A Study On The α-Methylacyl-CoA Racemase (AMACR) Elimination Reaction And Its Application To Inhibitor Testing

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Introduction

Branched-chain fatty acids are common in the diet and are found in medicines such as ibuprofen and related drugs. Metabolism of branched-chain fatty acids requires that the centres bearing the methyl groups possess $S$-stereochemical configuration, but centres with $R$-configuration are produced in the body and are found in the diet. Similarly, ibuprofen and related drugs require $S$-configuration for anti-inflammatory activity, but these drugs are often given as a mixture of isomers. The enzyme α-methylacyl-CoA racemase (AMACR) catalyses $R$- to $S$-conversion of 2-methylacyl-CoA derivatives of fatty acids, enabling $\beta$-oxidation. Similarly, acyl-CoA derivatives of ibuprofen and similar drugs undergo $R$- to $S$-conversion, resulting in pharmacological activation. 1,2

AMACR levels are increased in all prostate cancers, some colon cancers and other cancers. 1-3 In prostate cancer, higher AMACR levels result in higher rates of proliferation 4 and androgen-independent growth 5 and the enzyme is recognised as a novel drug target. However, few inhibitors have been identified, largely due to the difficulties in measuring enzyme activity which makes it difficult to quantify drug potency. 6 Recently, we showed that AMACR is able to catalyse the irreversible elimination of hydrogen fluoride from 3-fluoro-2-methylacryl-CoA (Scheme 1).

Scheme 1: The AMACR catalysed elimination reaction.

This new reaction allows easier measurement of AMACR activity by $\text{H}^1$ NMR since the signals for the substrate and product 2-methyl group appear in different places in the spectrum. 6 The reaction can potentially be adapted for colorimetric or fluorescent measurement of AMACR activity by the use of chromogenic substrates or fluorescent sensors. This study 7 focuses on using the elimination reaction to measure drug potency and investigates development of a colorimetric or fluorescent assay.

Results and Discussion

Recombinant human AMACR 1A was pre-incubated with a number of known AMACR substrates or inhibitors before addition of 3-fluoro-2-methylacryl-CoA substrate 1 at 100 μM final concentration. After 1 hour incubation at 20°C the reaction was terminated by heating and conversion of 1 determined by $\text{H}^1$ NMR. All known substrates and inhibitors reduced conversion of 1 compared to positive controls (Table 1).

Table 1: Inhibition of AMACR activity by known substrates and inhibitors. Results are normalised to conversion levels in positive controls in order to enable ranking of inhibitors. Values are means ($n=2$) ± SD.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Relative conversion in presence of inhibitor</th>
<th>Relative reduction compared to no inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>74.9 ± 0.4%</td>
<td>25.1%</td>
</tr>
<tr>
<td>91.6 ± 2.0%</td>
<td></td>
<td>8.4%</td>
</tr>
<tr>
<td>91.4 ± 1.2%</td>
<td></td>
<td>8.9%</td>
</tr>
<tr>
<td>92.0 ± 8.6%</td>
<td></td>
<td>8.0%</td>
</tr>
<tr>
<td>88.8 ± 4.0%</td>
<td></td>
<td>11.2%</td>
</tr>
<tr>
<td>8.8 ± 1.8%</td>
<td></td>
<td>83.2%</td>
</tr>
<tr>
<td>9.5%</td>
<td></td>
<td>&gt;95%</td>
</tr>
<tr>
<td>8.8 ± 5.4%</td>
<td></td>
<td>11.8%</td>
</tr>
<tr>
<td>10</td>
<td>&lt;5%</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>11</td>
<td>&lt;5%</td>
<td>&gt;95%</td>
</tr>
</tbody>
</table>

The presence of known substrates 3-7 in the assay all resulted in modest reductions in conversion of 1, due to competitive inhibition. In contrast, inhibitor 8 almost completely abolished conversion of 1, consistent with it being a known potent inhibitor (Figure 1). Protein modifying reagents 9-11 also reduced activity, in some cases abolishing enzyme activity. 3

![Figure 1: Reduction in conversion of 1 to 2 by the presence of 8. A. No inhibitor; B. With 100 μM 8.](image)

Synthesis of potential colorimetric substrates with aromatic side-chains was performed. 3 The acyl-CoA substrate 12 was converted to the expected unsaturated product 13 by AMACR (Figure 2), but the resulting chromophore had a maximum wavelength of ~340 nm. Conversion of the precursor acid to the acyl-CoA substrate was also difficult due to non-enzymatic elimination of the fluoride, and hence this approach was abandoned.

![Figure 2: Conversion of aromatic substrate 12 to eliminated product 13. A. Live enzyme; B. Negative control containing heat-inactivated enzyme.](image)

![Figure 3: Fluoride sensors identified to measure AMACR activity.](image)

Conclusions

Conversion of 3-fluoro-2-methylacyl-CoA substrates by AMACR is reduced in the presence of inhibitors. However the stability of colorimetric substrates limits their use in AMACR activity assays. The investigated fluoride sensors lacked sufficient reproducibility and sensitivity for use in an assay.

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References