Unexpected properties of NADP-dependent secondary alcohol dehydrogenase (ADH-1) in *Trichomonas vaginalis* and other microaerophilic parasites

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**Highlights**

- *Trichomonas vaginalis* NADPH-dependent alcohol dehydrogenase-1 (ADH-1) reduces acetaldehyde and acetone, and oxidizes 2-propanol.
- In addition to its canonical function, a strong reducing background activity was observed.
- All reactions catalyzed by ADH-1 are strongly inhibited by CoA.
- These observations also apply for the parasites *Entamoeba histolytica* and *Tritrichomonas foetus*, but not for *Giardia lamblia* which lacks ADH-1.

**Abstract**

Our previous observation that NADP-dependent secondary alcohol dehydrogenase (ADH-1) is down-regulated in metronidazole-resistant *Trichomonas vaginalis* isolates prompted us to further characterise the enzyme. In addition to its canonical enzyme activity as a secondary alcohol dehydrogenase, a pronounced, so far unknown, background NADPH-oxidising activity in absence of any added substrate was observed when the recombinant enzyme or *T. vaginalis* extract were used. This activity was strongly enhanced at low oxygen concentrations. Unexpectedly, all functions of ADH-1 were efficiently inhibited by coenzyme A which is a cofactor of a number of key enzymes in *T. vaginalis* metabolism, i.e. pyruvate:ferredoxin oxidoreductase (PFOR). These observations could be extended to *Entamoeba histolytica* and *Tritrichomonas foetus*, both of which have a homologue of ADH-1, but not to *Giardia lamblia* which lacks an NADP-dependent secondary alcohol dehydrogenase.

Although we could not identify the substrate of the observed background activity, we propose that ADH-1 functions as a major sink for NADPH in microaerophilic parasites at low oxygen tension.

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**1. Introduction**

*Trichomonas vaginalis* is a world-wide occurring microaerophilic human parasite that causes vaginitis in women and urethritis in men (*Nanda et al., 2006*). Like other microaerophilic parasites, such as *Entamoeba histolytica* and *Giardia lamblia*, *T. vaginalis* is incapable of oxidative phosphorylation but generates ATP by substrate level phosphorylation. In laboratory culture, the main energy source of *T. vaginalis* is glucose and the major metabolic end products are lactate, acetate, hydrogen and glycerol (*Chapman et al., 1985; Paget and Lloyd, 1990; Kulda, 1999*). A key intermediate of *T. vaginalis* metabolism is pyruvate:ferredoxin oxidoreductase (PFOR). These observations could be extended to *Entamoeba histolytica* and *Tritrichomonas foetus*, both of which have a homologue of ADH-1, but not to *Giardia lamblia* which lacks an NADP-dependent secondary alcohol dehydrogenase.
is decarboxylated to acetyl-CoA by pyruvate:ferredoxin oxidoreductase (PFOR) which uses ferredoxin as oxidant cofactor. Reduced ferredoxin, in turn, transfers electrons to hydrogenase resulting in the formation of hydrogen. In contrast to *E. histolytica* (Clark et al., 2007), *T. vaginalis* does not have a bifunctional alcohol/aldehyde dehydrogenase, such as alcohol dehydrogenase 2 in *E. histolytica*, which generates ethanol through reduction of acetyl-CoA. Instead, coenzyme A is recycled via succinyl-CoA synthetase and acetate:succinate CoA-transferase (ASCT) (Lindmark and Müller, 1973; van Grinsven et al., 2008), thereby releasing acetate which is not further utilised.

Nevertheless, ethanol is a metabolic end product of *T. vaginalis* metabolism (Ellis et al., 1992), albeit of comparably minor importance. Arguably, the most probable source of ethanol in *T. vaginalis* is acetaldehyde which is reduced by NADP-dependent alcohol dehydrogenase, also termed alcohol dehydrogenase 1 (ADH-1) (Leitsch et al., 2012). It was hypothesised that acetaldehyde is formed by PFOR as a side product under anaerobic condition (Leitsch et al., 2012) as described for PFOR from *Pyrococcus furiosus* (Ma et al., 1997). In accordance with this hypothesis, ethanol is only formed under anaerobic condition (Ellis et al., 1992). Further, expression of this enzyme is down-regulated in metabolic conditions as described before (Wassmann et al., 1999) by monitoring oxidase activity. Reduction of cytochrome c was followed at λ = 550 nm. Hydrogen peroxide formation was measured as described before (Wassmann et al., 1999) by monitoring oxidation of ferrous to ferric iron via formation of pentaaqua(thiocyanato-N)iron(III) complexes at λ = 450 nm after incubation of reactions for 5 min.

