Methionine catabolism in *Saccharomyces cerevisiae*

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Abstract

The catabolism of methionine to methionol and methanethiol in *Saccharomyces cerevisiae* was studied using 13C NMR spectroscopy, GC-MS, enzyme assays and a number of mutants. Methionine is first transaminated to a keto-(methylthio)butyrate. Methionol is formed by a decarboxylation reaction, which yields methional, followed by reduction. The decarboxylation is effected specifically by Ydr380wp. Methanethiol is formed from both methionine and a keto-(methylthio)butyrate by a demethiolase activity. In all except one strain examined, demethiolase was induced by the presence of methionine in the growth medium. This pathway results in the production of a keto-butyrate, a carbon skeleton, which can be re-utilized. Hence, methionine catabolism is more complex and economical than the other amino acid catabolic pathways in yeast, which use the Ehrlich pathway and result solely in the formation of a fusel alcohol.

Introduction

We have previously studied the catabolism of leucine, valine, isoleucine, phenylalanine and tryptophan to the corresponding fusel alcohols in yeast (Dickinson et al., 1997, 1998, 2000, 2003). In minimal media, in all cases examined, the reaction sequence is essentially the Ehrlich pathway (Ehrlich, 1907; Neubauer & Fromherz, 1911), in which the amino acid is first transaminated to an α-ketoacid. This is then decarboxylated to an aldehyde, which, in turn, is reduced to the alcohol end product. Biochemical specificity of the catabolic pathway is achieved by the selective use of one or more of the decarboxylases encoded by *PDC1* (YLR044c), *PDC5* (YLR134w), *PDC6* (YGR087c), *KID1* (YDL080c) and *ARO10* (YDR380w). In leucine catabolism, Ydl080cp is the major decarboxylase, accounting for approximately 94% of the decarboxylation of α-ketoisocaproate; the minor decarboxylase is Ydr380wp (Dickinson et al., 1997, 2003). In valine catabolism, any one of the three isozymes of pyruvate decarboxylase (Pdc1p, Pdc5p, Pdc6p) will decarboxylate α-ketoisovalerate (Dickinson et al., 1998) and, in isoleucine catabolism, any one of these five decarboxylases is sufficient for the conversion to the end product, active amyl alcohol (Dickinson et al., 2000). Ydl080cp has no role in the catabolism of the aromatic amino acids phenylalanine and tyrosine. The decarboxylation of 3-phenylpyruvate and 3-indolepyruvate can be accomplished by any one of the remaining four decarboxylases (Dickinson et al., 2003).

Although methionine will serve as a sole nitrogen source for *Saccharomyces cerevisiae*, the catabolism of this amino acid poses two important questions. First, the occurrence of the purported methionine catabolites, methionol and methanethiol (both of which are known to brewers of beers) cannot be explained by the Ehrlich pathway alone. It is conceivable that methionol could be produced by the Ehrlich pathway but the formation of methanethiol requires the involvement of the C-S-lyase-like enzyme(s) or γ-demethiolase. Certain fungi, including some yeasts, possess such activities (Helinck et al., 2000; Spinnler et al., 2001). Arfi et al. (2002) have mentioned this type of activity and methanethiol production has also been shown in *S. cerevisiae* (Perpète et al., 2002). Alternatively, methanethiol may be formed by entirely nonezymic processes. It is known that during the ‘mashing’ process in beer making (during which malt is heated in water), methionine is transformed to methional by purely
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chemical means (a so-called ‘Strecker’ degradation). Further oxidation can then lead to the formation of methanethiol (Balance, 1961; Yu & Ho, 1995). Second, even if the formation of methionol occurs entirely via the Ehrlich pathway, it needs to be established how the complement of α-ketoacid decarboxylases is deployed in methionine catabolism. This paper addresses both questions.

