Acid-base Catalysis in the Mechanism of Thioredoxin Reductase from *Drosophila melanogaster*

by

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Preface

This dissertation is composed of four chapters. The first chapter contains an introduction to the physiological functions of the thioredoxin (Trx) system, the various intracellular antioxidant systems, and the roles of the Trx system in toxicology. The Trx system is made up of Trx and thioredoxin reductase (TrxR) and comprises the major antioxidant system in dipteran insects such as *Drosophila melanogaster* and *Anopheles gambiae*, a vector of malarial parasites. Because TrxRs from *D. melanogaster* and *A. gambiae* are virtually identical, TrxR from *D. melanogaster* (DmTrxR) offers an excellent model for TrxRs from dipteran insects. The dithiol-disulfide interchange reaction is involved in the catalysis of DmTrxR. A dyad of His-464' and Glu-469' in DmTrxR is proposed to facilitate the formation of thiolate anion to initiate the interchange reaction. Thus, in the first chapter, the potential roles of His-464' and Glu-469' in DmTrxR are also discussed. The second chapter describes studies of the function of His-464' in the acid-base catalysis involved in DmTrxR. The results showed that this histidine residue is crucial to the catalysis of DmTrxR; it acts as the immediate base catalyst to facilitate the formation of thiolate anions and also stabilizes the thiolate anions by ionic interactions. His-464' is involved in both the reductive and oxidative half-reactions. The content of the second chapter is from a manuscript that is in press in *Biochemistry*. The third chapter describes studies of the function of Glu-469' in acid-base catalysis of DmTrxR. The results show that Glu-469' is important but not crucial;

the function of Glu-469’ is to facilitate the proper positioning of His-464’ toward the interchange thiol (Cys-57). The content of the third chapter will form the basis of a second manuscript when two further experiments have been completed. In the fourth chapter, the conclusions of this study and potential future work are addressed.

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List of Abbreviations

AP-1: activator protein 1

ASK-1: apoptotic signal kinase-1

CTC: charge-transfer complex (identified as donor-acceptor)

DmTrxR: thioredoxin reductase from *Drosophila melanogaster*

DTNB: 5,5'-dithiobis-(2-nitrobenzoic acid)

EH_2: 2-electron-reduced enzyme

EH_4: 4-electron reduced enzyme

FAD: flavin adenine dinucleotide

GPx: glutathione peroxidase

GSH: glutathione

GSSG: glutathione disulfide

GR: glutathione reductase

Grx: glutaredoxin

GST: glutathione-S-transferase

HIF-1α: hypoxia-inducible factor 1 α

LBHB: low-barrier hydrogen bond

MDS: mixed disulfide

NF-κB: nuclear factor- κB

PfTrxR: thioredoxin reductase from *Plasmodium falciparum*

Prx: perodiredoxin

Ref-1: redox factor-1

TNB: thionitrobenzoate

TNF: tumor necrosis factor

Trx: thioredoxin

TrxR: thioredoxin reductase

Abstract

Thioredoxin reductase (TrxR) catalyses the reduction of thioredoxin (Trx) by NADPH. Like other members of the pyridine nucleotide-disulfide family, TrxR is a homodimer. The catalytically active unit in the enzyme from *Drosophila melanogaster* (DmTrxR) consists of three redox centers: FAD and an N-terminal Cys-57/Cys-62 redox-active disulfide from one monomer, and a Cys-489'/Cys-490' C-terminal redox-active disulfide from the second monomer. Because dipteran insects such as *D. melanogaster* lack glutathione reductase, glutathione disulfide must be reduced by Trx, making DmTrxR particularly important in this organism. DmTrxR is used as a model for the enzyme from a malaria vector, *Anopheles gambiae*. Based on the structures and mechanisms of other family members, a dyad of His-464' and Glu-469' acts as the acid-base catalyst of the dithiol-disulfide interchange reactions required in the catalysis of DmTrxR.

The functions of His-464' and Glu-469' in the catalytic mechanism of DmTrxR were investigated. His-464' was shown to be critical to catalysis by DmTrxR; thus, H464'Q retains only 2% of the wild-type activity. The pH dependence of V_{max} for wild-type DmTrxR has apparent pK_a values of 6.4 and 9.3, whereas H464'Q DmTrxR has an observable pK_a only at 6.4, indicating that the pK_a at pH 9.3 is contributed by His-464'. The macroscopic pK_a at pH 6.4 has been assigned to Cys-57 and Cys-490'; the thiolate of Cys-57 is the nucleophile in the internal dithiol-disulfide interchange reaction and the

thiolate of Cys-490' is the nucleophile in the reduction of Trx. The rates of both the reductive and oxidative half reactions are markedly smaller in H464'Q DmTrxR than those of wild-type enzyme, indicating that His-464' is involved in both half reactions. The pH dependence of the steady-state kinetics shows that the basicity of His-464' decreases in the glutamate variants, as predicted. The reductive half-reactions of two glutamate variants are slower than those of wild-type enzyme.

Malaria causes serious public health problems in the world. It is hoped that differences among TrxRs from human, *Plasmodium falciparum* (the causative agent) and *Diptera* (the vector) will be useful for developing differential inhibitors, useful as prophylactics.