The conspicuously high expression of ADH-1 in *T. vaginalis*, and the potential link of ethanol production and metronidazole resistance, prompted us to further examine the function of this enzyme. To this end, ADH-1 was recombinantly expressed in *Escherichia coli* and used for biochemical characterisation, including determination of substrate specificity and kinetic parameters, and identification of inhibitors.

## 2. Materials and methods

### 2.1. Strains and cell culture

The *T. vaginalis* strains used were described previously (Leitsch et al., 2012). *Trichomonas foetus* K1 (ATCC 30924) was obtained from Caroline Frey (University of Berne, Switzerland) and *G. lamblia* WB clone C6 (ATCC 50803) was obtained from Norbert Müller (University of Berne, Switzerland). *E. histolytica* HM-1:IMSS (ATCC30459) had been in our possession before start of this study. *T. vaginalis* was described (Leitsch et al., 2012) in trypticase, yeast extract, maltose (TYM) medium (Diamond, 1957). *T. foetus* and *E. histolytica* were grown in TVY-S-33 medium (Diamond et al., 1978), and *G. lamblia* was grown in Keister's modified TVY-S-33 medium (Keister, 1983). Cultures were routinely grown in 40 ml culture polystyrene flasks (BD Biosciences). Trypticase was used for biochemical characterisation, including determination of substrate specificity and kinetic parameters, and identification of inhibitors.

### 2.2. Recombinant expression of ADH-1

The *adh-1* gene (XM_001580551) was amplified from genomic DNA of *T. vaginalis* strain G3 using the primers 5′-TAC GTA CGC ATA TGA CAT TCG AAC TTC CAA AG-3′ (forward) and 5′-TCA TCC AGG GAT CCT TAG TGA TGG TGA TGG AAC TTG TCG TTG TAT TCG ATG-3′ (reverse). PCR fragments were ligated into pET-17b (Novagen) via Ndel (forward primer) and BamHI (reverse primer) restriction sites. Expression of recombinant ADH-1 was performed in *E. coli* BL21-AI (Invitrogen) according to the manufacturer's protocol. The reverse primer encodes a hexahistidine tag for isolation on Ni-NTA spin columns (Qiagen).

### 2.3. Measurements of *T. vaginalis* ADH-1 activity with the purified recombinant enzyme

If not indicated otherwise, reduction of acetone and acetaldehyde was measured at 37°C in 100 mM potassium phosphate buffer pH 6.25, 200 μM NADPH, and 1 μg ml⁻¹ recombinant ADH-1 by determining oxidation of NADPH at λ = 340 nm (ΔA₃₄₀ = 6.2 mM⁻¹ cm⁻¹). Oxidation of 2-propanol was measured in the same way but at pH 6.75 and by replacing NADPH with 1 mM NADP⁺. For inhibition experiments appropriate amounts of coenzyme A, acetyl-coenzyme A, cysteine, or dithiothreitol (DTT) were added to reactions. All measurements were done in a Perkin Elmer Lambda 25 UV/VIS spectrophotometer, additionally equipped with a PTP A Peltier system. Kinetic parameters of ADH-1 and inhibitory concentrations of coenzyme A (CoA) and 2-propanol on acetaldehyde reduction or on enzyme activity in absence of added substrate were calculated using GraFit 7 software (Erithacus). NADPH, CoA, acetyl-CoA, DTT, cysteine, and acetaldehyde were obtained from Sigma–Aldrich. 2-propanol was obtained from Merck Chemicals.

For assessing ADH-1 potential role as an oxygen scavenging enzyme, following assays were performed: cytochrome c (50 μM) was added to reactions in order to visualise superoxide anion radical formation. Reduction of cytochrome c was followed at λ = 550 nm. Hydrogen peroxide formation was measured as described before (Wassmann et al., 1999) by monitoring oxidation of ferrous to ferric iron via formation of pentaaqua(thiocyanato-N)iron(III) complexes at λ = 450 nm after incubation of reactions for 5 min.

