Tellurite Reduction in *Schizosaccharomyces pombe*

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SUMMARY

The fission yeast *Schizosaccharomyces pombe* was grown in the presence of 10^{-3} to 10^{-6} M-sodium tellurite. Growth was inhibited by tellurite and at concentrations above 10^{-5} M a black reduction product (probably tellurium) was visible in sedimented cells. Electron microscopy of thin sections showed the reduction product to be on a localized system of membranous vesicles forming an area about 500 nm in diameter. Membrane systems of this type were not seen in control cells but they possibly resulted from the degeneration of some of the mitochondria.

INTRODUCTION

Tellurite (TeO_3^{2-}) is toxic to many organisms, both prokaryotes and eukaryotes, and is used in a variety of selective bacteriological media and differential tests. The mechanism of its toxicity is not clear but it has been attributed to an interaction with SH groups, in particular with those of NAD dehydrogenases (Siliprandi, de Meio, Toninello & Zoccarato, 1971). If an organism is able to grow in the presence of tellurite it frequently reduces the tellurite to black elemental tellurium. This is deposited within the cells and colonies appear black in colour. The deposition of elemental tellurium probably amounts to a detoxication of the tellurite.

When the yeasts *Saccharomyces cerevisiae* and *Rhodotorula mucilaginosa* are grown in the presence of K_2TeO_3 the reduction product is deposited mainly on specialized areas of endoplasmic reticulum (Corfield & Smith, 1970). The present report concerns the effect of tellurite on the fission yeast *Schizosaccharomyces pombe*.

METHODS

The organism *Schizosaccharomyces pombe* (strain 132, National Collection of Yeast Cultures, Nutfield, Surrey) was cultivated in a liquid medium containing (g/l distilled water): glucose, 30; yeast extract (Oxoid), 5; KH_2PO_4 , 2.1; final pH 5.6. Sterile solutions (1 ml amounts) of Na_2TeO_3 (BDH) were added to 57 ml culture medium in 250 ml side-arm flasks, to give final concentrations of tellurite in the range of 10^{-2} to 10^{-6} M.

Flasks were inoculated with 2 ml of a 48 h culture of *S. pombe* (giving an initial viable count of 0.17×10^7 /ml), shaken in a water bath at 30 °C and extinction measurements made hourly.

For electron microscopy cells were washed twice and then fixed in 1.5 % (w/v) KMnO_4 for 4 h. After postfixing with 0.5 % (w/v) uranyl acetate the sedimented material was dehydrated in tertiary butyl alcohol and embedded in Araldite. Sections were stained with alkaline lead citrate (Reynolds, 1963) before examination in a Siemens Elmiskop I electron microscope.

Table 1. *Effect of tellurite on Schizosaccharomyces pombe after 28 h growth*

	Concentration of Na_2TeO_3 (M)			
	0	10^{-6}	10^{-5}	10^{-3}
Approximate mean generation time (h)	3	9	11	> 11
Extinction ($\times$ dilution)	3.2	2.5	0.7	0.7
$10^{-7} \times$ Total count/ml	5.0	4.5	1.4	0.43
$10^{-7} \times$ Viable count/ml	4.0	2.5	0.9	0.13
Viability (%)	80	55	64	30
Average cell length (μm)	7.7	8.7	9.4	23.6
Final pH	4.5	4.9	5.2	5.6
Colour of sedimented cells	Cream	Cream	Pale grey	Black

Mean generation times were determined from the extinction measurement growth curves. Viable counts 28 h after inoculation were made on yeast extract glucose agar and total counts were determined with a Coulter Counter model F. Cell lengths were taken from micrographs produced with a Zeiss photomicroscope using phase contrast optics.

RESULTS AND DISCUSSION

Schizosaccharomyces pombe was very sensitive to tellurite: 10^{-6} M slowed the growth rate markedly even though no visible reduction product was produced. Extinction measurements became meaningless with higher concentrations of tellurite (10^{-3} M) because the reduction product gave a falsely high reading. Various parameters of the tellurite-grown and control cultures are shown in Table 1; it can be seen that tellurite increased the generation time and decreased the viability of the cells. Total cell counts of stationary phase cultures, made with a Coulter Counter, showed only an approximately threefold increase in number in the presence of 10^{-3} M-tellurite, whereas the control culture showed a 30-fold increase. The length of the *S. pombe* cells was increased about threefold by 10^{-3} M-tellurite and these cells also showed clearly-visible tellurium deposits when examined by light microscopy (Fig. 1). Cells from the control are shown in Fig. 2. The final pH of the medium was also higher in the presence of tellurite.

Electron microscopy revealed that, as previously reported in budding yeasts (Corfield & Smith, 1970), the reduction product after growth in tellurite was deposited on membranes in localized areas of the cytoplasm (Fig. 3). Several such areas, about 500 nm across, were found in each thin section. The apparently membranous structures on which the tellurite was reduced were never seen in untreated cells (Fig. 4) and therefore appeared to be produced in response to the tellurite: they are distinct in form from the Golgi bodies which have been reported in this yeast (Smith & Svoboda, 1972; Kopecka, 1972) although some Golgi-like vesicles could be seen in close association. In *S. pombe* these areas of reduction are more distinct from the normal endoplasmic reticulum than was the case with the budding yeasts. It is possible that these membranous clusters result from some mitochondrial disintegration since mitochondria (of mammalian heart tissue) have been reported to be sites of tellurite reduction (Barnett & Palade, 1957).

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Fig. 1. *S. pombe* after growth in the presence of 10^{-3} M-tellurite. The cells are elongated and show black deposits of tellurium.

Fig. 2. *S. pombe* after normal growth in the absence of tellurite.

Fig. 3. Thin section of *S. pombe* after growth in the presence of 10^{-3} M-tellurite. Tellurium is deposited on a localized system of membranes.

Fig. 4. Thin section of *S. pombe* after growth in tellurite-free medium.

REFERENCES

- BARNETT, R. J. & PALADE, G. E. (1957). Histochemical demonstration of the sites of activity of dehydrogenase systems with the electron microscope. *Journal of Biophysical and Biochemical Cytology* **3**, 577-588.
- CORFIELD, P. S. & SMITH, D. G. (1970). The endoplasmic reticulum as the site of potassium tellurite reduction in yeasts. *Journal of General Microbiology* **63**, 311-316.
- KOPECKA, M. (1972). Dictyosomes in the yeast *Schizosaccharomyces pombe*. *Antonie van Leeuwenhoek* **38**, 27-31.
- REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208-212.
- SILIPRANDI, D., DE MEIO, R. H., TONINELLO, A. & ZOCCARATO, F. (1971). The action of tellurite, a reagent for thiol groups, on mitochondrial oxidative processes. *Biochemical and Biophysical Research Communications* **45**, 1071-1075.
- SMITH, D. G. & SVOBODA, A. (1972). Golgi apparatus in normal cells and protoplasts of *Schizosaccharomyces pombe*. *Microbios* **5**, 177-182.