

t-Butyl-4-hydroxyanisole, a novel respiratory chain inhibitor

Effects on *Trypanosoma cruzi* epimastigotes

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t-Butyl-4-hydroxyanisole, an antioxidant food additive, inhibited the growth of *Trypanosoma cruzi* by almost 100% at 0.5 mM concentration. This compound inhibited 70% of oxygen consumption of epimastigotes. The redox level of NAD(P) was shifted to a more reduced state and inversely the redox level of cytochrome *b* changed to a more oxidized state. This hydroxyanisole thus is a new electron transport chain inhibitor. This compound and related ones, or the respiratory chain of *T. cruzi*, may be important in the design of antichagasic drugs.

(*Trypanosoma cruzi*) *t*-Butyl-4-hydroxyanisole Respiratory chain inhibition Chagas' disease

1. INTRODUCTION

Chagas' disease, the American trypanosomiasis, affects about 12 million people in Central and South America. The causative agent is a protozoan parasite, *Trypanosoma cruzi*.

Current therapy of the disease is unsatisfactory because the parasite is resistant to drugs in use. The possible involvement of detoxifying enzymes appears to be a plausible explanation for this phenomenon. Cytochrome P-450 [1], epoxide hydrolase [2], glutathione *S*-transferase [3], carboxylesterase [4] and phosphatase [5] have been shown to be part of the detoxication system of the parasite.

t-Butyl-4-hydroxyanisole (BHA) is a known antioxidant food additive [6,7] and is also an inducer of epoxide hydrolase and glutathione *S*-transferase [8]. Here, we report that BHA inhibits *T. cruzi* growth and respiration. BHA inhibited the respiratory chain at the NAD-cytochrome *b* segment.

2. MATERIALS AND METHODS

2.1. Materials

Tryptose, tryptone, yeast extract and fetal calf serum were obtained from Difco. All other chemicals were obtained from Sigma. BHA [2,3] was recrystallized from water-ethanol.

2.2. Parasites

T. cruzi epimastigotes (Tulahuen strain) were grown at 28°C in Diamond's monophasic medium [9] with blood replaced by 4 µM hemin; pH was adjusted to 7.2 before sterilization. Fetal calf serum was added at a 4% final concentration. *T. cruzi* epimastigote growth was followed by nephelometry using culture flasks with a side-arm tube. The parasites were harvested at the fifth day of growth by centrifugation at 500 × *g* for 10 min. They were washed twice with 0.17 M sodium chloride-0.052 M potassium phosphate, pH 7.5.

2.3. Assays

Oxygen uptake measurements were made polarographically with a Clark electrode no. 5331 (Yellow

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Springs Instrument) in a YSI model 53 O₂ monitor linked to a 100 mV mono-channel Goerz RE 511 recorder.

2.4. Spectrophotometry

Absorption changes in epimastigotes were recorded with an Aminco DW2a dual-wavelength spectrophotometer (American Instrument). Measuring and reference wavelengths were selected on the basis of data for mammalian and *T. cruzi* mitochondrial cytochromes [10]. NAD redox change was measured according to Lotsher et al. [11].

Results presented in this communication correspond to the average of 4–6 different experiments.

3. RESULTS AND DISCUSSION

Fig.1 shows the growth of *T. cruzi* epimastigotes with and without BHA. The chemical inhibited near 100% growth of the parasite at the concentration used. Concentration of BHA as low as 0.050 mM significantly inhibited the growth of the protozoan. BHA is an antioxidant widely used as a

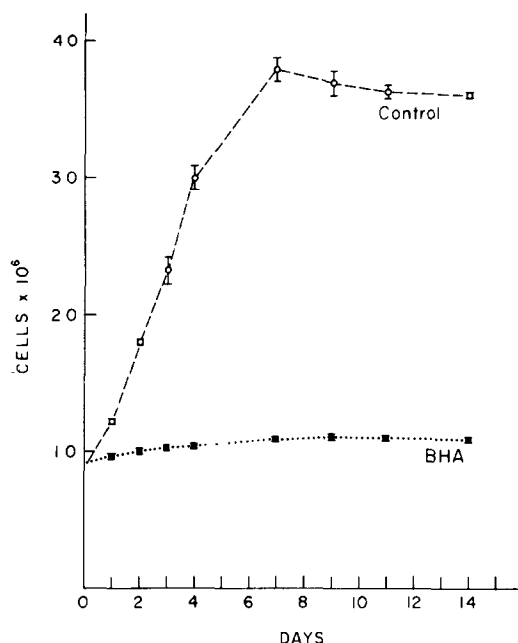


Fig.1. Effect of BHA on *T. cruzi* epimastigote growth. BHA was added at a final concentration of 0.5 mM. See section 2.

food additive and is noted for its low toxicity to mammals [6,7].

Fig.2 shows the effect of BHA on O₂ uptake by *T. cruzi* epimastigotes. Control parasites respired at a rate of 25 ngatom oxygen/min per mg protein. In parasites with BHA added the respiration decreased to 7.6 ngatom O/min per mg protein; this represents 70% inhibition. Respiration in control and BHA-treated parasites was linear at least for 10 min. Benznidazole, a drug currently used in the treatment of Chagas' disease, also inhibits the parasite O₂ consumption by a similar percentage at 1 mM [12].