**Materials and methods**

**Strains, media and cultural conditions**

The strains used are shown in Table 1. IWD72 is the wild-type strain, which we have used for all previous studies of amino acid catabolism and is the parent of mutant strains JRD815-1.2, ESH380w, 56.9.2 and 53.1.4, which harbour mutations in genes encoding the thiamine pyrophosphate-dependent decarboxylases or lipoamide dehydrogenase (53.1.4) (Dickinson et al., 1997, 1998, 2000, 2003). Strain X2180-1A is the parent of strains CD230, CD231, CD238, CD242-2A and CD278, which all harbour mutations in either known or putative genes of sulphur metabolism. Analyses of mutants were always made by comparison to their appropriate wild-type parent. The brewing strains BRAS212 and BRAS291 were included to evaluate whether their appropriate wild-type parent. The brewing strains BRAS212 and BRAS291 were included to evaluate whether there were differences in demethiolase activity between these and the ‘academic’ parent strain X2180-1A, because it has frequently been found in the past that brewing strains have important differences from academic strains. Starter cultures were grown in a medium comprising 1% (w/v) yeast extract (Difco, Oxford, UK), 2% (w/v) Bactopeptone (Difco) and 2% (w/v) carbon source. For studies of methionine catabolism, cells were grown in minimal medium containing (L⁻¹) 1.67 g of Yeast Nitrogen Base without amino acids and ammonium sulfate (Difco), 20 g of L-methionine and either 20 mL of ethanol or 20 g of glucose for the carbon source. Experiments involving ¹³C labelling used [U,¹³C₅]methionine (98 atom% ¹³C) that was obtained from Cambridge Isotope Laboratories (Cambridge, MA). Auxotrophic requirements were supplied as required at 20 µg mL⁻¹. Liquid cultures were grown in conical flasks filled to 40% nominal capacity in a gyrorotatory shaker. Agar (2%, w/v) was used to solidify media. Methionine (labelled and unlabelled) was sterilized by autoclaving in the growth media at 121 °C for 15 min. All cell cultures were at 30 °C unless specified otherwise.

**NMR and GC-MS analyses**

¹³C NMR spectra were recorded with Waltz-16 ¹H proton decoupling at 20 °C in culture supernatants [adjusted to pH 6 and containing 15% (v/v) ²H₂O and 50 mM potassium phosphate buffer] as described previously (Dickinson et al., 1997) using a Bruker (Coventry, UK) AMX360 NMR spectrometer operating at 90.5 MHz. Signals were identified by comparison with spectra of standard compounds recorded under identical conditions and by ‘spiking’ (addition of small amounts of bona fide compounds to experimental samples). Chemical shifts (δ, p.p.m) are reported relative to external tetramethylsilane in C²HCl₃; addition of sodium trimethylsilylpropanesulfonate gave a methyl signal at −2.6 p.p.m under the conditions used here. Methionol concentration was determined in culture filtrates using GC-MS as described before (Dickinson et al., 2000), except that the oven temperature was started at 100 °C followed by a temperature increase of 5 °C min⁻¹ to 150 °C; then a

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype or description</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>JRD815-1.2</td>
<td>MATA pdc1Δ::LEU2 pdc5::URA3 pdc6::TRP1 ydl080c::kanMX4</td>
<td>Dickinson et al. (1997)</td>
</tr>
<tr>
<td>ESH380w</td>
<td>MATA his3Δ1 pdc1Δ::LEU2 pdc5::URA3 pdc6::TRP1 ydr380w::kanMX4</td>
<td>Dickinson et al. (2003)</td>
</tr>
<tr>
<td>56.9.2</td>
<td>MATA pdc1Δ::LEU2 pdc5::URA3 pdc6::TRP1 ydl080c::kanMX4 ydr380w::kanMX4</td>
<td>Dickinson et al. (2000)</td>
</tr>
<tr>
<td>53.1.4</td>
<td>MATA lys2::hisG met8 ura3 lpd1::URA3</td>
<td>Dickinson et al. (2000)</td>
</tr>
<tr>
<td>IWD72</td>
<td>MATA</td>
<td>Dickinson &amp; Dawes (1992)</td>
</tr>
</tbody>
</table>
| CD230 | MATA ura3 yll058w::URA3 | Y. Surdin-Kerjanb)
| CD231 | MATA ura3 his3 leu2 trp1 ygr021w::HIS3 | Y. Surdin-Kerjanb)
| CD238 | MATA ura3 yhr112c::URA3 | Y. Surdin-Kerjanb)
| CD242-2A | MATA ura3 yml080w::URA3 | Y. Surdin-Kerjanb)
| CD278 | MATA ura3 yhr055w::kanMX4 | Y. Surdin-Kerjanb)
| X2180-1A | MATA | Y. Surdin-Kerjanb)
| BRAS212 | Bottom-fermenting Brewer’s yeast | UBIAc
| BRAS291 | Top-fermenting Brewer’s yeast | UBIAc

