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Methylthioadenosine and polyamine biosynthesis in a *Saccharomyces cerevisiae meu1Δ* mutant

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Abstract

As part of our studies on polyamine biosynthesis in yeast, the metabolism of methylthioadenosine was studied in a mutant that lacks methylthioadenosine phosphorylase (*meu1Δ*). The nucleoside accumulates in this mutant and is mainly excreted into the culture medium. Intracellular accumulation of the nucleoside is enough to account for the inhibition of spermidine synthase and thus to indirectly regulate the polyamine content of the *meu1Δ* cells. By comparing the results with this mutant with a *meu1Δ spe2Δ* mutant that cannot synthesize spermidine or spermine, we showed that >98% of methylthioadenosine is produced as a byproduct of polyamine synthesis (i.e., from decarboxylated *S*-adenosylmethionine). In contrast, in $MEU1^{+}$ $SPE2^{+}$ cells methylthioadenosine does not accumulate and is metabolized through the methionine salvage pathway. Using a *met15Δ* mutant we show that this pathway (i.e., involving polyamine biosynthesis and methylthioadenosine metabolism) is a significant factor in the metabolism of methionine, accounting for 15% of the added methionine.
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Keywords: Methionine; Methylthioadenosine; Methionine salvage pathway; Polyamines; Spermidine; Spermine; Yeast

Methylthioadenosine is formed from decarboxylated *S*-adenosylmethionine during the biosynthesis of spermidine and spermine [1–3], and is then converted back to methionine by the "methionine salvage" pathway [4–9]. The first step in the methionine salvage pathway is carried out by 5′-methylthioadenosine/*S*-adenosylhomocysteine nucleosidase in some bacteria and plants, and by methylthioadenosine phosphorylase in mammals ([10] and references therein) and yeast [7,8]. The biosynthesis, metabolism, and physiological role of methylthioadenosine have been of particular interest because of its regulatory effect on polyamine biosynthesis [11–13] and because a number of tumor cell lines [14–17] lack the first enzyme in the methionine salvage pathway (methylthioadenosine phosphorylase).

As part of our overall interest on polyamine biosynthesis in yeast, we felt that it was important to test whether the biosynthesis of polyamines is the only pathway for the synthesis of methylthioadenosine in yeast, since other possible pathways have been reported in several in vitro studies [18–20]. In addition, we wanted to carry out quantitative studies to evaluate whether this pathway is a significant component in the overall utilization of methionine by yeast cells.

To answer these questions, we have taken advantage of the availability of a mutant of *Saccharomyces cerevisiae* (*meu1Δ*) that is unable to metabolize methylthioadenosine [21,22]. Using this mutant we have shown that essentially all of the methylthioadenosine formed in yeast is a product of polyamine biosynthesis and that this pathway represents 10–15% of the methionine used by the cell. In addition, we have shown that the amount of methylthioadenosine accumulated in the *meu1Δ* mutant is sufficient to account for the inhibition of spermidine biosynthesis reported in our recent paper on the effect of the *meu1Δ* mutation on polyamine regulation in *S. cerevisiae* [23].

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Materials and methods

Yeast strains and media. Yeast strains Y607 (*MATα his3 leu2 lys2 ura3 Δmeu1*::KANMX) and Y610 (*MATα his3 leu2 lys2 ura3 MEU1*$^{+}$) were obtained from the yeast deletion bank. Y614 (*MATα his3 leu2 ura3 Δspe2*::*LEU2 Δmeu1*::KANMX) was obtained by crossing Y607 with the *spe2Δ* mutant (Y343) previously described [24]. The strains were maintained on YPAD plates (1% yeast extract, 2% Bactopeptone, 2% dextrose, and 2% agar). Cultures were inoculated from YPAD plates into SD medium (6.7 g yeast nitrogen base, 20 g dextrose, and necessary supplements per liter) and harvested in the logarithmic phase of growth (0.8–1.0 OD_{600}). The cell pellets were extracted with 5 volumes of 10% perchloric acid.

Estimation of methylthioadenosine and polyamines. Methylthioadenosine was quantitated by reverse-phase chromatography on a dC18 column as described by Kamatani and Carson [11]. One hundred to 200 μl of the above perchloric acid extract or 200 μl of the spent culture medium was injected into a dC18 column (4.6 × 150 mm, Atlantis column, Waters, 3 μm particle size, part No. 186001342). The elution buffer (8% acetonitrile, 10 mM potassium phosphate, pH 6.0) was run at 0.8 ml/min, and the UV absorbance of the eluate was measured at 254 nm. Methylthioadenosine was eluted at 32.5 min. For further characterization of the eluted material, fractions from 31–34 min of the HPLC chromatography were collected after a larger injection (2 ml), dried, and the HPLC was repeated. This material was then characterized by electrospray ionization mass spectrometry (LCT Premier time-of-flight (TOF) mass spectrometer, Waters) and UV absorbance spectra. The polyamine content of these extracts was determined by HPLC on a cation exchanger as previously described [25].