### 2.3.1. ¹H NMR and ¹³C analysis of product formation from NADPH oxidation by ADH-1

Phosphate buffer NaH₂PO₄–Na₂HPO₄ (0.1 M, pH 6.2) in D₂O (Sigma) was used as a solvent for NMR analysis. ADH-1 (40 μg ml⁻¹) and NADPH (5 mg ml⁻¹, equalling 6.7 mM) were added to 1 ml of D₂O buffer in 5 mm glass NMR tubes (Wilmad), along with a selection of other substrates including: sodium bicarbonate (Sigma), acetone (Fisher) or formaldehyde (Fisher) at concentrations of 100, 50 and 1 mM respectively. ¹³C and ¹H NMR spectra were recorded at 250 MHz (¹H) and 62 MHz (¹³C) on a Bruker Avance 250 MHz spectrometer equipped with a 5 mm ¹H/¹³C probe and operating at a temperature of 298 K. Spectra were acquired with 16 pulses (¹H) and 256 pulses (¹³C) over a spectral width of 14 ppm (¹H) and 235 ppm (¹³C). An acquisition time of 2 s and 0.8 s and a relaxation delay of 1.5 s and 2 s for ¹H and ¹³C spectra respectively, were employed. The solvent D₂O was used as an internal lock and chemical shifts are expressed as parts per million (ppm). Compounds were identified by comparison with spectra of standard solutions.

### 2.4. Mass spectrometric analysis

Membrane-inlet mass spectrometry for the detection of H₂ was performed as previously described (Millet et al., 2010).

### 2.5. Measurements of ADH-1 activity in cell extracts

Fully grown parasite cultures (40 ml) were harvested by centrifugation at 800g for 5 min. Pellets were washed once in 20 ml 1× PBS followed by another round of centrifugation at 800g for 5 min. Cell pellets were resuspended in 500 μl of 100 mM potassium phosphate buffer pH 6.25 and lysed with 25 strokes in a Dounce homogeniser. Cell debris and large organelles were removed by centrifugation at 20,000g for 10 min. Protein concentrations of lysates were measured using Bradford assay. Enzyme activity was measured in 100 mM potassium phosphate pH 6.25.
after adding cell extract (10 μg protein/ml assay buffer) and 200 μM NADPH, as oxidation of NADPH at λ = 340 nm (ΔAbs = 6.2 - mM⁻¹ cm⁻¹) either without added substrate or with 1 mM acetaldehyde. Inhibition experiments were performed by adding 100 μM coenzyme A or 200 mM 2-propanol. Oxygen-depleted enzyme buffer was obtained by exposition to an anaerobic atmosphere (i.e. oxygen undetectable and 18% CO₂ according to the manufacturer’s specifications) as generated by Anaerocult A (Merck Chemicals) in an anaerobic jar (Merck Chemicals). If not indicated otherwise, all measurements were repeated at least twice.

3. Results

3.1. Enzymatic parameters of ADH-1

The T. vaginalis adh-1 gene (XM_001580551) was amplified by PCR from genomic DNA and cloned into a pET17-b expression vector. Recombinant ADH-1 was expressed and used for the determination of the enzymatic parameters with the substrates acetaldehyde, acetone, and 2-propanol (Table 1). Potassium phosphate buffer, pH 6.25, was found to be optimal for reduction of acetaldehyde and acetone. Oxidation of 2-propanol was slightly faster at pH 6.75. The enzyme showed a marked preference for the reduction of acetaldehyde and acetone as compared to oxidation of 2-propanol. The Km for acetone was found to be several-fold lower than that for acetaldehyde (Table 1). Very much to our surprise, however, we noticed strong oxidation of NADPH even in the absence of any added substrate. This background activity represented a substantial proportion of the maximal enzyme activities measured (3.4 μmol min⁻¹ mg⁻¹) and was not due to spontaneous oxidation of NADPH in the enzyme buffer, as determined by measuring oxidation of NADPH in the absence of enzyme. A Coomassie-stained gel from the enzyme preparation (Supplementary Fig. 1) showed only very minor impurities of possibly bacterial origin. Assays were repeated using ultrapure water from two other suppliers (Sigma, Merck Chemicals) but the background activity remained. Also different buffers, including 100 mM Tris pH 7.5 and 100 mM KCl, and ultrapure water alone were tested, giving similar results (but far lower enzyme activities as such). Further, NADPH batches from two different sources (Sigma, AppliChem) gave identical results. Consequently, the Vmax and Km as determined for acetaldehyde and acetone were corrected by subtracting background oxidation of NADPH (Table 1).

