Trypanothione-Dependent Synthesis of Deoxyribonucleotides

by Trypanosoma brucei Ribonucleotide Reductase

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Running title

Trypanosoma brucei Ribonucleotide Reductase
SUMMARY

Trypanosoma brucei, the causative agent of African sleeping sickness, synthesizes deoxyribonucleotides via a classical eukaryotic class I ribonucleotide reductase. The unique thiol metabolism of trypanosomatids in which the nearly ubiquitous glutathione reductase is replaced by a trypanothione reductase prompted us to study the nature of thiols providing reducing equivalents for the parasite synthesis of DNA precursors. Here we show that the dithiol trypanothione [bis(glutathionyl)spermidine] – in contrast to glutathione – is a direct reductant of T. brucei ribonucleotide reductase with a K_m-value of 2 mM. This is the first example of a natural low molecular mass thiol directly delivering reducing equivalents for ribonucleotide reduction. At submillimolar concentrations, the reaction is strongly accelerated by tryparedoxin, a 16 kD parasite protein with WCPPC active site motif. The K_m-value of T. brucei ribonucleotide reductase for T. brucei tryparedoxin is about 4 µM. The disulfide form of trypanothione is a powerful inhibitor of the tryparedoxin-mediated reaction which may represent a physiological regulation of deoxyribonucleotide synthesis by the redox state of the cell. The trypanothione/tryparedoxin system is a new system providing electrons for a class I ribonucleotide reductase, in addition to the well known thioredoxin and glutaredoxin systems described in other organisms.
INTRODUCTION

Ribonucleotide reductases (E.C. 1.17.4.1) catalyze the rate limiting step in the de novo synthesis of DNA precursors and thus are key enzymes for the replication of an organism (1). African trypanosomes possess a typical eukaryotic class I ribonucleotide reductase (2-4). The genes encoding the Trypanosoma brucei R1\(^1\) and R2 proteins have been cloned and overexpressed in Escherichia coli. Class I enzymes are tetrameric proteins composed of two R1 and R2 molecules each. The large R1 protein harbours the active site as well as regulatory sites, the small R2 protein contains a µ-oxo-bridged diiron cluster and a tyrosyl radical essential for catalysis (1, 5).

T. brucei ribonucleotide reductase is regulated via the R2 subunit. Whereas the R1 protein is present throughout the life cycle of the parasite, the R2 protein is not found in cell-cycle arrested short stumpy trypanosomes (6).

Reduction of the 2'-OH group of ribonucleoside diphosphates to the corresponding deoxynucleotides requires external electron donors. For class I enzymes, small thiol proteins with an active site CXXC motif like thioredoxin (CGPC) and glutaredoxin (CPYC) are well known hydrogen donors (7, 8). Oxidized thioredoxin is subsequently reduced by thioredoxin reductase at the expense of NADPH (8). The dithiol form of glutaredoxin is spontaneously regenerated by glutathione. Glutathione disulfide formed in the reaction is then reduced by NADPH and glutathione reductase (9, 10).

Trypanosomatids are the causative agents of tropical diseases such as South American Chagas' disease (Trypanosoma cruzi), African sleeping sickness (T. brucei rhodesiense and T. b. gambiense), Nagana cattle disease (T. congolense and T. b. brucei) and the three manifestations of Leishmaniasis. All these parasitic protozoa have in common that the ubiquitous glutathione/glutathione reductase system is replaced by a trypanothione/trypanothione reductase system. Monogluthathionylspermidine (Gsp) and
trypanothione \([N^1,N^8\text{-bis(glutathionyl)spermidine}; \text{T(SH)}_2]\) are the main low molecular mass thiols and are responsible for the redox balance of the cell (11, 12). These glutathionyl spermidine conjugates are kept reduced by the flavoenzyme trypanothione reductase (TS\(_2 + \) NADPH + H\(^+\) → T(SH)_2 + NADP), an essential enzyme of the parasite (13, 14).

