The effect of excess mevalonic acid on ubiquinone and tetrahymanol biosynthesis in *Tetrahymena pyriformis*

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(Received 13 March 1985/30 April 1985; accepted 14 May 1985)

When *T. pyriformis* is grown in the presence of 10^{-2} M-mevalonic acid, the uptake exceeds the cell's requirement for this biosynthetic intermediate. The majority of the excess mevalonic acid is diverted into ubiquinone-8 biosynthesis whereas the biosynthesis of tetrahymanol, the major product of the mevalonic acid pathway, is unchanged. In the presence of excess external mevalonic acid, the biosynthesis of mevalonic acid by the cell is inhibited. It is proposed that ubiquinone biosynthesis is normally regulated by mevalonic acid availability, whereas tetrahymanol biosynthesis is regulated primarily at a later point in the pathway.

In eukaryotic organisms membrane sterols or triterpenoids and the polyisoprene side chain of ubiquinone are the two major products of mevalonic acid metabolism. Attention has been directed to the regulation of this branched pathway from mevalonic acid in mammalian cells (reviewed by Brown & Goldstein, 1980). The eukaryotic protozoan *Tetrahymena pyriformis* is of particular interest because its membrane triterpenoid alcohol is biosynthesized as the result of the direct proton-initiated cyclization of squalene (Zander *et al.*, 1970) and hence is a much simpler pathway than cholesterol biosynthesis in mammalian cells. In addition, the organism contains high levels of ubiquinone-8 (Vakirtzi-Lemonias *et al.*, 1963) because of the large number of mitochondria in this free-swimming ciliated protozoan.

I have shown previously that this organism is able to convert added tracer amounts of radioactive mevalonic acid into both tetrahymanol and ubiquinone-8, and the incorporation of radioactivity reflected the amounts of these two compounds in the cell (Wilton, 1983). Moreover, when tetrahymanol biosynthesis was inhibited by growing the organism in the presence of cholesterol, there was inhibition of radioactive incorporation into tetrahymanol and a corresponding increase of radioactive incorporation into ubiquinone-8. It should be noted that this tracer amount of radioactivity makes no significant contribution to the mevalonic acid flux within the organism, and indeed the total ubiquinone-8 biosynthesis and amounts within the organism remain unchanged in the presence of cholesterol (Wilton, 1983).

I have now investigated the effect of growing the organism in the presence of very high concentrations of external mevalonic acid (10 mM) so that uptake into the cell will make the major contribution to total mevalonic acid metabolism. I wished to investigate how this branched pathway copes with the increased mevalonic acid load in terms of tetrahymanol and ubiquinone-8 biosynthesis.

Materials and methods

All methods have been described previously (Beedle *et al.*, 1974; Warburg *et al.*, 1982; Wilton, 1983) and are summarized.

Cultures (100 ml) were grown at 28°C aerobically with shaking in bacteriological peptone supplemented with yeast extract and glucose. Mevalonic acid lactone was neutralized with NaOH prior to addition to the culture. After overnight inoculation, cultures were incubated with the appropriate additions for 24 h. Incubations were terminated with conc. HCl (0.2 ml) and the cells were harvested and homogenized in methanol/water (1:1, v/v). β -Amyrin (500 μ g) was added as an internal standard for g.l.c.

After removal of a sample for determination of dry weight, the homogenate was extracted with chloroform and half of the total lipid extract was separated by t.l.c. in order to isolate tetrahymanol and ubiquinone-8 for radioactivity determinations. Samples of the remaining half were used for tetrahymanol estimation by g.l.c. and ubiquinone-8 estimation by spectrophotometric analysis at 275 nm. Lipid saponification was

carried out by refluxing in 10% (w/v) KOH in ethanol under N₂ for 2 h. After adding water and extracting the neutral lipids with diethyl ether the mixture was acidified (HCl) and the fatty acids were extracted with petroleum spirit (b.p. 40–60°C).

