Supporting Information

METHYLARSENICALS AND ARSINOTHIOLS ARE POTENT INHIBITORS OF MOUSE LIVER THIOREDOXIN REDUCTASE

Shan Lin, William R. Cullen, and David J. Thomas

Experimental Procedures

Purification of TR. The purification of TR from mouse liver homogenate followed the general scheme outlined by Luthman and Holmgren [1] and Zhong and associates [2]. Male C6B3F1 mice (4 to 6 weeks old) were obtained from Charles River Laboratories (Raleigh, NC) and maintained at 23°C in a 12-hour light-12 hour dark photocycle with free access to rodent chow (Purina Mills, Richmond, IN) and tap water. Mice were killed by cervical dislocation and livers were rapidly excised and washed in ice-cold 50 mM tris HCl, 1 mM Na_4EDTA (pH 7.5) (Sigma, St. Louis). All subsequent steps in the purification scheme were carried at 0 to 4° C. A homogenate (1:3 w/v) was prepared in 50 mM tris HCl, 1 mM Na_4EDTA (pH 7.5), using a glass-teflon homogenizer. The homogenate was centrifuged at 10,000 x*g* for 15 minutes and the supernate collected. Supernate was adjusted to pH 5 by dropwise addition of ice-cold 1 M CH_3COOH (Fisher, Fairlawn, NJ) and centrifuged at 10,000 x*g* for 15 minutes. The supernate was adjusted to pH 7.5 by the addition of 1M NH_4OH (Sigma), then brought to 40% saturation with $(NH_4)_2SO_4$ (Sigma) and precipitated proteins were removed by centrifugation. This supernate was brought to 80% saturation with $(NH_4)_2SO_4$. Precipitated proteins were collected by centrifugation at 10,000 x*g* for 15 minutes, dissolved in 50 mM tris HCl, 1 mM Na_4EDTA (pH 7.5) and were dialyzed overnight against this buffer. Dithiothreitol was added to dialysate at a final concentration of 2 mM and this mixture was incubated at 37°C for 30 minutes. The treated

dialysate was applied to a column (32 x 1.5 cm) of DEAE Sepharose (Pharmacia, Piscataway, NJ) equilibrated with 50 mM tris HCl, 1 mM Na_4EDTA (pH 7.5). A linear gradient of 0 to 0.3 M KCl was applied and active fractions were pooled and dialyzed overnight against 50 mM tris HCl, 1 mM Na_4EDTA (pH 7.5). The dialysate was then applied to a column (16 x 0.8 cm) of 2',5'-ADP agarose (Sigma) and TR activity was eluted with 10 mM $NADP^+$ (Sigma) in 50 mM tris HCl, 1 mM Na_4EDTA (pH 7.5). To remove higher molecular weight contaminants, active fractions from 2',5'-ADP agarose chromatography were pooled and chromatographed on a Sepharcyl S-200 (Sigma) column (96 x 1.6 cm) that was eluted with 50 mM tris HCl, 1 mM Na_4EDTA (pH 7.5). Fractions with the highest specific activity for TR were pooled and stored at -70°C. The DTNB reductase activity of fractions obtained in the purification scheme was determined using the assay of Luthman and Holmgren [1]. Various fractions from the purification scheme were electrophoresed on 4 to 20% polyacrylamide gels by the method of Laemmli [3], using the Mark 12 wide range protein standard set (Novex, San Diego, CA) for a calibration. Proteins were visualized by staining with Coomassie Brilliant Blue G.

Results

Purification and Characterization of Mouse Liver TR. The coupling of anion exchange and affinity chromatography with acidification and ammonium sulfate fractionation provided a facile method for the purification of TR from mouse liver cytosol. Yield and fold purification of TR from the livers of adult male B6C3F1 mice by this scheme are summarized in Table S1. The filtrate prepared from pooled active fractions from Sepharcyl S-200