Trypanothione spontaneously reduces dehydroascorbate (15) and hydrogen peroxide (16). The latter reaction is catalyzed by an enzyme cascade composed of trypanothione, trypanothione reductase, tryparedoxin, and a tryparedoxin peroxidase (17, 18). Tryparedoxin is a 16 kD protein with an active site WCPPC motif (19, 20). The gene encoding the \(T. \text{brucei}\) protein has been cloned and overexpressed. Tryparedoxin functions as a trypanothione-dependent thiol-disulfide oxidoreductase with catalytic properties intermediate between those of classical thioredoxins and glutaredoxin (20).

The known dependence of eukaryotic ribonucleotide reductases on external thiols prompted us to study if trypanothione is able to provide the electrons for the parasite synthesis of deoxyribonucleotides. Here we will show that the trypanosomatid specific dithiol trypanothione – in contrast to the monothiol glutathione - is a direct donor of reducing equivalents for \(T. \text{brucei}\) ribonucleotide reductase and that the reaction is catalyzed by tryparedoxin.

EXPERIMENTAL PROCEDURES

Materials – \([^{3}\text{H}]\text{GDP}\) was purchased from Amersham Pharmacia, GDP and dTTP from Sigma, trypanothione disulfide (TS\(_2\)) and glutathionylspermidine disulfide (Gsp\(_{ox}\)) from Bachem, Switzerland, and NaBH\(_4\) from Fluka. All chemicals were of the highest available purity. C\text{18}-Cartridges were obtained from Millipore, the Aminex A9 anion exchange resin was from BioRad. The plasmids encoding \(T. \text{brucei}\) R1 and R2 were kindly provided by Drs. Anders Hofer and Lars Thelander, Umeå, Sweden. Recombinant \(T. \text{brucei}\) tryparedoxin (20),
ribonucleotide reductase (2, 3) and \textit{T. cruzi} trypanothione reductase (21, 22) were purified as described. Alkaline phosphatase from calf intestine was purchased from Roche Molecular Biochemicals. Human glutathione reductase was a kind gift of Dr. R. Heiner Schirmer, Biochemie-Zentrum Heidelberg.

Ribonucleotide Reductase Assay – Ribonucleotide reductase activity was determined from the rate of conversion of $[^3\text{H}]$GDP into $[^3\text{H}]$dGDP essentially as described for CDP reduction (23). The assay mixture contained in a total volume of 200 µl 50 mM Hepes, pH 7.6, 500 µM GDP (including 1.25 µCi $[^3\text{H}]$GDP), 100 µM dTTP, 100 mM KCl, 6.4 mM MgCl$_2$, and variable concentrations of thiols and tryparedoxin. In the standard assay 1 U \textit{T. brucei} R1 (about 40 µg protein $\approx$ 0.4 nmol) with a 5-fold molar excess of R2 was used (1 unit corresponds to 1 nmol dGDP formation/min). The reaction mixture was incubated at 37°C for 10 and 20 min, respectively, the reaction was stopped by boiling for 10 min and the precipitated protein was removed by centrifugation. The reaction components were dephosphorylated by 45 min incubation with 10 U alkaline phosphatase. Guanosine, deoxyguanosine, and guanine were separated isocratically by HPLC on an Aminex A9 column (250 × 4 mm) in 100 mM ammonium borate, pH 8.3, and quantified by scintillation counting (24).