Fig.3 shows the effect of BHA on the redox level of NAD(P) and cytochrome *b*. BHA shifted the redox level of NAD(P) towards a more reduced state and that of cytochrome *b* to a more oxidized state. The redox states of cytochromes *c* and *a* (not shown) were also shifted to more oxidized states. Control experiments done with rotenone showed similar results to those obtained with BHA. These experiments suggest that BHA inhibited the electron transport chain at a point between NAD and cytochrome *b*. At present we do not have any evidence that correlates the antioxidant property of BHA with its capacity to inhibit the respiratory chain of *T. cruzi*.

The inhibition of the *T. cruzi* respiratory chain and prevention of the reoxidation of NADH resulting from substrate oxidation may prevent the

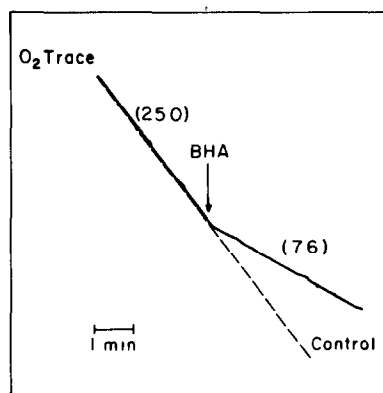


Fig.2. Effect of BHA on *T. cruzi* respiration. Epimastigotes were resuspended in culture medium at a concentration of 120×10^6 cells per ml. BHA was added at a final concentration of 0.5 mM. Temperature was 25°C. Figures in parentheses correspond to O₂ consumption in ngatom O/min per mg protein. See section 2.

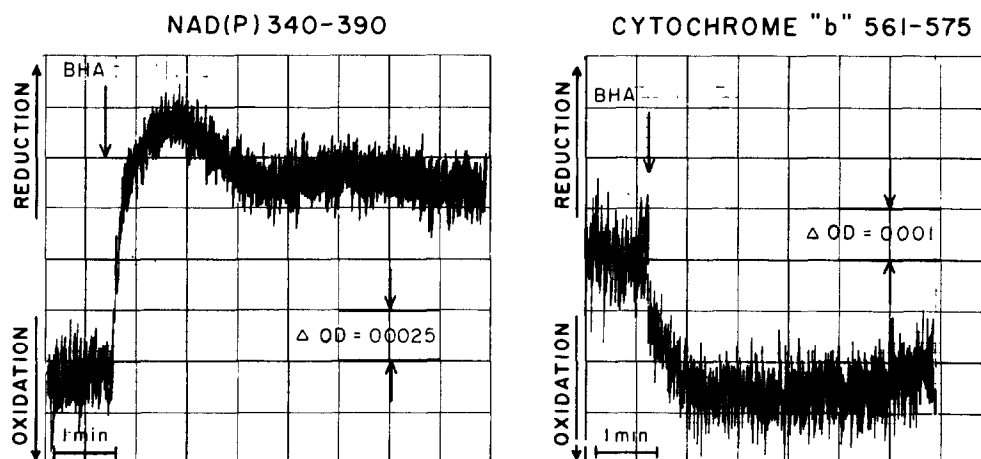


Fig.3. Effect of BHA on the redox level of *T. cruzi* NAD(P) and cytochrome *b*. Epimastigotes were resuspended at a concentration of 120×10^6 cells per ml in 0.107 M sodium chloride-0.052 M potassium phosphate, pH 7.5. BHA was added at a final concentration of 1.0 mM. Absorption was measured at the indicated wavelength pairs. Temperature was 25°C. See section 2.

operation of the tricarboxylic acid cycle, oxidative phosphorylation, and/or related anabolic processes. In summary, our results indicate that BHA inhibits growth and respiration of *T. cruzi* epimastigotes because it interferes with the respiratory chain at a point between NAD and cytochrome *b*.

These results suggest the possibility of using BHA and related compounds as experimental antichagasic drugs. Also, the respiratory chain of the protozoa may constitute a suitable target for drugs.

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REFERENCES

- [1] Agosin, M., Naquira, C., Paulin, J. and Capdevila, J. (1976) *Science* 194, 195-197.
- [2] Yawetz, A. and Agosin, M. (1979) *Biochim. Biophys. Acta* 585, 210-219.
- [3] Yawetz, A. and Agosin, M. (1980) *Comp. Biochem. Physiol.* 66C, 265-267.
- [4] Repetto, Y., Aldunate, J. and Morello, A. (1983) *Comp. Biochem. Physiol.* 76B, 61-64.
- [5] Letelier, M.E., Repetto, Y., Aldunate, J. and Morello, A. (1985) *Comp. Biochem. Physiol.* 81B, 47-51.
- [6] Brannen, A.L. (1975) *J. Am. Oil Chem. Soc.* 52, 59-63.
- [7] Hathway, D.E. (1966) *Adv. Fd Res.* 15, 1-56.
- [8] De Pierre, J.W., Seidegard, J., Morgenstern, R., Balk, L., Meijer, J. and Astrom, A. (1981) in: *Mitochondria and Microsomes* (Lee, C.P. et al. eds) pp. 585-610, Addison-Wesley, MA.
- [10] Stoppani, A.O.M., Docampo, R., DeBoiso, J.F. and Frasch, A.C.C. (1980) *Mol. Biochem. Parasitol.* 2, 3-21.
- [11] Lötscher, H.R., Winterhalter, K.H., Carafoli, E. and Richter, C. (1979) *Proc. Natl. Acad. Sci. USA* 76, 4340-4344.
- [12] Docampo, R. and Moreno, S.N.J. (1984) in: *Oxygen Radicals in Chemistry and Biology*. (Bors, W. et al. eds) pp. 749-752, De Gruyter, New York.