BRIEF DESCRIPTION OF PROJECT

The long term goals of this research are to define the substrate oxidation pathways, the electron transport mechanisms, and the modes of energy conservation employed during the dissimilatory oxidation of sulfur practised by various species of the thiobacilli. In accordance with these long term aims, the specific aims for the current grant period are as follows:

(1) To purify known sulfur-transformation enzymes to electrophoretic homogeneity. The enzymes selected include, but are not limited to

a) APS reductase from Thiobacillus denitrificans,

b) bisulfite reductase, also from T. denitrificans,

c) sulfite oxidase from T. novellus, and

d) sulfur oxygenase from Acidianus brierleyi;

(2) To perform immunochemical analyses of protein expression using cell-free extracts and polyclonal antibodies directed against each protein purified in Specific Aim #1. This will include

a) preparation of antisera,

b) gel electrophoresis and immunoblot analysis, and

c) immunoprecipitation of sulfur oxidation activities.

B. SUMMARY OF PROGRESS

1. We have purified APS reductase to electrophoretic homogeneity from cell-free extracts of Thiobacillus denitrificans. Sufficient protein is available to initiate the production of polyclonal antibodies and to perform the kinetic experiments discussed below.

2. We have acquired purified rhodanese (thiosulfate:cyanate sulfur transferase) from commercial sources.

3. Efforts to purify a thiosulfate oxidase from cell-free extracts of Thiobacillus neapolitanus are currently in progress. A purification scheme that consists of ammonium sulfate fractionation, heat denaturation, and column chromatography on DEAE-cellulose and hydroxylapatite has yielded a partially purified preparation that couples the oxidation of thiosulfate to the reduction of ferricyanide. When SDS polyacrylamide gel electrophoresis was performed on the thiosulfate oxidase preparation thus obtained, a disappointing pattern of 5 bands that stained with Coomassie Blue was observed. It is clear that significant further purification of the protein is necessary. We

will concentrate on gel filtration and column chromatography with other materials in our attempts to further our existing purification.

Efforts to purify other known sulfur transformation enzymes from various species of the thiobacilli continue and are currently in progress.

4. Additional kinetic experiments

It should be emphasized that the experiments in the goals stated above do not lend themselves to rapid publication. Protein purification and antibody preparation and characterization are long-term projects that take time. It is much more likely that visible progress in the form of manuscripts for publication will be generated in the additional experiments described below. Now that electrophoretically homogeneous enzymes are available, steady state and rapid mixing kinetic experiments have been initiated on the purified enzymes to characterize the functional behavior of each catalyst. These studies were not detailed in the original grant proposal.

Two separate studies are currently underway. First, an initial velocity investigation of the kinetic mechanism of purified APS reductase has been initiated. The kinetic mechanism appears to be a Bi Uni Uni Uni Ping Pong with AMP and sulfite adding first. The product APS then dissociates, leaving two electrons behind on the enzyme-bound flavin. The oxidized electron acceptor (cytochrome c or ferricyanide) then adds and subsequently leaves in the reduced form. Once we have established the order with which substrates bind and products dissociate from the enzyme by initial velocity studies, we will conduct a stopped flow spectrophotometric investigation of the rates of oxidation and reduction of the enzyme-bound flavin. It is anticipated that several peer-reviewed publications could be generated from these studies.

An initial velocity and stopped flow spectrophotometric investigation of the sulfur-transfer reactions catalyzed by the purified rhodanese is also in progress. Several novel chromogenic and fluorimetric substrates were synthesized and their kinetic properties with the rhodanese were investigated. Rhodanese catalyzes the transfer of the outer sulfur atom of organic thiosulfonates to the corresponding organic thiosulfinates. For each thiosulfonate/thiosulfinate conjugate pair, the following kinetic experiments are in progress:

a. The equilibrium constant for the interaction of each conjugate pair with the rhodanese is under investigation by steady state absorbance and (where applicable) fluorescence spectroscopy.

b. The individual rate constants for the transfer of sulfur from each thiosulfonate to the free enzyme and the transfer of sulfur from the enzyme-sulfur complex to each thiosulfinate are under investigation by stopped-flow spectrofluorometry.

c. Steady-state kinetic studies are in progress (using both fluorescence and absorbance spectroscopy) to establish whether the rate constants assigned to the transient enzyme species from the stopped-flow experiments agree with the numerical values for the steady-state kinetic coefficients.

As a result of the kinetic experiments outlined above, a comprehensive table of equilibrium and rate data regarding rhodanese-catalyzed sulfur transfer will be compiled. The Marcus theory of group transfer will then be applied to this set of equilibria and rate data to see if we can provide any additional insight into the activation process for sulfur transfer.

C. RENEWAL PROPOSAL

The experimental plan remains intact relative to the original proposal.

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