Ribonucleotide Reductase Activity in the Presence of Different Thiols – The thiols were generated \textit{in situ} in the ribonucleotide reductase reaction mixture containing all components except R1, R2, and radiolabeled GDP. Glutathione disulfide was reduced by 200 mU human glutathione reductase, glutathionylspermidine disulfide and trypanothione disulfide by 200 mU \textit{T. cruzi} TR in the presence of a 2.5-fold molar excess of NADPH. The mixture was incubated for 15 min at 37°C and the ribonucleotide reductase reaction was started by adding R1, R2, and $[^3\text{H}]$GDP.
Chemical Reduction of Trypanothione Disulfide – 10 mM Trypanothione disulfide in 1 ml water was incubated on ice with 100 mM NaBH₄ for 1 h. The solution was acidified with 1 M HCl to pH 3.0 in order to prevent reoxidation of the thiol after decomposition of excess hydrid. A C₁₈-cartridge was washed with 4 ml acetonitrile followed by 10 ml water. The reaction mixture was applied and the cartridge was washed with 3 ml 0.1% trifluoroacetic acid (TFA). Trypanothione was eluted with 1.5 ml 80% acetonitrile in 0.1% TFA, lyophilized, dissolved in 50 mM Hepes, pH 7.6 to a final concentration of 25 mM and immediately used. The concentration of free thiols was determined by reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, 25).

Carboxamidomethylation of Tryparedoxin – In a final volume of 100 µl 50 µM tryparedoxin were incubated with 1 mM T(SH)₂ in 50 mM Hepes, pH 7.6 for 15 min under argon atmosphere. 3 µl 200 mM Iodoacetamide in water were added. After 60 min incubation at room temperature in the dark, the reaction was stopped by adding 15 µl 200 mM DTE in water. A control reaction contained tryparedoxin and iodoacetamide but no T(SH)₂ and was not stopped by DTE. The low molecular mass components were removed by centrifugation in a Centricon 3 concentrator (Millipore) and the modified protein was washed several times with 50 mM Hepes, pH 7.6. This procedure resulted in a homogeneous protein sample (see below) which represents tryparedoxin specifically modified at the first cysteine residue (Cys40) of the WCPPC motif as described for C. fasciculata tryparedoxin (19). For the alkylation of both active site cysteinyl residues, the reaction was carried out in the presence of 6 M guanidinium chloride.

Oxidized, reduced, mono-, and bis-carboxamidomethylated tryparedoxin were separated by HPLC on a VYDAAC 208 TP column at a flow rate of 0.2 ml/min by a linear gradient from 38.5% to 45.5% acetonitrile in 0.1% TFA within 1 h. The proteins were detected at 214 nm. The content of free SH-groups was determined with DTNB (25). The thiol content of
unmodified tryparedoxin was determined after reduction with NaBH₄ at room temperature for 5 min. HCl was added to destroy excess NaBH₄ and an aliquot of the sample was immediately analyzed for free SH-groups.

RESULTS

Different Thiols as Hydrogen Donors of T. brucei Ribonucleotide Reductase – Formation of [³H]dGDP from [³H]GDP by T. brucei ribonucleotide reductase was followed in the presence of trypanothione, glutathionylspermidine, glutathione and the non-physiological dithiol DTE. T(SH)₂, Gsp, and GSH were generated and kept reduced by NADPH/TR and NADPH/glutathione reductase, respectively. Control assays revealed that the activity of ribonucleotide reductase is only slightly affected by millimolar concentrations of NADPH and NADP (data not shown).

(please insert Figure 1 here)

