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Discussion Letter

A comment on the absence of calcium regulation of human thioredoxin reductase

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It has been previously suggested that human thioredoxin reductase activity is regulated by calcium. However, the activity of a purified form of human placental thioredoxin reductase was found to not be affected by mM concentrations of calcium, well above intra- and extracellular physiological levels. Furthermore, the suggestion that an E–F hand is present in *Escherichia coli* thioredoxin reductase is strongly contested. These current results suggest that human thioredoxin reductase is not regulated by calcium.

Flavoenzyme; Human; Calcium; Redox

1. INTRODUCTION

The proteins thioredoxin (Trx) and thioredoxin reductase (TR) comprise a ubiquitous redox system present in both prokaryotic and eukaryotic organisms (for a review, see [1]). Schallreuter and colleagues have reported that purified human TR is regulated in an allosteric manner by Ca^{2+} at physiological levels [2–4]. On this basis, Ca^{2+} fluxes between tumor and surrounding epidermis have been suggested to account for differences in TR activity between primary human melanomas and their surrounding normal skin [5].

The issue of Ca^{2+} regulation of TR activity is an important biochemical point to clarify. The Trx/TR redox system has been recognized to be important in regulating gene expression through modulation of transcription factor activity [6,7]. Furthermore, human Trx has been shown to be identical to the human adult T-cell leukemia-derived factor (ADF) [8,9], an autocrine growth factor produced by leukemic cells [10], and a recombinant form of human Trx has been shown to stimulate cellular proliferation in a redox specific manner [11]. TR is found on the surface of some cancer cells and could be responsible for the reductive activation of extracellular Trx [5]. Thus, Ca^{2+} regulation of TR activity, if it occurs, could have significant consequences for the biological effects of the Trx and TR redox system.

We have recently reported the purification and characterization of human TR from placental tissue [12]. In contrast to the earlier reports, we have now determined that human TR activity is not affected by Ca^{2+} up to mM levels.

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2. RESULTS

TR was purified from human placental tissue and was shown to have the same isoelectric point, size, and K_m values as those reported for rat liver TR [12,13]. TR activity was measured by two separate spectrophotometric assays [13]. The first assay monitored the reduction of DTNB by TR as the change in absorbance at 412 nm over time. The second assay measured the oxidation of NADPH by the change in absorbance at 340 nm during the linked reduction of insulin by TR recombinant human Trx [9,12]. Incubation of human TR with increasing concentrations of Ca^{2+} up to 5 mM for 10 min had no effect on either the rate of DTNB reduction or NADPH oxidation (Fig. 1). Furthermore, incubation of human TR in medium without added Ca^{2+} and 250 μM EGTA had no effect on the rate of DTNB reduction compared to medium with mM concentrations of Ca^{2+} (data not shown).

A Ca^{2+} binding EF-hand domain was speculated to be present in both *E. coli* TR and human TR by Schallreuter and Wood [3,4], based on their identification of a potential domain in *E. coli* TR with suggested homology to one of the four EF-hands in calmodulin. However, upon examination of the speculated domain, it is evident that a critical aspartic acid and glycine residue are missing at positions 3 and 6, and 2 residues at the end of the 12 residue E–F hand loop domain required for chelation of the Ca^{2+} ion are absent [14]. The tertiary structure of *E. coli* TR has been solved recently by X-ray crystallography [15]. The authors of this study did not identify the presence of an EF-hand in *E. coli* TR. Using an updated version of the same computer sequence analysis system used by Schallreuter

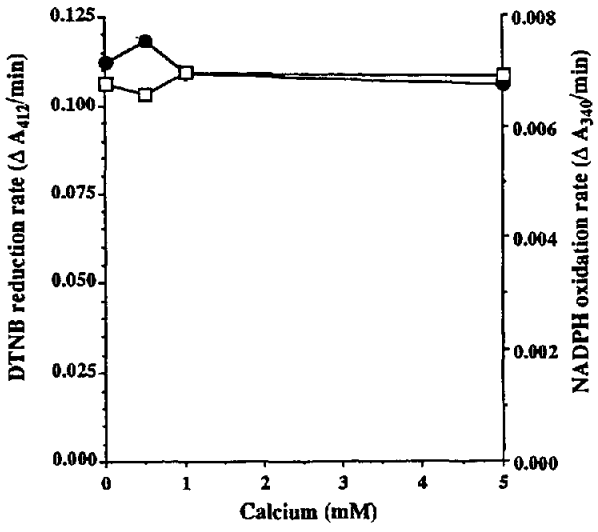

Fig. 1. Effect of Ca^{2+} on human placental thioredoxin reductase activity. Human thioredoxin reductase was purified from placental tissue as previously described (Oblong et al. [12]). Aliquots of thioredoxin reductase were incubated with increasing concentrations of Ca^{2+} for 10 min and then assayed in either the DTNB reduction assay (left axis, closed circles) or insulin reduction assay (right axis, open circles) as previously described [12,13].

and Wood [3] we found there is no EF-hand domain present in *E. coli* TR ('PROSITE' program in the 1991 PC/GENE software package (Version 6.6) from Intelligenetics, Inc., Mountain View, CA). This version of the software package includes identification of different families of calcium-binding proteins from all published sequences to date. The domain in the *E. coli* enzyme suggested by Schallreuter and Wood to be homologous to the EF-hand domain in calmodulin did not reveal the presence of a Ca^{2+}-binding site when analyzed by 'PROSITE'.

3. DISCUSSION

The question of whether Ca^{2+} can regulate human TR activity is of considerable biological significance given the reported growth stimulatory activity of Trx for both normal and cancer cells [10,11]. Growth stimulation by Trx requires the redox activity of Trx [11] and Trx could be reduced by TR that is present on the outside of some cancer cells [5]. Thus, alterations in extracellular Ca^{2+} levels might affect TR activity and, indirectly, the growth stimulation by Trx. Schallreuter and Wood [3] have attempted to explain differences in TR activity of melanoma and normal cells by differences in tissue Ca^{2+} concentrations. However, in our studies we could find no evidence to substantiate the claim that human TR is a Ca^{2+} regulated flavoenzyme by either μM or mM levels of the cation.

The work by Schallreuter and colleagues used a partially purified preparation of TR from human melanoma cells [2,3]. The specificity of this preparation for Trx as a substrate was not reported and the flavin binding properties of the enzyme appear atypical for flavoenzyme reductases. The reported absorbance spectra for the melanoma TR had an absorbance maximum at 450 nm [3]. This is the absorbance maximum of free FAD [16]. The characteristic absorbance maxima of the FAD group in flavoprotein reductases occurs at 464 nm [17,18], as occurs in human placental and rat liver TR [12,13]. We have found, however, that irreversible denaturation of human placental TR results in a shifting of the absorbance maxima from 464 nm to 450 nm [12].

Since we found no Ca^{2+} effect on the activity of purified human TR from placenta and could find no E–F hand motif in the sequence of *E. coli* TR, as claimed, it seems highly unlikely that TR is a Ca^{2+}-regulated enzyme. We suggest that the ambiguity in the literature concerning the Ca^{2+} regulation of TR activity may be due to the purification by Schallreuter and Wood of either an isoform of TR that is less stable than placental TR, or to another reductase.

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