chromatography yielded a single major protein band on SDS-PAGE with an estimated molecular weight of about 57 kDa (Figure S1). However, because earlier work suggests that polyacrylamide gel electrophoresis under denaturing conditions overestimates the mass of structurally-related disulfide reductases by 5 to 10% [4], the mass of mouse liver TR may range from about 51 to 54 kDa. This estimate is similar to, albeit smaller, than that reported for TR purified from rat liver (58 kDa) [1]. The purified enzyme catalyzed the reduction of DTNB over broad pH range (Figure S2a) and its activity was greatest at 50°C at pH 7.5 (Figure S2b). Analysis of data on the rate of DTNB reduction by TR over the range of 18 to 50°C indicated that the energy of activation for this reaction was 97.2 kJ/mol (Figure S3) which is similar to the energy of activation recently reported for purified human placental TR (53.2 kJ/mol) [5]. Like TR purified from other mammalian tissues, mouse liver TR was inhibited by aurothioglucose (Sigma) with an IC_{50} of about 800 nM (data not shown). This finding is consistent with a role for a Secys residue in its catalytic action. All studies of the effects of arsenicals and arsinothiols on the activity of purified mouse liver TR were performed using the material obtained from chromatography on Sepharose S-200 in which the DTNB reductase activity has been enriched 5800-fold.

References

1. Luthman, M., and Holmgren, A. (1982) Rat liver thioredoxin reductase: Purification and characterization. *Biochemistry*. **21**, 6628-6633.

2. Zhong, L., Arnér, E.S.J., Ljung, J., Åslund, F., and Holmgren, A. (1998) Rat and calf

thioredoxin reductase are homologous to glutathione reductase with a carboxyl-terminal elongation containing a conserved catalytically active penultimate selenocysteine residue. *J.Biol. Chem.* **273**, 8581-8591.

3. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage.

4. Arscott, L.D., Gromer, S., Schirmer, R.H., Becker, K., and Williams, C.H. Jr. (1997) The mechanism of thioredoxin reductase from human placenta is similar to the mechanisms of lipoamide dehydrogenase and glutathione reductase and is distinct from the mechanism of thioredoxin reductase from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **94**, 3621-3626.

5. Gromer, S., Arscott, L.D., Williams, C.H., Jr., Schirmer, R.H., and Becker, K. (1998) Human thioredoxin reductase. Isolation of the selenoenzyme, steady state kinetics, and inhibition by therapeutic gold compounds. *J. Biol. Chem.* **273**, 20096-20101.

Table S1 - Purification and Yield of Thioredoxin Reductase From Mouse Liver

Fraction	Total Protein (mg)	Specific Activity[a] (units/mg protein)	Total Activity (units)	Yield (%)	Fold Purification
10000 xg Supernate	17050	0.0025	41.8	100	-----
Acidified Extract	5400	0.007	37.8	91	2.8
$(NH_4)_2SO_4$ Precipitate	1600	0.023	36.8	88.1	9.2
DEAE-Sepharose Eluate	54	0.35	18.9	45.2	140
2′, 5′-ADP-Agarose Eluate	1.2	11.2	13.9	33.3	4480
Sephacryl S-200 Eluate	0.8	14.5	11.6	27.8	5800

a. A unit of activity is 1 µmol of NADPH consumed per minute per mg of protein.