Trypanothione is an efficient reductant of the parasite enzyme. At a fixed concentration of 2 mM thiol groups, the activity of ribonucleotide reductase with trypanothione amounts to 30% of that observed with DTE (Fig. 1, light grey columns). In contrast, the monothiol Gsp showed very low activity and GSH was completely inactive at this concentration. The Kₘ-values of T. brucei ribonucleotide reductase for T(SH)₂ and DTE were determined by varying the concentration of the dithiol from 0.5 to 4 mM and 2 to 12 mM, respectively. The reactions followed Michaelis-Menten kinetics and yielded Kₘ-values of 2.1 ± 0.4 mM for T(SH)₂ and 6.9 ± 1.2 mM for DTE (Table 1). Since the external electron donors for ribonucleotide reductase interact with the R1 protein (26, 27) the assays contained a molar excess of R2 and the specific activity refers to the amount of R1 protein. The maximum reaction rates were calculated by extrapolating to saturating concentrations of dithiol. The Vₘₐₓ-value of ribonucleotide reductase with DTE was about sixfold higher than that with T(SH)₂ (Table 1).
Effect of Tryparedoxin on Ribonucleotide Reductase Activity – The activity of T. brucei ribonucleotide reductase with DTE, T(SH)₂, Gsp, and GSH was followed in the absence and presence of T. brucei tryparedoxin. The protein stimulated the rate of dGDP formation in the presence of all four thiols. At 1 mM low molecular mass dithiol, the trypanothione/tryparedoxin couple yielded about 50% activity compared to DTE/tryparedoxin. With tryparedoxin in the reaction mixture, also the monothiols glutathionylspermidine and glutathione caused a pronounced dGDP formation (Fig. 1, dark grey columns). When the ribonucleotide reductase activities in the presence of thiol and tryparedoxin were corrected for the respective activity with the thiol alone, DTE (0.185 nmol/min), trypanothione (0.21 nmol/min) and glutathionylspermidine (0.205 nmol/min) resulted in nearly identical rates of GDP reduction. Only with GSH (0.1 nmol/min) the activity of ribonucleotide reductase was significantly lower in accordance with a weak reduction of tryparedoxin by GSH (20). As shown in Fig. 1, at millimolar concentrations, trypanothione is an efficient direct hydrogen donor for ribonucleotide reductase. At lower T(SH)₂ concentrations, stimulation of the reaction by tryparedoxin becomes pronounced. For instance, at 100 µM trypanothione, 4 µM tryparedoxin increased dGDP formation by a factor of 14 (data not shown).

The Kₘ-value of T. brucei ribonucleotide reductase for tryparedoxin was determined in the presence of a constant concentration of 2.5 mM T(SH)₂. The dependence of the reaction rate on the tryparedoxin concentration showed saturation kinetics and yielded an apparent Kₘ-value of 3.7 ± 0.5 µM (Fig. 2).

Alkylation of Tryparedoxin Abolishes Reduction of Ribonucleotide Reductase – Tryparedoxin was carboxamidomethylated with iodoacetamide under non-denaturing conditions which, in analogy to E. coli thioredoxin (28) and C. fasciculata tryparedoxin (19), should result in the

(insert Table 1 here)
exclusive modification of Cys40, the first cysteine residue of the WCPPC motif. Analysis of
the modified protein with Ellman’s reagent yielded a total of 5 nmol thiol groups per 6 nmol
protein whereas the control contained 8.6 nmol free thiol groups per 5.6 nmol protein. The
relatively low thiol content of the control sample may be due to formation of covalent dimers
which were observed when storing the NaBH4-reduced protein at pH 7.6 (not shown). HPLC
analysis of the carboxamidomethylated protein revealed a single peak in accordance with the
specific modification of one cysteine residue. As expected, the monoalkylated tryparedoxin
did not catalyze the trypanothione dependent reduction of GDP by ribonucleotide reductase
(Table 2).

(insert Table 2 here)

The Activity of Tryparedoxin is Inhibited by Trypanothione Disulfide – The effect of
trypanothione disulfide, TS₂, on the activity of T. brucei ribonucleotide reductase was studied
in the reaction with trypanothione as sole reductant and in the trypanothione/tryparedoxin
system (Fig. 3). Trypanothione disulfide showed only a minor effect on the activity of
ribonucleotide reductase. At 1 mM trypanothione, 2.5 mM trypanothione disulfide diminished
the rate of GDP reduction by about 40% (Fig. 3a). In contrast, the tryparedoxin-mediated
reaction proved to be much more sensitive. 2.5 mM TS₂ in the presence of 1 mM T(SH)₂ and
4 µM tryparedoxin inhibited the rate of deoxyribonucleotide formation by 90% (Fig. 3b). The
residual activity of ribonucleotide reductase was identical with that observed with
trypanothione alone indicating that it is tryparedoxin and not ribonucleotide reductase which
is strongly regulated by the thiol/disulfide ratio of trypanothione. The IC₅₀-value of
tryparedoxin for trypanothione disulfide is about 50 µM in the presence of 1 mM T(SH)₂.