3.2. Inhibition of ADH-1 by 2-propanol and background NADPH-oxidising activity can also be detected in T. vaginalis cell extracts

Previously (Leitsch et al., 2012) we had determined acetaldehyde reduction rates in cell extracts of four metronidazole-sensitive and five metronidazole-resistant T. vaginalis isolates. We wanted to assess whether the enzyme in T. vaginalis cell extracts showed similar characteristics as the recombinant enzyme and added 2-propanol to the reactions. Indeed, 200 mM 2-propanol inhibited reduction of 1 mM acetaldehyde by T. vaginalis G3 extract by 82% (Table 2). In contrast to our observations with the recombinant enzyme, however, the NADPH-oxidising background activity was not detectable in G3 cell extract in this experimental setup. This changed when the enzyme buffer was exposed to an anaerobic atmosphere overnight, as background activity was now also readily measurable using T. vaginalis cell extract (Fig. 1). When we measured background activities in cell extracts of all nine strains, we observed that, in almost perfect correlation to previous measurements in normal buffer and with 1 mM acetaldehyde, metronidazole-resistant isolates tended to have lower background activities (Fig. 1). Moreover, background NADPH oxidation rates nearly matched acetaldehyde reduction rates as published recently (Leitsch et al., 2012). One of the metronidazole-resistant strains, LA1, had been previously found to display low acetaldehyde reduction activity due to an intracellular lack of zinc, which is a cofactor of ADH-1. Accordingly, addition of 0.5 mM ZnCl₂ to the reactions increased background NADPH-oxidising activity in LA1 cell extract from 258 to 735 nmol min⁻¹ mg⁻¹. Addition of ZnCl₂ to cell extracts of other strains only had a minimal effect, if any, on background NADPH-oxidising activity (data not shown). As determined with G3 cell extract, addition of 200 mM 2-propanol resulted in a decrease of background activity of roughly 66% (Table 2), which mirrors the result previously obtained with recombinant ADH-1.

3.3. ADH-1 is efficiently inhibited by coenzyme A

In our previous publication on ADH-1 in T. vaginalis (Leitsch et al., 2012) we had speculated that the enzyme might have a role in scavenging acetaldehyde which can be a by-product of pyruvate-ferredoxin oxidoreductase (PFOR) under strictly anaerobic condition (Ma et al., 1997). We planned to determine whether T. vaginalis PFOR produces acetaldehyde and wanted to couple a PFOR assay with measurements of NADPH-oxidation in the presence of ADH-1. However, when we conducted preliminary experiments in order to check whether components of the PFOR assay buffer inhibit ADH-1, we identified CoA, an essential cofactor of PFOR, as a potent inhibitor of ADH-1 (Table 3). Both, acetaldehyde reduction and background NADPH-oxidation were strongly inhibited by CoA when applied in concentrations of 60–100 μM. Inhibi-

Table 1
Kinetic parameters of ADH-1 were determined with the substrates acetone, acetaldehyde, and 2-propanol. All measurements were repeated twice, values are given with SEM.

| Substrate   | Vmax (μmol min⁻¹ mg⁻¹) | Km (μM)   | Vmax modified (μmol min⁻¹ mg⁻¹) | Km modified (μM) |
|-------------|------------------------|-----------|--------------------------------|------------------|
| Acetaldehyde| 10.03 ± 0.63           | 180 ± 59  | 680 ± 131                      | 6.9 ± 0.41       |
| Acetone     | 9.33 ± 0.78            | 31.3 ± 10.5| 169 ± 15                      | 7.01 ± 0.23      |
| 2-Propanol  | 5.33 ± 0.2             | 11570 ± 1250| NA                            | NA               |
| NADPH       | 3.4 ± 8.6              | 34.2 ± 4.4| NA                            | NA               |
| NADPH*      | 3.4 ± 7               | 34.2 ± 4.4| NA                            | NA               |