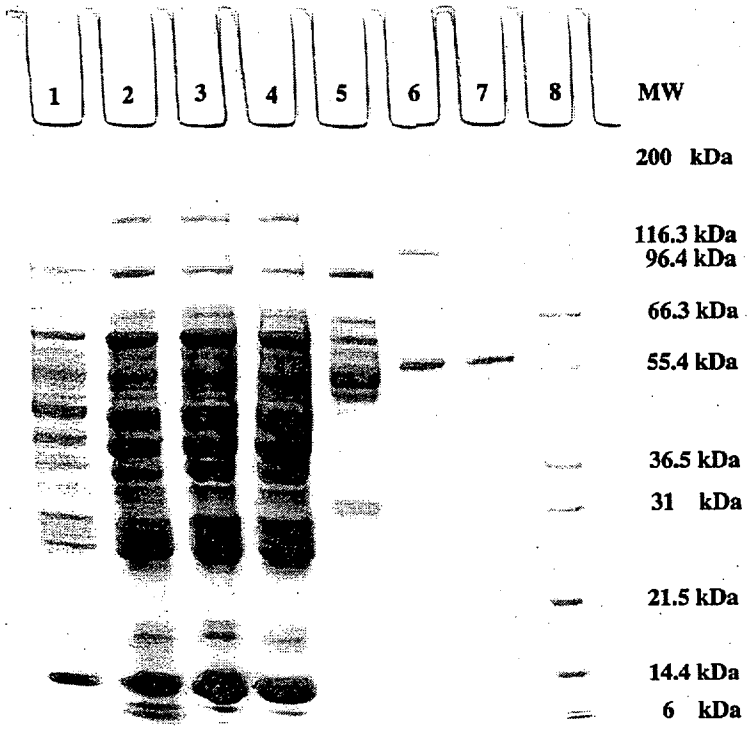
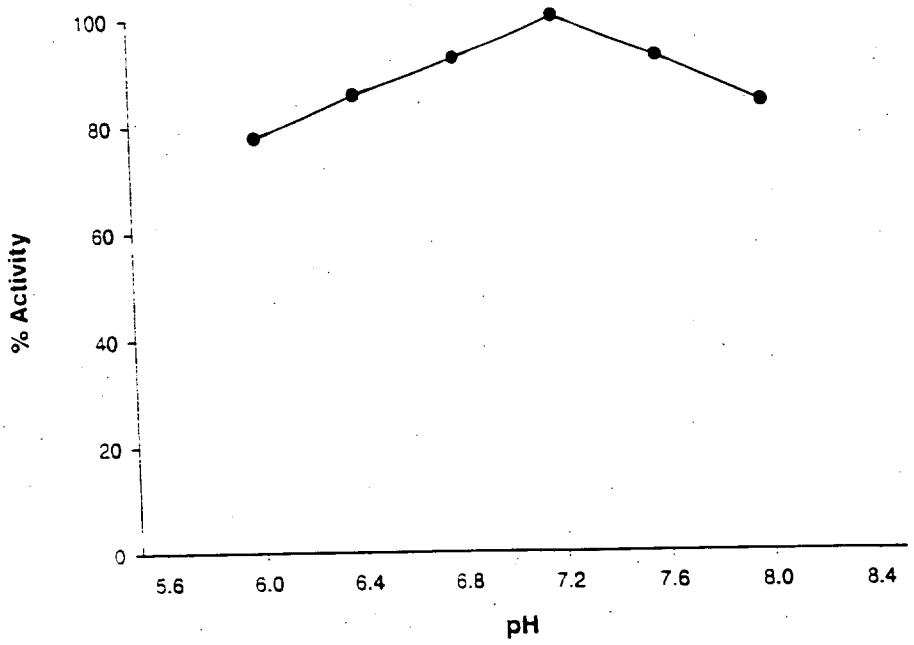


Figure S1 - Purification of TR from Mouse Liver. Fractions from the purification scheme resolved by 4%-20% SDS-PAGE. Lane 1 - 10000 xg supernate; Lane 2 - Acidified supernate; Lane 3 - 40% $(NH_4)_2SO_4$ supernate; Lane 4 - 80% $(NH_4)_2SO_4$ pellet; Lane 5 - Active fractions from DEAE-sepharose chromatography; Lane 6 - Active fractions from 2',5'-ADP- agarose chromatography; Lane 7 - Active fractions from Sephacryl S-200 chromatography; Lane 8 - Molecular weight (mass in kDa) markers (Mark 12 wide range protein standard set, Novex, San Diego, CA) for calibration. PAGE stained with Coomasie Brillant Blue.

Supplemental Information- METHYLARSENICALS AND ARSINOTHIOLS ARE POTENT INHIBITORS OF MOUSE LIVER THIOREDOXIN REDUCTASE - Shan Lin, William R. Cullen, and David J. Thomas