(insert Figure 3 here)

The pronounced sensitivity of tryparedoxin towards trypanothione disulfide became also
evident when NADPH and trypanothione reductase were added to the assays (second column
in Figs. 3a and b). In the trypanothione/ribonucleotide reductase assay the rate of dGDP formation increased by only 10% whereas in the trypanothione/tryparedoxin/ribonucleotide reductase system the activity was doubled. The sample of trypanothione used in these experiments contained about 4% disulfide as revealed by an end point determination in a trypanothione reductase assay. This corresponds to a concentration of 40 µM TS₂ at the beginning, in addition to trypanothione disulfide formed during the reaction, and explains the pronounced effect of trypanothione reductase/NADPH.

DISCUSSION
The discovery of the trypanothione system in Kinetoplastida raised the question as to the specific functions of the dithiol. The pivotal role of trypanothione in the antioxidant defense mechanisms of the parasites is well established (11, 13-17). As shown here, trypanothione is also involved in the parasite synthesis of DNA precursors (Fig. 4). The dithiol serves as direct donor of reducing equivalents for *T. brucei* ribonucleotide reductase. In contrast, mono-glutathionylspermidine and glutathione result in a very low and no activity, respectively, in accordance with other ribonucleotide reductases where DTE and lipoate are hydrogen donors whereas monothiols are inactive (29). The ability of trypanothione – but not of glutathione - to reduce ribonucleotide reductase directly is not related to the redox potentials of the thiols that are very similar (−242 and −230 mV for T(SH)₂ and GSH, respectively; 11). In contrast, the pK-values of the thiols differ significantly. A pK-value of 7.4 has been reported for trypanothione which is more than one pH unit lower than the pK 8.7 of GSH (30). Since second order rate constants for thiol-disulfide exchanges exhibit an optimum when the thiol pK is equal to the pH of the solution, T(SH)₂ is expected to be much more reactive than GSH under physiological conditions. In addition, as reductants for intramolecular disulfides as in the R1 protein, dithiols are kinetically superior to monothiols (31). Trypanothione is the first
example of a natural low molecular mass dithiol that is a direct reductant of ribonucleotide reductase.

*(insert Figure 4 here)*

The trypanothione-dependent synthesis of deoxyribonucleotides by *T. brucei* ribonucleotide reductase is catalyzed by *T. brucei* tryparedoxin. This thioredoxin-like protein has been found exclusively in trypanosomatids and its first elucidated role was as component of a trypanothione-dependent peroxidase cascade (17-19). The apparent $K_m$-value of *T. brucei* ribonucleotide reductase for *T. brucei* tryparedoxin (3.7 µM) is higher than those of *E. coli* ribonucleotide reductase for glutaredoxins 1 and 3 (0.13 and 0.35 µM, respectively) (32). It is comparable to that for thioredoxin (1.3 µM) in the *E. coli* system (10). The maximum activity of recombinant *T. brucei* ribonucleotide reductase in the trypanothione/tryparedoxin system is 24 nmol/min•mg R1. This value is in the same order of magnitude as the varying activities reported for the *E. coli* enzyme with thioredoxins 1 or 2 and glutaredoxin 1 (10, 33) and thus significantly higher than that of *E. coli* ribonucleotide reductase with glutaredoxin 3 as hydrogen donor where the $V_{max}$ is only 5% that of glutaredoxin 1 (32).

