Inhibition of thiamine transport in baker's yeast by methylene blue

A. Iwashima, H. Nishimura and H. Nishino

Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamikyoku, Kyoto (Japan), 22 January 1980

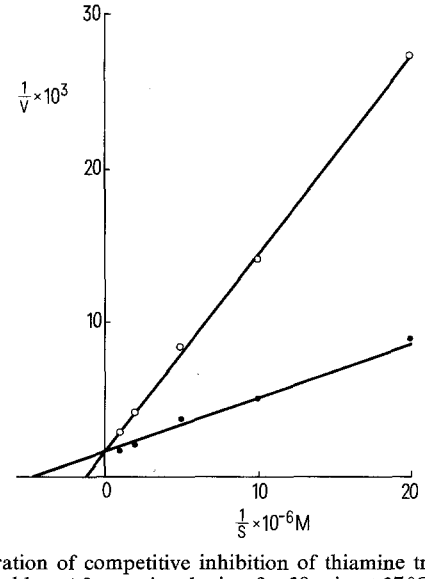
Summary. Methylene blue was found to inhibit thiamine transport competitively ($K_i = 0.63$ μM) in baker's yeast. The dye was also effective in abolishing the growth inhibition of *Saccharomyces cerevisiae* by pyrithiamine which is known to be taken up by a common transport system for thiamine in yeast cells. A possible mechanism for the inhibition by methylene blue of the thiamine transport system in baker's yeast is discussed.

It has been demonstrated that a specific transport system for thiamine is present in baker's yeast (*Saccharomyces cerevisiae*[1-3]). The experimental inhibition of thiamine transport by various analogs of thiamine suggested that an intact pyrimidine moiety of the thiamine molecule is necessary to bind to some component of the transport system, but this does not necessarily indicate transport[2,4]. Among the analogs tested pyrithiamine and dimethialium, which have a quaternary nitrogen atom adjacent to the pyrimidine moiety, were found to be accumulated as much as thiamine in yeast cells[3,5]. Since dimethialium, in particular, has a methyl group in place of a hydroxyethyl group at the 5-position of the thiazole moiety of thiamine and is not a substrate of thiamine pyrophosphokinase, these results strongly suggested that thiamine can be transported and accumulated without obligatory phosphorylation in yeast cells. Consequently it was of interest to investigate the effect of compounds with a quaternary nitrogen atom, other than thiamine derivatives, on yeast thiamine transport.

Methylene blue is an aromatic dye containing a quaternary nitrogen atom in the molecule and is known to be taken up by yeast cells. The present paper describes the evidence showing that methylene blue is a specific inhibitor of the thiamine transport system in baker's yeast.

As shown in table 1 thiamine transport in baker's yeast was markedly inhibited by micromolar concentrations of methylene blue added to the uptake medium simultaneously with [^{14}C]thiamine (1 μM) after preincubation for 30 min at 37 °C. It has been demonstrated that thiamine transport in baker's yeast is dependent upon the presence of energy sources such as glucose and is inhibited by several metabolic inhibitors as 2,4-dinitrophenol, KCN and iodoacetate[6]. As shown in table 2 the addition of these inhibitors to the uptake medium during preincubation caused stronger inhibition of thiamine transport than did simultaneous addition with thiamine to the medium. On the other hand, methylene blue differed from these inhibitors in that its inhibition was not increased by preincubation with it, but it was similar to the effect of pyrithiamine, an antagonist of thiamine. These results suggest that methylene blue might not inhibit energy production in the cells for thiamine transport but affects the binding of thiamine to some component of the thiamine transport system. The figure shows the results of kinetic studies of the inhibition of thiamine transport by methylene blue. As seen in the figure methylene blue behaved as a competitive inhibitor: apparent K_m for thiamine is 0.20 μM and apparent K_i for methylene blue is 0.63 μM.

In the previous paper we showed evidence suggesting that pyrithiamine is taken up by yeast cells by a common transport system for thiamine[3]. Pyrithiamine uptake by yeast cells was also inhibited 32.5% and 80.8% by methylene blue at the concentration of 2 μM and 5 μM, respectively,



Demonstration of competitive inhibition of thiamine transport by methylene blue. After preincubation for 30 min at 37 °C, yeast cell suspensions (80 μg dry wt/ml) were incubated for 1 min with varying concentrations of [^{14}C]thiamine in the absence (—●—) or presence of 2 μM methylene blue (—○—).

Table 1. Effect of methylene blue on thiamine transport in baker's yeast

Addition	[^{14}C]Thiamine uptake (nmole/mg dry weight/2 min)	Inhibition (%)
None	0.72	-
Methylene blue		
1 μM	0.63	12.5
2 μM	0.33	54.2
5 μM	0.12	83.3
10 μM	0.04	94.4

The uptake of [^{14}C]thiamine was assayed as previously described[2]. Methylene blue was added to yeast cell suspensions simultaneously with 1 μM [^{14}C]thiamine.