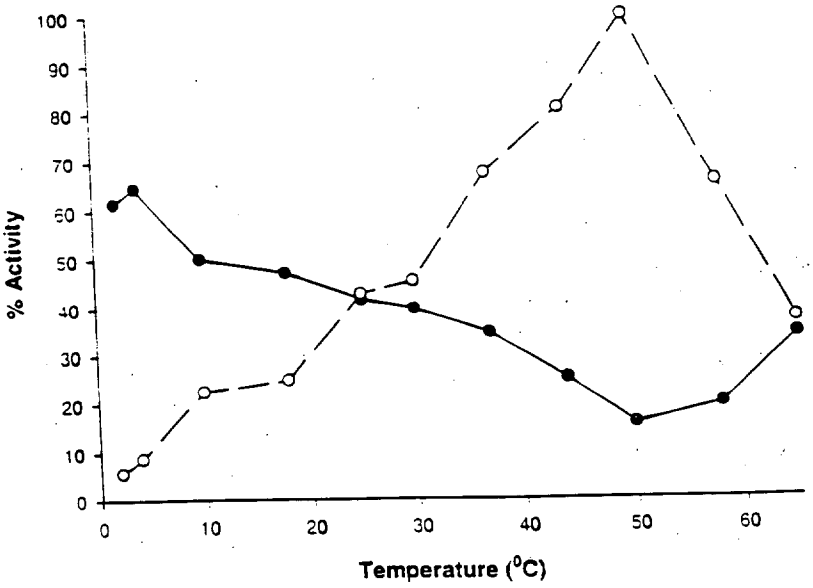


Figure S2 - Characterization of Purified TR. a) pH dependence of DTNB reductase activity. b) Temperature dependence of TR in the absence (○) or presence of 1μM CH_3AsI_2 (●). Effect of pH measured at 25°C and effect of temperature measured at pH 7.5. Activities expressed as percentages of maximum activities observed as a function of pH or temperature.

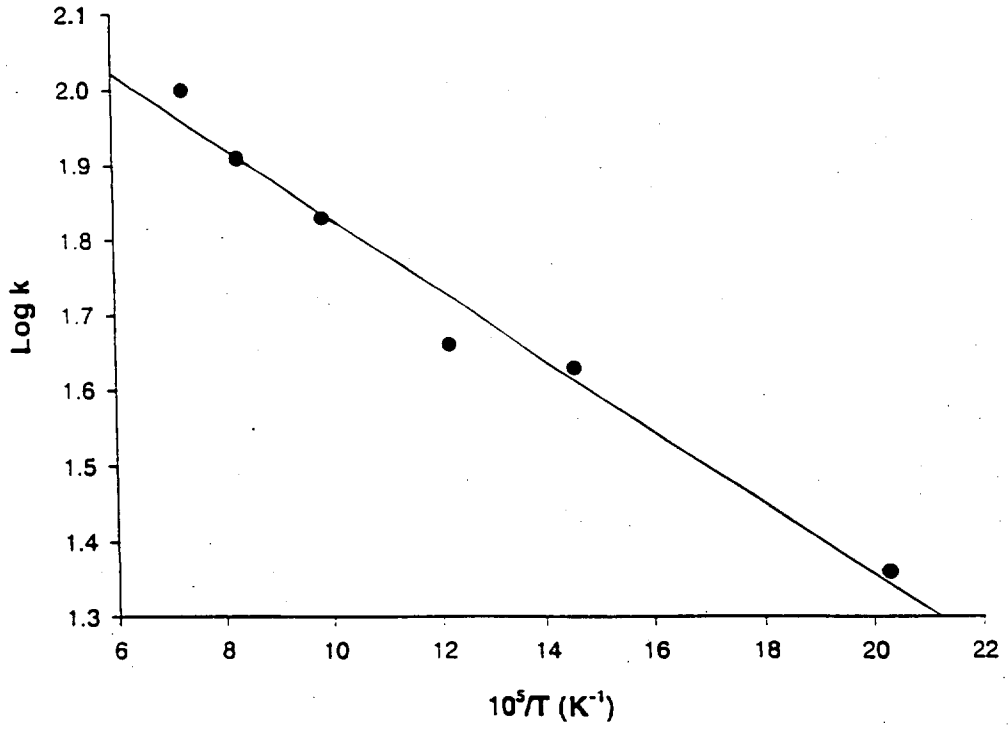


Figure S3 - Effect of Temperature on the Rate of DTNB Reduction by Purified TR Presented as an Arrhenius Plot. Rate of DTNB reduction (log k) by purified TR as a function of the absolute temperature (°K). Range of temperature tested is 18° to 50°C.