* As determined with acetaldehyde (1 mM) as substrate.
* As determined with 2-propanol (100 mM) as substrate.
* Not applicable.
* Of all measured values, a background NADPH-oxidising activity of 3.4 μmol min⁻¹ mg⁻¹ were subtracted.
Inhibitory effect of ADH-1 could be observed with the recombinant enzyme and with *T. vaginalis* cell extract alike (Table 3). We further observed that the extent of inhibition depended on the NADPH concentration in the buffer (Fig. 2a), suggesting competitive binding of NADPH and CoA to ADH-1. Interestingly, also oxidation of 2-propanol was inhibited by CoA (Fig. 2b). Congruently, the extent of inhibition depended on the NADP+ concentration in the buffer (Fig. 2b). Inhibition was not due to the thiol group as such, as 10 mM cysteine did not inhibit ADH-1 at all (not shown) and 10 mM β-mercaptoethanol resulted only in a modest inhibition of acetaldehyde reduction of about 25%. However, acetyl-CoA had no inhibitory effect on ADH-1 (not shown).

### 3.4. ADH-1 activity can also be measured in cell extracts from *E. histolytica* and *T. foetus*

In order to assess whether our novel findings on ADH-1 do only apply for *T. vaginalis* ADH-1 or also for homologous enzymes in other organisms, we assayed NADPH-dependent alcohol dehydrogenase activity in cell extracts of *E. histolytica* and *T. foetus*. As expected, NADPH-dependent acetaldehyde reduction could also be measured in cell extracts from these two parasites (Table 3). In accordance with our observations in *T. vaginalis*, this enzyme activity was inhibited by 2-propanol and CoA (Table 4). Furthermore, considerable background NADPH-oxidation, which was also inhibited by 2-propanol and CoA, could be measured in reaction buffer exposed to an anaerobic atmosphere overnight (Table 4). In cell extracts of *G. lamblia*, which lacks an NADPH-dependent secondary alcohol dehydrogenase, no NADPH-dependent acetaldehyde reduction or background NADPH-oxidation in anaerobic buffer could be detected (Table 4).

### 3.5. Failed attempts to identify of the substrate of the background NADPH-oxidising activity

Based on our observation that background NADPH-oxidising activity occurs even in aqueous 100 mM KCl solution, we speculated that only following compounds could be substrates of ADH-1: (1) molecular oxygen, resulting in the formation of water or reactive oxygen species such as hydrogen peroxide or the superox-
ide radical anion; (2) water, resulting in the formation of hydrogen gas; or (3) carbon dioxide, resulting in the formation of formic acid or formaldehyde.

In order to address hypothesis 1, we added cytochrome c (50 μM) to reactions and monitored cytochrome c reduction in a spectrophotometer. In the presence of superoxide radicals, cytochrome c is reduced, resulting in increased absorption at λ = 550 nm. Further, hydrogen peroxide formation was tested by monitoring oxidation of ferrous to ferric iron via formation of pentaqua(thiocyanato-N)iron(III) complexes at λ = 450 nm. Both assays gave a negative result. In order to test, whether ADH-1 can reduce oxygen directly to water, reactions were allowed to proceed for 5 min. Afterwards, 0.5 mM of fresh NADPH, 10 μM FMN, and 1 μg ml⁻¹ of T. vaginalis flavin reductase 1 (Leitsch et al., in preparation), an enzyme that reduces oxygen to hydrogen peroxide via FMN or other flavins were added and hydrogen peroxide formation was measured as described. No altered hydrogen peroxide formation as compared to controls could be observed, indicating that the oxygen concentration had remained unaffected by ADH-1. In order to address hypothesis 2, hydrogen gas formation was measured by membrane-inlet spectrometry. However, no hydrogen gas formation could be observed. Hypothesis 3 was tested using NMR spectroscopy upon addition of sodium bicarbonate (10–100 mM) as a carbon dioxide donor. Again, no products could be detected. In contrast, formation of 2-propanol from acetone was readily observed (Fig. 3). Furthermore, addition of acidified sodium bicarbonate did not result in increased NADPH oxidation, arguing against carbon dioxide as a substrate.