*Genotypes are given for all of the academic strains; a description of the yeast type is given for the commercial strains.

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cUBIA. The collection of Brewer’s yeasts, Unité de Brasserie et des Industries Alimentaires, Université Catholique de Louvain, Louvain-la-Neuve, Belgium.
Assay of methionine aminotransferase activity

Cultures of wild-type strain IWD72 were grown in minimal medium with 2% (w/v) glucose as the carbon source and 2% (w/v) methionine as nitrogen source to OD_{600nm} 3.0, which corresponds to late-exponential phase (see Fig. 3). The cells were then harvested by centrifugation (2000 g at 25 °C for 5 min), the growth medium was decanted and the cells were resuspended in 3 mL of ice-cold buffer A (50 mM potassium phosphate buffer, pH 7.4, containing 2 mM EDTA) and 6.5 U of glutamate dehydrogenase in a total volume of 25 mL. The formation of NADPH was measured spectrophotometrically at 340 nm. Standard solutions of monosodium glutamate gave a linear response over the range 0–1000 /µM per assay. The methionine aminotransferase activity was calculated from the difference in glutamate between 10 min and zero time samples.

Assay of demethiolase activity

Starter cultures were grown in a complete medium (YPD) containing 1% (w/v) yeast extract, 2% (w/v) Bactopeptone and 2% (w/v) glucose. For studies of demethiolase activity, cells were grown both in minimal medium with 2% (w/v) methionine as nitrogen source [with additional auxotrophic supplements at 0.1% (w/v) where required] and in YPD medium. After cultivation for 36 h at 28 °C, the cells were harvested by centrifugation [5 min at 4080 g in a Sorvall (Stevenage, UK) RC5b centrifuge using a GSA rotor] and washed twice. A crude extract was obtained using a Braun MSK homogenizer following the method of Dufour et al. (1988). Demethiolase activity was determined using a modified protocol developed from those of Laasko & Nurminsko (1976) and Ferchichi et al. (1985). Enzymically produced methanethiol was trapped in situ by 5,5'-dithiobis (2-nitrobenzoic acid) and the resulting complex was monitored at 595 nm using a Shimadzu (Kyoto, Japan) UV-102-02 spectrophotometer. Assay blanks contained a substrate but no cell extract. To calculate specific activities, protein content was measured using the method of Bradford (1976).