Results

The effect of growing cultures of *T. pyriformis* in the presence of high concentrations (10 mM) of mevalonic acid is shown in Table 1 (expt. 1). Cultures were grown for 24 h in the presence of [2-¹⁴C]mevalonic acid and [methyl-³H]methionine. The cultures grew more slowly in the presence of the high concentration of mevalonic acid; the effect was most noticeable in early exponential phase. However, these cultures did normally reach the same final density as control cultures after longer incubations.

All the ¹⁴C taken up from the medium was incorporated into the total lipid fraction, and this value was the same in the absence and presence of 10 mM-mevalonic acid. This indicates that uptake involved a concentration-dependent simple permeability process.

In the presence of 10 mM-mevalonic acid the incorporation into total lipid was 14 µmol (50 µmol/g dry wt.). This value exceeds the normal mevalonic acid requirement of the organism for tetrahymanol and ubiquinone-8 biosynthesis (approx. 30 µmol/g dry wt.).

The tetrahymanol content as measured per g dry wt. was the same in both cultures and illustrates the importance to the organism of assembling its membranes with the correct tetrahymanol content for optimal physiological activity. This regulation of tetrahymanol biosynthesis must be occurring at a point after mevalonic acid in the pathway.

It would appear that the majority of the excess mevalonic acid is converted to ubiquinone, the concentration of which in the cell rises more than 3-fold. This ubiquinone was confirmed as ubiquinone-8 (Vakirtzi-Lemonias *et al.*, 1963) by reverse phase t.l.c. and h.p.l.c. The increased biosynthesis of ubiquinone-8 was confirmed by a study of the incorporation of ³H from [methyl-³H]methionine, which gave a more than 3-fold greater incorporation in cells grown in the presence of mevalonic acid. [methyl-³H]Methionine is the source of the methyl group in the quinone ring of ubiquinone.

When the specific radioactivities of the tetrahymanol (six mevalonic acid units) and ubiquinone-8 (eight mevalonic acid units) were calculated, the respective values were 2700 and 3000 d.p.m./µmol of mevalonic acid incorporated compared with 2900 d.p.m./µmol for the 10 mM-mevalonic acid in the medium. Therefore, under these conditions, both cellular lipids have been biosynthesized primarily from externally derived mevalonic acid and endogenous mevalonic acid biosynthesis must have been inhibited. This prediction was confirmed in a separate experiment (expt. 2 of Table 1) where [methyl-³H]acetate was substituted for the

Table 1. *Effect of mevalonic acid on tetrahymanol and ubiquinone biosynthesis*

Expt. 1: cultures (100 ml) of *T. pyriformis* were inoculated overnight and then grown for 24 h in the absence or presence of mevalonic acid (10 mM). In addition [2-¹⁴C]mevalonic acid (1.25 µCi) and [methyl-³H]methionine (10 µCi) were added to each flask. Incubations were terminated by the addition of conc. HCl (0.2 ml) and the cells were harvested. The dry weight, ubiquinone and tetrahymanol content, together with the radioactive incorporation into total lipids, ubiquinone and tetrahymanol, were determined as described in the Materials and methods section. All results are the mean of at least duplicate inoculations varying by not more than 10%. Expt. 2: as for expt. 1 except that [methyl-³H]acetate (100 µCi, 100 µmol) was used instead of [methyl-³H]methionine.

	Control	+ Mevalonic acid
Expt. 1		
Dry weight (mg)	320	280
Tetrahymanol (µmol/g dry wt.)	4.0	3.9
Ubiquinone-8 (µmol/g dry wt.)	0.64	2.35
[2- ¹⁴ C]Mevalonic acid uptake into total lipid (% of added radioactivity)	1.5	1.4
[2- ¹⁴ C]Mevalonic acid (µmol) incorporated into:		
Total lipid	—	14
Tetrahymanol	—	6.6
Ubiquinone-8	—	5.3
[methyl- ³ H]Methionine incorporated into ubiquinone (total d.p.m.)	37800	131000
Expt. 2		
[methyl- ³ H]Acetate incorporated into tetrahymanol (total d.p.m.)	186000	37000

radioactive methionine. The total incorporation into tetrahymanol from acetate and hence from biosynthesized mevalonic acid was inhibited over 80% in cells grown in the presence of mevalonic acid. Radioactive acetate incorporation into fatty acids was not inhibited.