4. Discussion

In this study we characterised recombinantly expressed NADP-dependent secondary alcohol dehydrogenase, or alcohol dehydrogenase 1 (ADH-1), from T. vaginalis. While we were preparing the present manuscript, a study was published by Sutak et al. describing purification of ADH-1 from T. vaginalis cell extract and its characterisation (Sutak et al., 2012). Interestingly, the determined kinetic parameters of the enzyme differ in various aspects from those presented in this study. The most conspicuous differences are the determined Kₘ's for 2-propanol and NADP⁺.

|                | Aldehyde reduction (nmol min⁻¹ mg⁻¹) | Background NADPH oxidationa (nmol min⁻¹ mg⁻¹) | Inhibition 2-propanolb aldehyde reduction | Inhibition 2-propanolb background NADPH oxidation | Inhibition CoAc² aldehyde reduction | Inhibition CoAc² background NADPH oxidation |
|----------------|-------------------------------------|---------------------------------------------|------------------------------------------|-----------------------------------------------|-----------------------------------|------------------------------------------|
| E. histolytica  | 300 ± 119                           | 236 ± 21                                    | 80 ± 11%                                 | 70 ± 4%                                       | 62 ± 22%                          | 52 ± 3%                                  |
| T. foetus      | 136                                 | 128                                         | 60%                                      | 65%                                           | 60%                               | 67%                                      |
| G. lamblia     | 0                                   | 0                                           | NA²                                      | NA²                                           | NA²                               | NA²                                      |

a Under anaerobic condition.

b At a concentration of 200 mM.

c At a concentration of 100 μM.

d Not applicable.

e Only measured once.

Fig. 3. ¹H NMR spectrum of 2-propanol ((CH₃)₂ doublet peak at 1 ppm) produced by the reduction of acetone, substrate concentration 50 mM acetone in D₂O/100 mM Na phosphate, pH 7.2 buffer. Chemical shifts of the single and hydroxyl proton of 2-propanol are indicated by an asterisk. Reducing power derived from NADPH (at 8–8.25 ppm).
T. vaginalis extract (Sutak et al., 2012). Since the measured Km's for acetaldehyde, acetone, and NADPH are much more congruent between both studies, it is likely that the observed discrepancies are brought about by post-translational modifications, either in E. coli or in T. vaginalis. However, the conclusion by Sutak et al. that reduction is favoured to oxidation by ADH-1, could be confirmed by us.

A novel observation was the marked background activity of ADH-1 in absence of any added substrate (3.4 μmol min⁻¹ mg⁻¹). We recalculated the Km's determined for acetaldehyde and acetone accordingly by subtracting the observed background activity from the measured values with added substrate. This raised Km's significantly: from 31 to 169 μM in case of acetone, and from 180 to 680 μM in case of acetaldehyde (Table 1). Although we were unable to identify the substrate of this reaction, it can be ruled out for several reasons that background activity of ADH-1 is due to impurities in the enzyme buffer or due E. coli proteins stemming from the purification procedure of the recombinant enzyme: background activity was consistently observed with buffers and solutions without any organic component (potassium phosphate buffer, aqueous KCl solution) which had been prepared with ultrapure water from three different suppliers. Furthermore, this enzyme activity was observed in two different laboratories (Medical University of Vienna and University of Cardiff) utilising different chemicals and instruments. As background activity of ADH-1 was also observable with cell extracts of T. vaginalis (Fig. 1), and as this activity was inhibited by coenzyme A and 2-propanol, regardless of whether purified enzyme or T. vaginalis cell extract was used (Table 3), it is highly improbable that co-isolated E. coli proteins caused the observed effect. Moreover, NADPH-oxidising background activity in the absence of any added substrate could also be observed in E. histolytica and T. foetus (Table 4), both having a NADP-dependent secondary alcohol dehydrogenase. In accordance with our findings with T. vaginalis, NADPH oxidation was inhibited by CoA and 2-propanol (Table 4). In contrast, when using cell extracts of G. lambia, which lacks a homologue of ADH-1, no NADPH-oxidising background activity could be measured.