Results

Methionine catabolism in a wild-type strain

[U-13C5]methionine is chemically and biochemically equivalent to unlabelled methionine (in which the natural abundance of 13C atoms is only 1.1%). The use of a 13C-enriched substrate allows the detection of carbon atoms in metabolites whose concentrations would otherwise be too small to be observed by 13C NMR. Figure 1 shows the 13C NMR spectrum of a culture supernatant of wild-type strain IWD72 that had been cultured for 24 h in a minimal medium in which glucose was the carbon source and [U-13C5]methionine was the sole nitrogen source. All of the carbon atoms of methionine were observed because this substrate was uniformly labelled. All of the other resonances observed except for terminal CH3 groups were at least doublets because the uniformly labelled substrate gives rise to intermediates that are uniformly labelled; hence, every carbon atom is adjacent to a labelled carbon atom. Important resonances observed include: the C-2 of α-ketoglutarate (potassium salt), 0.1 mM pyridoxal phosphate, 50 mM potassium phosphate buffer pH 7.8 and cell extract in a total volume of 0.9 mL. The assay was initiated by the addition of methionine (6.67 µM in 0.1 mL) and was run for 10 min, after which time it was terminated by plunging the tubes into boiling water for 3 min. The tubes were then cooled in ice for 10 min. Aliquots were removed and the glutamate that had been formed was measured using Proteus NADP-dependent glutamate dehydrogenase (Sigma, Poole, UK). The glutamate dehydrogenase assay was run at 25 °C and comprised 0.1 M imidazole buffer, pH 7.9, containing 0.9 mM EDTA, 0.12 mM ADP, 0.24 mM NADP and 6.5 U of glutamate dehydrogenase in a total volume of 3.0 mL. The formation of NADPH was measured spectrophotometrically at 340 nm. Standard solutions of monosodium glutamate gave a linear response over the range 10–150 µmol per assay. The methionine aminotransferase activity was calculated from the difference in glutamate between 10 min and zero time samples.
Fig. 1, indicating that similar amounts of the compound were formed in the two experiments. Hence, the formation of methionine sulfoxide from methionine is an abiotic process, that requires moderate heat (30 °C) and aeration.

Another minor compound that could not be identified and displayed a multiplet at 62.5 p.p.m was also abiotic in origin. Neither of these needs to be considered in schemes of the catabolism of methionine by yeast.

There were no natural abundance signals due to glucose, indicating that it had all been consumed, but the singlets at 57.5 and 16.8 p.p.m (C-1 and C-2, respectively, of ethanol produced from the glucose) were evident.

The metabolites identified and the positions that were labelled with 13C indicate that in yeast the catabolism of methionine to methionol proceeds as shown in Fig. 2. The 13C NMR results do not reveal whether the conversion of methionine to KMBA is accomplished by transamination or deamination, because both would lead to identical labelling of KMBA. However, enzyme assays established that transamination is involved as methionine aminotransferase activity was readily detectable in cells that had been grown in glucose minimal medium with methionine as sole nitrogen source. The mean specific activity of duplicate determinations performed on three separate cultures was 43 (SD = 1.7) nM methionine consumed mg protein⁻¹ s⁻¹. The KMBA is then presumably decarboxylated to β-(methylthio)propionaldehyde (‘methional’), that is subsequently reduced to methionol. It is also apparent that KMBA is reduced to α-hydroxy-γ-(methylthio)butyrate in a fashion analogous to the reduction of α-ketoisocaproic acid to α-hydroxyisocaproic acid in leucine catabolism (Dickinson et al., 1997) and α-ketoisovaleric acid to α-hydroxyisovaleric acid in valine catabolism (Dickinson et al., 1998) in this yeast.

**Branched-chain α-ketoacid dehydrogenase is not involved in methionine catabolism**

In higher eukaryotes, catabolism of the branched-chain amino acids leucine, isoleucine and valine involves an initial transamination with α-ketoglutarate to produce glutamate and the corresponding α-ketoacids. These α-ketoacids are then oxidatively decarboxylated by branched-chain α-ketoacid dehydrogenase.