Not all the excess uptake of mevalonic acid can be accounted for in ubiquinone, and analysis of the lipids after t.l.c. has revealed other ^{14}C -labelled material, and in particular material that stays at the baseline under the conditions of t.l.c. separation. This material, which does not contain ^3H from [*methyl*- ^3H]methionine, has not been identified.

Discussion

The results presented in Table 1 clearly demonstrate that when *T. pyriformis* is grown in high concentrations of mevalonic acid the uptake of this compound exceeds the cell's biosynthetic requirements. Normally it would be expected that mevalonic acid formation within the organism from acetyl-CoA would be precisely regulated to meet the demands of the cell, and indeed the uptake of excess mevalonic acid from the medium resulted in inhibition of mevalonic acid biosynthesis. However, in terms of metabolizing this excess mevalonic acid from the medium it would appear that the major route of mevalonic acid metabolism, namely to tetrahymanol, is further regulated at a later stage unique to tetrahymanol biosynthesis in order to prevent excess tetrahymanol formation. I suggest that the enzyme squalene synthetase may be the site of this further regulation. We have already shown that squalene synthetase is the primary site of inhibition of tetrahymanol biosynthesis in cells grown in the presence of cholesterol, and that this enzyme appears to have a short half-life consistent with the enzyme having an important regulatory role (Warburg *et al.*, 1982).

The ubiquinone branch of the pathway appears not to be regulated beyond mevalonic acid formation under the conditions of these experiments, and

as a result the excess mevalonic acid is to a large extent metabolized by this route. Under normal conditions ubiquinone biosynthesis must be regulated by mevalonic acid formation within the cell and the relative activities of the first enzyme unique to tetrahymanol and ubiquinone formation.

It is interesting to note that *T. pyriformis* behaves very differently to mammalian cells in responding to high levels of mevalonic acid. Incubation of cultured human fibroblasts in the presence of high concentrations of mevalonic acid had little effect on ubiquinone biosynthesis, but dramatically increased cholesterol biosynthesis (Faust *et al.*, 1979). It is pertinent to note that in *T. pyriformis* tetrahymanol is not metabolized or esterified. However, in mammalian cells, cholesterol can be converted to other products, and more particularly excess cholesterol is normally esterified so that the concentration of free cholesterol within the cell remains relatively constant. Thus cholesterol esters within the cell represent an expandable pool of physiological inert cholesterol.

The biosynthesis of other mevalonic acid-derived cellular components, e.g. the dolichols, remains to be investigated.

I would like to thank Mrs. K. Platt for g.l.c. analysis and Mrs. J. S. Elliott for h.p.l.c. analysis.

References

- Beedle, A. S., Munday, K. A. & Wilton, D. C. (1974) *Biochem. J.* **142**, 57–64
- Brown, M. S. & Goldstein, J. L. (1980) *J. Lipid Res.* **21**, 505–517
- Faust, J. R., Goldstein, J. L. & Brown, M. S. (1979) *Arch. Biochem. Biophys.* **192**, 86–99
- Vakirtzi-Lemonias, C., Kidder, G. W. & Dewey, V. C. (1963) *Comp. Biochem. Physiol.* **8**, 331–334
- Warburg, C. F., Wakeel, M. & Wilton, D. C. (1982) *Lipids* **17**, 230–234
- Wilton, D. C. (1983) *Biochem. J.* **216**, 203–206
- Zander, J. M., Greig, J. B. & Caspi, E. (1970) *J. Biol. Chem.* **245**, 1247–1253