It is not possible to determine the $K_m$-value of tryparedoxin for T(SH)$_2$ using formation of dGDP as indicator reaction. The reaction catalyzed by ribonucleotide reductase is three orders of magnitude slower than the preceding reduction of the enzyme by tryparedoxin when assuming that the latter reaction occurs at a rate similar to the reduction of tryparedoxin peroxidase by tryparedoxin (17, 34). $K_m$-values of different tryparedoxins for T(SH)$_2$ between 30 and 150 µM have been estimated using the tryparedoxin peroxidase/hydroperoxide system or GSSG as final electron acceptor (19, 20, 35). The trypanothione concentration in *T. brucei* is 400-800 µM (11) which should be adequate to keep tryparedoxin predominantly in the reduced state. In addition, tryparedoxin is a very abundant protein. In the insect parasite *C. fasciculata*, it represents 5% of the total soluble protein of the cell (17). Taken together, these
data indicate that reduction of ribonucleotide reductase by the trypanothione/tryparedoxin system is not a limiting factor in the parasite synthesis of deoxyribonucleotides. The tryparedoxin-mediated activity of ribonucleotide reductase was highest with DTE and trypanothione, but in the presence of tryparedoxin the monothiols Gsp and GSH – which fail as direct hydrogen donors - also yielded a significant dGDP formation. The increase of ribonucleotide reductase activity caused by tryparedoxin was comparable for trypanothione and mono-glutathionyl spermidine but much lower with GSH as hydrogen donor. Since the glutathionyl spermidine conjugates are the main low molecular mass thiols in the parasites (11) they are most probably the physiological electron donors in the parasite synthesis of DNA precursors.

Trypanothione disulfide proved to be a powerful inhibitor of the tryparedoxin-mediated ribonucleotide reduction. At a T(SH)2/TS2 ratio of 10:1, the activity of T. brucei ribonucleotide reductase is lowered by more than 60%. Inhibition of tryparedoxin by TS2 may be a physiological control mechanism. For E. coli glutaredoxin a respective observation has been made. Glutaredoxin is strongly inhibited in the presence of GSSG (10) indicating a relationship between the rate of DNA synthesis and the redox state of the cell.

In the reaction with ribonucleotide reductase, tryparedoxin resembles mechanistically glutaredoxin. The ultimate reductant is a low molecular mass thiol and the reaction is inhibited by the disulfide form of the respective thiol (10). In contrast, with respect to the protein sequence as well as thiol-disulfide exchange reactions tryparedoxin is more similar to thioredoxins (20). Obviously, the parasite dithiol protein has properties intermediate between those of classical thioredoxins and glutaredoxins.

In all organisms with class I ribonucleotide reductases investigated so far different hydrogen donor systems occur simultaneously. For instance, E. coli contains two thioredoxins and two glutaredoxins that are able to deliver electrons for ribonucleotide reductase (32, 33). Recently
we have cloned the gene encoding a classical thioredoxin from *T. brucei* (36). The recombinant protein is also a hydrogen donor for the trypanosomal ribonucleotide reductase when applied together with DTE or NADPH and human thioredoxin reductase2. The thioredoxin gene is expressed throughout the life cycle of *T. brucei* but the protein concentration in the parasites is unusually low3. Therefore the trypanothione/tryparedoxin system described here is supposed to be the main donor of reducing equivalents for the parasite synthesis of deoxyribonucleotides.

REFERENCES


ACKNOWLEDGMENTS

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FOOTNOTES

1The abbreviations used are: DTE, dithioerythritol; DTNB, 5,5'-dithiobis(2-nitrobenzoate); GSH, glutathione; GSSG, glutathione disulfide (oxidized glutathione); Gsp, (mono)glutathionylspermidine; R1 and R2, large and small subunit, respectively of
ribonucleotide reductase; TFA, trifluoroacetic acid; T(SH)$_2$, trypanothione [N$^{1,11}$-bis(glutathionyl)spermidine]; TS$_2$, trypanothione disulfide (oxidized trypanothione); T. brucei, *Trypanosoma brucei brucei*.


$^3$A. Schmidt, and R. L. Krauth-Siegel, unpublished observations.
Figure Legends

Fig. 1. **Activity of *T. brucei* ribonucleotide reductase in the presence of different thiols and tryparedoxin.** Formation of dGDP was followed as described under “Experimental Procedures”. The assay mixtures contained 1 mM DTE, 1 mM T(SH)₂, 2 mM Gsp red, and 2 mM GSH, respectively (light grey columns) and in addition, 4 µM *T. brucei* tryparedoxin (dark grey columns). The reactions were started by adding 62 µg R1 and 92 µg R2 protein. The data represent a typical experiment out of five, the values of which differed by less than 10 %.