*Saccharomyces cerevisiae* possesses branched-chain α-ketoacid dehydrogenase activity, which is very active when the cells are growing in a complex medium with glycerol as the carbon source (Dickinson & Dawes, 1992) but is not involved in the catabolism of leucine, valine, isoleucine, phenylalanine or tryptophan in minimal media when any
one of these amino acids is the sole nitrogen source (Dickinson et al., 1997, 1998, 2000, 2003). Hence, it seemed worthwhile examining whether this enzyme is involved in methionine catabolism. If branched-chain α-ketoacid dehydrogenase were involved in the catabolism of methionine to methionol, the oxidative decarboxylation of KMBA would yield β-(methylthio)propionyl CoA, which on subsequent hydrolysis by acyl CoA hydrolase would produce β-(methylthio)propionate. This intermediate was not observed in the NMR spectrum of the culture medium of the wild-type strain (Fig. 1) nor of the mutant 53.1.4 (data not shown), which is defective in lipoamide dehydrogenase and hence lacks branched-chain α-ketoacid dehydrogenase, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and glycine decarboxylase (Dickinson et al., 1986; Repetto & Tzagoloff, 1991; Sinclair & Dawes, 1995; Lanterman et al., 1996). The lpd1Δ mutant produced wild-type levels of methionol when grown in minimal medium in which methionine was the sole source of nitrogen (Table 2). These results are consistent with the notion that branched-chain α-ketoacid dehydrogenase is not involved in the catabolism of methionine to methionol.

**Ydr380wp is specifically required for the decarboxylation of α-keto-γ-(methylthio)butyrate**

The wild-type strain and a variety of mutants were grown in minimal medium with methionine as the sole nitrogen source. Each strain grew with different kinetics (Fig. 3).
The wild-type strain (IWD72) grew fastest with glucose as the carbon source but was much slower when ethanol was the carbon source as it displayed a diauxic lag of over 100-h duration before restarting proliferation to reach a stationary-phase density, that was slightly higher than when growing on glucose. Strain ESH380w (pdc1 pdc5 pdc6 ydr380w) grew slowest, only reaching stationary phase by about 800 h. Despite these differences, all of the strains were clearly capable of growth using methionine as the sole source of nitrogen (Fig. 3). Methionol concentrations in the growth media were recorded when each strain had reached stationary phase. The wild-type strain produced approximately half the amount of methionol (measured both as the simple concentration in the medium and on a per cell basis) when using ethanol as a carbon source compared with glucose (Table 2). It is informative to compare the amounts of methionol produced by the three strains JRD815-1.2, ESH380w and 56.9.2, which all lack the three pyruvate decarboxylases Pdc1p, Pdc5p and Pdc6p as well as Ydl080c and/or Ydr380wp. Both ESH380w and 56.9.2 produced no methionol. Thus, when Ydr380wp was inactive, no methionol was produced. From this, we conclude that Ydr380wp is required specifically for the decarboxylation of KMBA to methional.

Table 2. The amount of methionol produced by various yeast strains growing in minimal medium with methionine as the sole source of nitrogen

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carbon source</th>
<th>Relevant genotype</th>
<th>Methionol (µg mL⁻¹)</th>
<th>Methionol (µg cell⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JRD815-1.2</td>
<td>Ethanol</td>
<td>pdc1 pdc5 pdc6 ydl080c</td>
<td>163</td>
<td>1.3</td>
</tr>
<tr>
<td>ESH380w</td>
<td>Ethanol</td>
<td>pdc1 pdc5 pdc6 ydr380w</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>56.9.2</td>
<td>Ethanol</td>
<td>pdc1 pdc5 pdc6 ydl080c ydr380w</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>53.1.4</td>
<td>Glucose</td>
<td>lpd1</td>
<td>742</td>
<td>13.5</td>
</tr>
<tr>
<td>IWD72</td>
<td>Ethanol</td>
<td>Wild-type</td>
<td>592</td>
<td>6.4</td>
</tr>
<tr>
<td>IWD72</td>
<td>Glucose</td>
<td>Wild-type</td>
<td>1094</td>
<td>11.1</td>
</tr>
</tbody>
</table>

All pdc1 pdc5 pdc6 triple mutants cannot utilize glucose; these had ethanol (2%) as the carbon source. A lpd1 mutant cannot use ethanol; this strain had glucose (2%) as the carbon source. Methionol was determined by GC-MS when each strain had reached stationary phase. The values are the means of two separate experiments. Differences between samples were < 5%.