For the experiments on growth in limiting methionine, strain Y606 (*MAT*a *his3 leu2 met15 ura3 meu1Δ*::KANMX) was obtained from the yeast deletion bank; this mutant is blocked in the utilization of sulfur for the synthesis of methionine and cysteine, Y606 inoculated in the same SD medium as above, but with the addition of 5–10 μM (final concentration) of L-methionine. Cultures were harvested after growth stopped (OD_{600} = 0.3–0.5). Aliquots of the spent medium were then assayed for methylthioadenosine as above. For [^{35}S]methionine incorporation, Y606 cultures were grown in 10 μM unlabeled L-methionine and 0.25 μCi of L-[^{35}S]methionine per milliliter of culture medium (GE Amersham, 1000 Ci/mmol, SJ1015) until the growth stopped (0.5 OD_{600}). The cells were harvested and treated with 10% perchloric acid as above. Aliquots of this extract and of the spent medium were fractionated on the dC18 column. The peak fractions (31–33 min) were collected and aliquots were counted in a scintillation counter. The perchloric acid-insoluble pellet was washed and dissolved in 0.1 M Tris–HCl (pH 9.5), prior to counting.

Results

Methylthioadenosine accumulates in meu1Δ SPE2^{+} *cultures and is absent in meu1Δ spe2Δ cultures*

An *meu1Δ* culture (Y607) was grown to an optical density of 0.9. Analysis of the cell extracts and of the culture medium showed that these cultures accumulated methylthioadenosine, and that most of the accumulated material was excreted into the medium (Figs. 1 and 2). The elution profile showed a clear peak of methylthioadenosine, which is well separated from other components present in either the culture medium or in the cell extract (Fig. 1). Even though most of the accumulated methylthioadenosine was excreted into the culture medium (1155 nmol in 385 ml of culture medium), about 2% of the total accumulation was found in cells (23 nmol in 754 mg of wet weight of cells or approximately 680 μl of cell volume) giving a calculated intracellular concentration of 3×10^{-5} M.

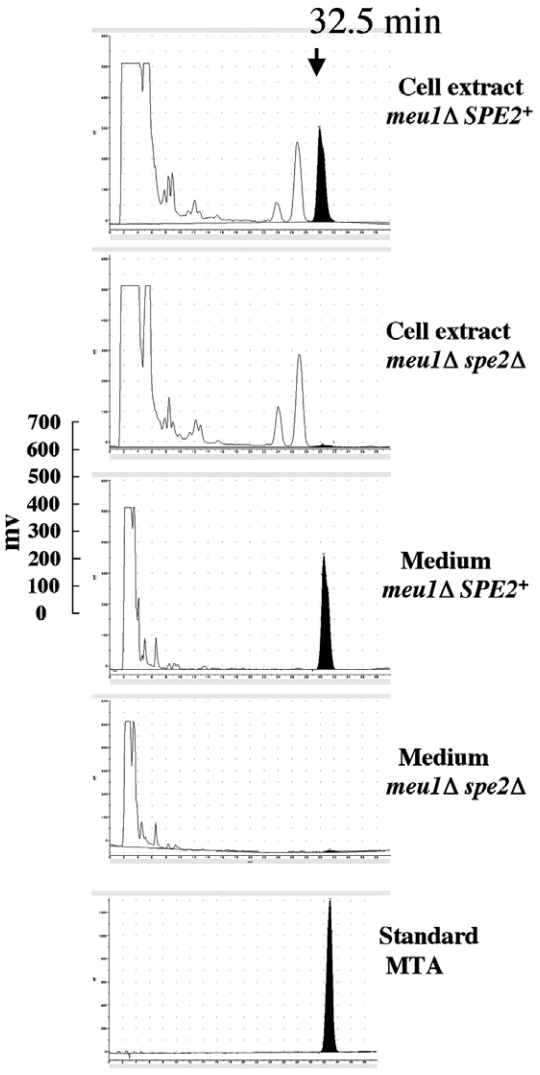

Fig. 1. Methylthioadenosine accumulates in *meu1ΔSPE2*$^{+}$ cultures and is absent in *meu1Δ spe2Δ* cultures. Cultures of Y607 (*meu1Δ SPE2*$^{+}$) and of Y614 (*meu1Δ spe2Δ*) were grown to an OD of 0.9. Cells were harvested and perchloric acid extracts of the cells were prepared as described in Materials and methods. Aliquots of the cell extract (100 μl) or of the spent medium (200 μl) were then assayed by HPLC dC18 chromatography as described in Materials and methods. These chromatograms are representatives of several assays. The arrow (32.5 min) indicates the peak of standard methylthioadenosine, and the scale is shown on the left side.

As opposed to the results with the *meu1Δ* cultures, only traces of methylthioadenosine were found in the cells or medium of *meu1Δ spe2Δ* (Fig. 1) or *MEU1*$^{+}$ *SPE2*$^{+}$ cultures (data not shown).

Methylthioadenosine was identified by its position on the HPLC chromatograph (32.5 min), by UV absorption spectrum (peak: 257 nm), by mass spectrometry (found 298.0978 Da; calculated for M + 1 298.0974 Da)[1], and by labeling with L-[^{35}S]methionine (see below).

[1] We thank Dr. John Lloyd, Proteomics and Mass Spectrometry Facility, NIDDK, for performing the mass spectrometric analysis.