Presently, the physiological function of this enzyme remains unresolved. In our previous paper on ADH-1 (Leitsch et al., 2012), we speculated that that acetaldehyde might be formed by PFOR in the absence of oxygen which, in turn, would be reduced to ethanol by ADH-1. Indeed, ethanol is only formed under strictly anaerobic condition (Ellis et al., 1992) and the inhibitory effect of CoA on ADH-1 activity also suggests a link to hydrogenosomal pathways. Accordingly, Sutak et al. (2012), observed a major shift in the composition of metabolic end products when acetone was added to the growth medium, production of acetate being enhanced and that of lactate being decreased. However, as T. vaginalis hardly produces any acetone (Sutak et al., 2012) and as ethanol is only a minor metabolite product, it is difficult to put into perspective the extremely high expression level of ADH-1, making up to 2% of total protein visualised on 2D-gels from T. vaginalis extracts (Leitsch et al., 2012). Sutak et al. speculated that ADH-1 has a protective role against elevated acetone levels in urine during ovulation but E. histolytica, which colonises the large intestine, also expresses very high levels of this enzyme (Kumar et al., 1992; Leitsch et al., 2005).

Interestingly, we only observed significant NADPH-oxidising activity in cell extracts after exposure to an anaerobic atmosphere (Fig. 1). Although the nature of the substrate of this reaction remains unresolved, it is reasonable to assume that it is also present in the cell. In this case, ADH-1 would function as a major sink for NADPH at low oxygen tension. In the presence of oxygen, this function might be redundant as NADPH is utilised by flavin reductase (Leitsch et al., 2012) in order to form hydrogen peroxide. In this context, it is interesting to note that ADH-1 is down-regulated in metronidazole-resistant T. vaginalis strains (Leitsch et al., 2012) which have impaired oxygen scavenging mechanisms (Yarlett et al., 1986) and, consequently, higher intracellular oxygen levels. Thus, ADH-1 might have an essential function, i.e. recycling NADP⁺, only under anaerobic condition. Supposedly, this also applies for T. foetus and the non-related parasite E. histolytica. In light of the potential importance of this pathway, further work on secondary alcohol dehydrogenases in microaerophilic parasites is imperative.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.exppara.2013.03.034.

References

Carlton, J.M., Hirt, R.P., Silva, J.C., Delcher, A.L., Schatz, M., Zhao, Q., Wortman, J.R., Bidwell, S.L., Alsmark, U.C.M., Besteiro, S., Sichertscht-Ponten, T., Noel, C.J., Dacks, J.B., Foster, P.G., Simillion, C., Van de Peer, Y., Miranda-Sawad Sen, D., Barton, C.J., Westrop, G.D., Müller, S., Dessi, D., Fiori, P.L., Ren, Q.J., Zhang, H., Bastida-Corcuera, F.D., Simones-Barbosa, A., Brown, M.T., Hayes, R.D., Mukherjee, M., Okumura, C.H., Schneider, R., Smith, A.J., Vanacova, S., Villalvazo, M.J., Haas, R.J., Peraza, M., Feldhyaun, T.V., Utterback, R.T., Shu, C.L., Gioeagaw, K., de Jong, P.J., Hryd, I., Horvathova, L., Zubacova, Z., Dolezal, P., Malik, S.B., Logsdon Jr., J.M., Henze, K., Gupta, A., Wang, C.C., Dunne, R.L., Upcroft, J.A., Upcroft, P., White, O., Salzberg, S.L., Tang, P., Chiw, C.H., Lee, Y.S., Embley, T.M., Coons, G.H., Mottram, J.C., Tachezy, J., Fraser-Litggett, C.M., Johnson, P.J., 2007. Draft genome sequence of the sexually transmitted pathogen Trichomonas vaginalis. Science 315, 207–212.

Chapman, A., Linde, D.J., Lloyd, D., Williams, J., 1985. 13C-NMR reveals glycerol as an unexpected major metabolite of the protozoan parasite Trichomonas vaginalis. FEBS Lett. 191, 287–292.

Clark, C.G., Alsmark, U.C., Tazreiter, M., Saito-Nakano, Y., Ali, V., Marion, S., Weber, C., Sutak, G., Chapman, D., van Hellemon, J.J., Tielens, A.G., Henze, K., 2010. Acetate:succinate CoA-transferase in the hydrogenosomes of Trichomonas vaginalis: identification and characterisation. J. Biol. Chem. 283, 1411–1418.