The wild-type strain (IWD72) grew fastest with glucose as the carbon source but was much slower when ethanol was the carbon source as it displayed a diauxic lag of over 100-h duration before restarting proliferation to reach a stationary-phase density, that was slightly higher than when growing on glucose. Strain ESH380w (pdc1 pdc5 pdc6 ydr380w) grew slowest, only reaching stationary phase by about 800 h. Despite these differences, all of the strains were clearly capable of growth using methionine as the sole source of nitrogen (Fig. 3). Methionol concentrations in the growth media were recorded when each strain reached stationary phase. The wild-type strain produced approximately half the amount of methionol (measured both as the simple concentration in the medium and on a per cell basis) when using ethanol as a carbon source compared with glucose (Table 2). It is informative to compare the amounts of methionol produced by the three strains JRD815-1.2, ESH380w and 56.9.2, which all lack the three pyruvate decarboxylases Pdc1p, Pdc5p and Pdc6p as well as Ydl080c and/or Ydr380wp. Both ESH380w and 56.9.2 produced no methionol. Thus, when Ydr380wp was inactive, no methionol was produced. From this, we conclude that Ydr380wp is required specifically for the decarboxylation of KMBA to methional.
Methanethiol is produced by demethiolation of both methionine and α-keto-γ-(methylthio)butyrate

The results described so far have not revealed the origin(s) of methanethiol. Due to its high volatility, this compound would not be expected to be retained in the culture medium that was subjected to $^{13}$C NMR analysis. For similar reasons, the GC-MS analytical protocol used to measure methionol would not detect methanethiol. However, experiments in which crude extracts of yeast cells were assayed for demethiolase revealed that this activity is genuinely present in S. cerevisiae. Considerable quantities of demethiolating activities were measured when either methionine or KMBA were used as the substrate (Table 3). Besides the two industrial strains (bottom-fermenting strain BRAS212 and top-fermenting strain BRAS291), several mutant strains (see Table 1 for details) were used to try to discover the gene(s) responsible for this activity. However, only slight differences were observed between the mutants. Further experiments are thus needed to elucidate the gene(s) required for methanethiol production. The data obtained were analysed by one-way analysis of variance (ANOVA) and Tukey's pairwise comparisons. This confirmed that when the cells were grown with methionine as the sole source of nitrogen, the bottom-fermenting strain BRAS212 had the highest activity of all the strains tested using methionine ($F = 18.85; P < 0.001$) and KMBA as the substrate ($F = 47.8; P < 0.001$).

Performing overall two-way ANOVA comparisons of the activities in cell-free extracts obtained from cells grown in different media (Table 3) revealed significant increases in the demethiolase activities of all of the strains except BRAS291 when they were grown with methionine as the sole nitrogen source, compared with growth in a complete YPD medium. This was true when using both methionine as the substrate in the assay ($F = 274.79; P < 0.001$) or KMBA as the substrate ($F = 311.23; P < 0.001$). The exceptional demethiolase activity of strain BRAS291 (higher in complex medium) seen only when using KMBA as the substrate was also statistically significant ($T = -13.11; P = 0.006$). It may be worthwhile examining whether this is a general property of top-fermenting yeasts.

### Discussion

This study has revealed the way in which methionine is catabolized to methionol and methanethiol. The routes can be considered as an elaboration of the Ehrlich pathway with branches at methionine and at KMBA (Fig. 2). A ‘conventional Ehrlich pathway’ would involve the initial transamination of methionine to KMBA. The KMBA is then decarboxylated to methional, which is subsequently reduced to methionol. The $^{13}$C NMR analysis indicated a branch in which KMBA is reduced to α-hydroxy-γ-(methylthio) butyrate. Similar reductions of α-ketoisocaproic acid to α-hydroxyisocaproic acid, α-ketoisovaleric acid to α-hydroxyisovaleric acid and 3-phenylpyruvate to 3-phenyllactate (in leucine, valine and phenylalanine catabolism, respectively) have been observed previously in *Saccharomyces cerevisiae* (Dickinson et al., 1997, 1998, 2003). However, the significance of these reactions is not understood because analogous processes have not been observed in the catabolism of isoleucine and tryptophan (Dickinson et al., 2000, 2003). Branches at methionine and KMBA comprise demethiolase activity. The demethiolation of KMBA is especially noteworthy because, besides yielding methanethiol, it would also...