Table 2. Comparison of inhibitory effect of methylene blue with those of several metabolic inhibitors and pyrithiamine on thiamine transport

Addition	[^{14}C]Thiamine uptake (nmole/mg dry weight/2 min)
None*	0.01
None	0.66
2,4-Dinitrophenol, 0.2 mM	0.46 (0.08)
KCN, 2 mM	0.44 (0.21)
Iodoacetate, 0.2 mM	0.62 (0.46)
Pyrithiamine, 2 μM	0.18 (0.22)
Methylene blue, 2 μM	0.30 (0.31)
5 μM	0.10 (0.12)

The data in parentheses show [^{14}C]thiamine uptake when methylene blue, pyrithiamine or each metabolic inhibitor was added to the uptake medium during preincubation for 30 min at 37 °C. * Glucose was removed from the uptake medium.

whereas the uptake of biotin, which has been demonstrated to occur by an active process as well as thiamine[7], was not affected by the dye at all under the same conditions. Furthermore, it was found that the effect of methylene blue is exerted on growing yeast. As shown in table 3 the growth inhibition of *S. cerevisiae* by pyrithiamine was abolished by the addition of methylene blue to the growth medium. The abolition of the inhibition by the dye was apparently competitive with pyrithiamine and the ratio of the concentration of methylene blue to pyrithiamine for a half maximal growth inhibition was 35–40.

From the results described above, methylene blue appears to be a specific inhibitor of thiamine transport in baker's yeast. Although the mechanism of its inhibition is unknown it might be suggested that there may be competition between a quaternary nitrogen atom of methylene blue and that of thiamine for a negatively charged group of some surface component of the cell membrane which is involved in thiamine transport in baker's yeast.

Table 3. Effect of methylene blue on growth inhibition of *Saccharomyces cerevisiae* by pyrithiamine

Addition to growth medium		Growth
Pyrithiamine (μM)	Methylene blue (μM)	(optical density at 560 nm)
0	0	0.350
1.0	0	0.015
1.0	10	0.015
1.0	20	0.065
1.0	40	0.170
1.0	100	0.350
2.5	100	0.200
5.0	100	0.100
10.0	100	0.040

Growth studies were carried out using Wickerham's synthetic medium with thiamine omitted as previously described[3]. Growth was measured turbidimetrically at 560 nm. The cells grown with methylene blue were washed twice with water, resuspended and measured.

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Protein anabolism in endometrium and myometrium during the growth of induced deciduoma in rats

U. Tarachand, R. Sivabalan[1] and J. Eapen

Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay 400 085 (India), 17 December 1979

Summary. Protein anabolism in the endometrium and myometrium was studied during the growth of induced deciduoma in terms of incorporation of [^{14}C]-leucine into proteins. The data show that the specific activity of the proteins was highest 2 days after decidualisation. Protein synthesis in the endometrium studied on that day as a function of time after injection of the labelled amino acid showed a steady increase during the first 2 h. Cycloheximide (2 mg/kg b.wt) administration produced nearly 95% inhibition of protein synthesis in endometrium as well as in myometrium.

A variety of biochemical changes precede nidatory response in the uterus[2]. Proliferation and transformation of endometrial stromal cells occur as a consequence of the entry of blastocysts. The artificially induced decidual cell reaction forms a convenient model for observing changes during implantation[3]. This reaction in a primed uterus, however, is not dependent on the blastocyst alone but can be elicited by uterine trauma[4]. With a view to understanding some of the changes associated with biochemical transformations during the morphogenesis of deciduoma, a study on protein anabolism in the endometrium and myometrium was carried out. The present paper reports the results of in vivo incorporation of a labelled amino acid into proteins of endometrium and myometrium during growth and regression of deciduoma. In addition, data on the sensitivity of endometrium and myometrium to cycloheximide, a potent inhibitor of protein synthesis, are presented.

Materials and methods. 75-day-old virgin female Wistar rats weighing approximately 280 g were allowed to mate with proven males of the same strain. The presence of spermatozoa in the vaginal smear the next morning was taken as an indication of successful mating and was designated as day 1 of pregnancy. Deciduoma was induced with arachis oil on day 5 of pregnancy, as reported earlier[5]. Animals were maintained on a balanced laboratory diet and water ad libitum.

DL-Leucine-[1-^{14}C] (sp. act. 53.7 mCi/mmole, obtained from the Isotope Division, Bhabha Atomic Research Centre) was injected i.p. at a dose of 0.2 μCi/g b.wt. Cyclohex-

Effect of cycloheximide on incorporation of [^{14}C]-leucine into proteins of endometrium and myometrium on day 7 of pseudopregnancy

Status	Endometrium cpm/mg protein	% inhibition	Myometrium cpm/mg protein	% inhibition
Control	7584 ± 209 (5)*	–	4717 ± 99 (10)	–
Cycloheximide-treated	377 ± 30 (7)	95.3	237 ± 26 (7)	94.08

* Mean ± SEM (n).