Keister, D.B., 1983. Axenic culture of Giardia lamblia. J. Biol. Chem. 283, 1411–1418.

Kumar, A., Shen, P.S., Descoteaux, S., Pohl, J., Bailey, G., Samuelson, J., 1992. Cloning and expression of an NADP⁺-dependent alcohol dehydrogenase gene of Trichomonas vaginalis. Mol. Biochem. Parasitol. 56, 9–88.

van Grinsven, K.W., Rosnowsksy, S., van Weelden, S.W., Pütz, S., van der Geien, M., Martin, W., van Hellemon, J.J., Tielen, A.G., Henze, K., 2008. Acetate: succinate CoA-transferase in the hydrogenosomes of Trichomonas vaginalis: identification and characterization. J. Biol. Chem. 283, 1411–1418.

Keister, D.B., 1983. Axenic culture of Giardia lamblia in TV-5-3 medium supplemented with bile. Trans. R. Soc. Trop. Med. Hyg. 77, 487–488.

Kulda, J., 1999. Trichomonads, hydrogenosomes and drug resistance. Int. J. Parasitol. 29, 199–212.

Kumar, A., Shen, P.S., Descoteaux, S., Pohl, J., Bailey, G., Samuelson, J., 1992. Cloning and expression of an NADP⁺-dependent alcohol dehydrogenase gene of Entamoeba histolytica. Proc. Natl. Acad. Sci. USA 89, 10188–10192.

Leitsch, D., Radauer, C., Paschinger, K., Wilson, I.B.H., Brettiner, H., Schier, O., Duchene, M., 2005. Entamoeba histolytica: analysis of the trophozoite proteome by two-dimensional polyacrylamide gel electrophoresis. Exp. Parasitol. 110, 19–95.

Leitsch, D., Drinic, M., Kolari, D., Duchene, M., 2012. Down-regulation of flavin reductase and alcohol dehydrogenase-1 (ADH-1) in metronidazole-resistant isolates of Trichomonas vaginalis. Mol. Biochem. Parasitol. 183, 177–183.

Lindmark, D.G., Müller, M., 1973. Hydrogenosome, a cytosolic organelle of the anaerobic flagellate Trichonymna foetus, and its role in pyruvate metabolism. J. Biol. Chem. 248, 7724–7728.

Ma, K., Hutchins, A., Sung, S.J., Adams, M.W., 1997. Pyruvate ferredoxin oxidoreductase from the hyperthermophilic archaeon, Pyrococcus furiosus,
functions as a CoA-dependent pyruvate decarboxylase. Proc. Natl. Acad. Sci. USA 94, 9608–9613.
Millet, C.O.M., Cable, J., Lloyd, D., 2010. The diplomonad fish parasite Spironucleus vortens produces hydrogen. Eukaryot. Microbiol. 57, 400–404.
Nanda, N., Ross, G.M., Kurdgelashvili, G., Wendel, K.A., 2006. Trichomoniasis and its treatment. Expert. Rev. Anti Infect. Ther. 4, 125–135.
Paget, T.A., Lloyd, D., 1990. Trichomonas vaginalis requires traces of oxygen and high concentrations of carbon dioxide for optimal growth. Mol. Biochem. Parasitol. 41, 65–72.
Sutak, R., Hrdy, I., Dolezal, P., Cabala, R., Sedinová, M., Lewin, J., Harant, K., Müller, M., Tachezy, J., 2012. Secondary alcohol dehydrogenase catalyzes the reduction of exogenous acetone to 2-propanol in Trichomonas vaginalis. FEBS J 279, 2768–2780.
Wassmann, C., Hellberg, A., Tannich, E., Bruchhaus, I., 1999. Metronidazole resistance in the protozoan parasite Entamoeba histolytica is associated with increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase. J. Biol. Chem. 274, 26051–26056.
Yarlett, N., Yarlett, N.C., Lloyd, D., 1986. Metronidazole-resistant clinical isolates of Trichomonas vaginalis have lowered oxygen affinities. Mol. Biochem. Parasitol. 19, 111–116.