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Methionine</th>
<th>α-Keto-γ-(methylthio)butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methionine as the sole nitrogen source</td>
<td>Complete YPD medium</td>
</tr>
<tr>
<td>Strain</td>
<td>Specific activity</td>
<td>SEM</td>
</tr>
<tr>
<td>BRAS212</td>
<td>43.90</td>
<td>0.54</td>
</tr>
<tr>
<td>BRAS291</td>
<td>17.30</td>
<td>1.05</td>
</tr>
<tr>
<td>X2180-1A</td>
<td>21.63</td>
<td>0.54</td>
</tr>
<tr>
<td>CD230</td>
<td>31.24</td>
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</tr>
<tr>
<td>CD231</td>
<td>22.44</td>
<td>0.62</td>
</tr>
<tr>
<td>CD238</td>
<td>27.61</td>
<td>0.80</td>
</tr>
<tr>
<td>CD242-ZA</td>
<td>22.06</td>
<td>0.54</td>
</tr>
<tr>
<td>CD278</td>
<td>19.44</td>
<td>1.05</td>
</tr>
</tbody>
</table>

*aThe specific activity is expressed as nmol of methanethiol formed mg protein$^{-1}$ s$^{-1}$.

Demethiolase activity was measured using both methionine and α-keto-γ-(methylthio)butyrate as the substrate. The results are the means of three experiments.

SEM, standard error of measurement.
result in the formation of α-ketobutyrate. The advantage of this is that α-ketobutyrate is a useable carbon skeleton because it serves as the precursor in the biosynthesis of isoleucine. The 'conventional Ehrlich pathway' seen in yeast yields only fusel alcohols whose carbon skeletons cannot be further metabolized. Despite the fact that α-ketobutyrate is a product of methionine catabolism in yeast, this pathway is very different from that used by mammals. In mammals, methionine is first demethylated to homocysteine, which then reacts with serine to form cystathionine. The cystathionine is subsequently cleaved to yield cysteine, α-ketobutyrate and NH$_4^+$. In vitro enzyme assays have indicated the demethiolation of methionine and KMBA.

In both the wild-type and ipd1 mutant, the $^{13}$C NMR analyses detected no β-(methylthio)propionate and the levels of methionol were similar in both strains. This clearly indicated that branched-chain α-ketoacid dehydrogenase is not involved in the catabolism of methionine to methanol. However, work in Daran and Pronk's laboratory has indicated the existence of β-(methylthio)propionate in culture supernatants of cells grown on methionine in glucose-limited and ethanol-limited chemostats (Vuralhan et al., 2005). The β-(methylthio)propionate formed in these highly respiratory conditions derives from the oxidation of methional and not from the oxidative decarboxylation of KMBA by branched-chain α-ketoacid dehydrogenase to β-(methylthio)propionyl CoA and subsequent hydrolysis by acyl CoA hydrolase (Vuralhan et al., 2005).