Fig. 2. **Determination of the Kₘ-value of *T. brucei* ribonucleotide reductase for tryparedoxin.** A constant concentration of 2.5 mM T(SH)₂ was generated enzymatically by NADPH and trypanothione reductase. The concentration of tryparedoxin was varied between 1 and 20 µM. Kₘ- and Vₘₐₓ-values were derived from the Lineweaver-Burk plot (inset). The assays were performed as described under Experimental Procedures. The activities were corrected for the spontaneous rate observed at 2.5 mM trypanothione. The data represent a typical experiment out of 4 which differed by less than 15 %.

Fig. 3. **Influence of TS₂ on a) the trypanothione- b) the T(SH)₂/tryparedoxin-mediated synthesis of dGDP by *T. brucei* ribonucleotide reductase.** The assays were performed as described under Experimental Procedures. All reaction mixtures contained 1 mM trypanothione, 40 µg R1 and 60 µg R2 protein and variable concentration of TS₂ as indicated. Columns marked by TR/NADPH represent assays which contained also 100 µM NADPH and 100 mU trypanothione reductase. A typical experiment out of 3 is given which differed by less than 10 %.
Fig. 4. Scheme for the two trypanothione-dependent reactions of *T. brucei* ribonucleotide reductase. Left. Spontaneous reduction of ribonucleotide reductase by trypanothione. Right. Tryparedoxin mediated reduction of ribonucleotide reductase by trypanothione. TR, trypanothione reductase.
Table I

Kinetic parameters of T. brucei ribonucleotide reductase

The assays were carried out as described under Experimental Procedures and the kinetic data were derived from Lineweaver Burk plots. Mean values of at least three independent measurements together with the standard deviations are given. aThe assays contained a constant concentration of 2.5 mM trypanothione.

<table>
<thead>
<tr>
<th></th>
<th>Kₘ (µM)</th>
<th>Vₘₐₓ (nmol/min·mg)</th>
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<tbody>
<tr>
<td>DTE</td>
<td>6900 ± 1200</td>
<td>60</td>
</tr>
<tr>
<td>T(SH)₂</td>
<td>2100 ± 400</td>
<td>9.6</td>
</tr>
<tr>
<td>Tryparedoxina</td>
<td>3.7 ± 0.5</td>
<td>24</td>
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Table II

Effect of carboxamidomethylation of T. brucei tryparedoxin on ribonucleotide reductase activity

All reaction mixtures contained 250 µM trypanothione. The tryparedoxin concentration was 4 µM. The assays were performed as described under Experimental Procedures.

<table>
<thead>
<tr>
<th>Ribonucleotide reductase (nmol dGDP/ min)</th>
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<tr>
<td>T(SH)₂                                  0.04</td>
</tr>
<tr>
<td>T(SH)₂ + tryparedoxin                   0.33</td>
</tr>
<tr>
<td>T(SH)₂ + monoalkylated tryparedoxin     0.06</td>
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</tbody>
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nmol dGDP/ min

- DTE
- Trypanothione
- Glutathionyl-spermidine
- Glutathione
Figure 2

The graph illustrates the relationship between Tryparedoxin concentration (µM) and the rate of dGDP production (nmol/min). The x-axis represents the concentration of Tryparedoxin in µM, ranging from 0 to 20. The y-axis shows the rate of dGDP production in nmol/min, ranging from 0 to 0.3. The data points are plotted on both axes, with an additional inset graph that demonstrates a linear relationship between the reciprocal of the concentration of Tryparedoxin and the reciprocal of the rate of dGDP production (1/v).
Trypanothione-dependent synthesis of deoxyribonucleotides by trypanosoma brucei ribonucleotide reductase
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