The specific use of Ydr380wp to accomplish the decarboxylation of KMBA is a further illustration of the fact that S. cerevisiae deploys its five decarboxylases (Pdc1p, Pdc5p, Pdc6p, Ydl080cp and Ydr380wp) highly selectively according to the nitrogen source that is being catabolized. In leucine catabolism, Ydl080cp was deduced to be the major decarboxylase and Ydr380wp the minor enzyme (Dickinson et al., 1997, 1999, 2000). In valine catabolism, any one of the three isozymes of pyruvate decarboxylase (Pdc1p, Pdc5p, Pdc6p) is sufficient to decarboxylate α-ketoisovalerate (Dickinson et al., 1998) and, in isoleucine catabolism, any one of the five enzymes can be used (Dickinson et al., 2000). In catabolism of the aromatic amino acids phenylalanine and tryptophan, Ydl080cp has no role. The decarboxylation of 3-phenylpyruvate and 3-indolepyruvate, respectively, can be accomplished by any one of Pdc1p, Pdc5p, Pdc6p or Ydr380wp (Dickinson et al., 2003). Very recent work has shown that YDR380w encodes an α-ketoacid decarboxylase activity with broad substrate specificity (Vuralhan et al., 2005). This is an important insight into amino acid catabolism in yeast. Transcript analysis has revealed that of the family of decarboxylases comprising Pdc1p, Pdc5p, Pdc6p, Ydl080cp and Ydr380wp, only YDR380w was up-regulated when phenylalanine, leucine or methionine was the sole nitrogen source compared with growth on ammonia, proline or asparagine (Vuralhan et al., 2005). This allows a simple explanation for our observation reported here that only Ydr380wp is used in methionine catabolism. It also indicates that the provision of adequate activities of the other relevant decarboxylases in the catabolism of leucine (mainly by Ydl080cp) and phenylalanine (by any of Pdc1p, Pdc5p, Pdc6p and Ydr380wp) have more complicated explanations.

The ability of strains with mutations in YDR380w to grow in a minimal medium with methionine as the sole nitrogen source demonstrates that the initial transamination step is sufficient to enable yeast to produce all of the other nitrogen-containing metabolites needed for growth. In this respect, methionine catabolism in yeast is similar to the catabolism of leucine, valine, isoleucine, phenylalanine and tryptophan, where the 'conventional Ehrlich pathway' operates and whose sole purpose seems to be the provision of nitrogen catabolites whereas the resulting carbon skeletons are apparently wasted (Dickinson et al., 1997, 1998, 2000, 2003). Strains carrying a ydr380w mutation presumably grow more slowly compared with strains lacking this mutation, due to their increased levels of both KMBA and α-hydroxy-γ-(methylthiobutyrate).

No attempt was made in this study to identify the enzyme(s) involved in the reduction of β-(methylthio)propionaldehyde (methional) to methionol. Previous work had already shown that, as long as S. cerevisiae has one enzyme out of Adh1p, Adh2p, Adh3p, Adh4p, Adh5p or Sfa1p, it is capable of growth on a minimal medium with a single amino acid as the nitrogen source and the concomitant formation of the appropriate fusel alcohol (Dickinson et al., 2003). In addition, mutants of this type are not expected to offer a straightforward metabolomic approach to inferring the enzyme(s) involved in the reduction of methional to methionol, because an earlier study had shown that an adh1 adh2 adh3 adh4 quadruple mutant had reduced levels of aldehydes due to an increased NADPH-dependent aldehyde reductase (Evellin et al., 1999).

We assayed a variety of mutants defective in either known or putative genes of sulphur metabolism. This included strain CD230 (with a disruption in ORF YLL058w, which encodes cystathionine γ-synthase), strain CD231 (disrupted in YGR021w, which encodes a hypothetical mitochondrial cysteine synthase), strain CD238 (disrupted in YHR112c, which encodes cystathionine β-lyase), strain CD242-2A (disrupted in the ORF YML082w, which encodes an integral membrane protein containing a novel cysteine motif) and strain CD278 (disrupted in YFR055w, which encodes a hypothetical cystathionine β-lyase). Despite these attempts, we were unable to assign a gene to the demethiolase activity. Our expectation had been that the absence of demethiolase activity in a specific mutant would enable us to infer the gene that encodes that activity. Perhaps this failure is explained by genetic redundancy: maybe two or more genes

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must be mutated together in a single strain to completely negate activity. Constructing strains with combinations of mutations may provide the answer. As an alternative approach, work is also underway to purify the activity so that the gene(s) can be identified via proteomics. Identifying the structural genes and purifying the enzyme will both help to explain how the increased demethiolase activity (which was up to fourfold higher when methionine was the sole nitrogen source compared with that in cells grown on YPD) is achieved. Microarray data comparing the transcriptomes of methionine-grown and YPD-grown cells may also provide a way to identify candidate genes.

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