Plant biotechnology

Caffeine synthase gene from tea leaves

Caffeine is synthesized from purine nucleotides. The two final steps of the pathway, in which two methyl groups are added successively to 7-methylxanthine to produce theobromine and then caffeine, are catalysed by caffeine synthase, a bifunctional enzyme comprising two N-adenosylmethionine-dependent N-methyltransferase activities. It has been hard to purify and isolate caffeine synthase and other enzymes of this pathway because they are extremely labile, but the amino-terminal sequence of caffeine synthase from young tea leaves has now been reported.

We used the RACE (rapid amplification of complementary DNA ends) technique to degenerate gene-specific primers based on the amino-terminal sequence of caffeine synthase to obtain a 1.31-kilobase cDNA. The 5’ untranslated sequence of the cDNA fragment was isolated by 5’ RACE. The isolated cDNA, termed TCSI (GenBank accession no. AB031280), consists of 1,438 base pairs and encodes a protein of 369 amino acids. The deduced amino-acid sequence of TCSI shares a small amount of sequence similarity with other N-, S- and O-methyltransferases from plants and microorganisms, but considerably more with the salicylic acid O-methyltransferase (41.2%).

To determine whether our cDNA encodes an active caffeine synthase enzyme, we expressed TCSI in Escherichia coli and incubated lysates of the bacterial cells with a variety of xanthine substrates in the presence of S-adenosylmethionine as methyl donor. We found that the substrate specificity of the recombinant enzyme was very similar to that of the native enzyme purified from young tea leaves (Table 1), with the recombinant enzyme mainly catalysing 3-N-methylation and 1-N-methylation of the purine ring of mono- and dimethylxanthines. There was no 7-N-methylation activity when xanthosine was the methyl acceptor. These results indicate that TCSI encodes caffeine synthase.

The cloning of the caffeine synthase gene is an important advance towards the development of transgenic caffeine-deficient Camellia sinensis and Coffea arabica plants through antisense messenger RNA technology or by gene silencing. It is possible that the health benefits of tea11, whose catechins and related polyphenols are thought to help protect against heart disease56, may be enhanced without the potentially hypertensive effects of caffeine44. Misako Kato*, Kouichi Mizuno†,*

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Table 1 Substrate specificity of recombinant and native caffeine synthase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Methylated product</th>
<th>N-methylation position</th>
<th>Recombinant CS</th>
<th>Native CS*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monomethylxanthines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Methylxanthine</td>
<td>Theobromine</td>
<td>3</td>
<td>100†</td>
<td>100</td>
</tr>
<tr>
<td>5-Methylxanthine</td>
<td>Theophylline</td>
<td>1</td>
<td>1.0</td>
<td>17.6</td>
</tr>
<tr>
<td>1-Methylxanthine</td>
<td>Theophylline</td>
<td>3</td>
<td>12.3</td>
<td>4.2</td>
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<tr>
<td><strong>Dimethylxanthines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theobromine</td>
<td>Caffeine</td>
<td>1</td>
<td>18.5</td>
<td>26.8</td>
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<tr>
<td>Theophylline</td>
<td>Caffeine</td>
<td>7</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Paraxanthine</td>
<td>Caffeine</td>
<td>3</td>
<td>230</td>
<td>210</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthosine</td>
<td>7-Methylxanthine</td>
<td>7</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

† Taken from ref. 5.

The full-length coding region for the tea leaf caffeine synthase (CS) protein was amplified into pET32at mammalian and the mouse embryonic extract inserted into E. coli (BL21). Recombinant CS protein was extracted by sonication of the transformed cells in 50 mM Tris-HCl pH 7.5 containing 1 mM EDTA, NaCl, and 0.1 M NaCl. The substrate specificity of recombinant CS was determined according to ref. 5. CS activity is expressed as a percentage of the activity on 7-methylxanthine.

*Caffeine synthase activity of the recombinant enzyme with 7-methylxanthine (100%) was 5.4 pmol mg⁻¹ protein; values represent the average of duplicate samples.

for the conservation of this threatened species5. During the breeding season, females favour the subtropical waters north of Crozet where tuna fisheries are located, whereas males prefer the colder waters at higher latitudes where fisheries for toothfish have been developed11. Birds spending their sabbatical year in sectors where long-line fisheries occur are at risk of being killed. This bizarre selection pressure, dependent perhaps on the whims of juvenile choice, means that only those wintering in zones without fisheries will survive in the long term. We now need to find out what factors lead to selection of sabbatical areas in the first place. In the meantime, we have to accept that even during their sabbatical adult birds are unlikely to change their habits to avoid the dangers from fishing.

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Kainate receptors and synaptic plasticity

Bortolotto et al. report that the kainate subtype of glutamate receptor is essential for the plasticity of certain types of synaptic transmission in the brain, which is of interest as these receptors were previously not thought to initiate plastic processes. In particular, a new antagonist (LY382884) was shown to act selectively against the GluR5 type of kainate receptor: in the presence of the GluR5 subunit of kainate receptors, only weakly expressed in the dentate gyrus and in area CA3 of the hippocampus, and that most (about 90%) of the cells generating the weak signal are GABAergic interneurons, thought not to be directly involved in the induction of mossy-fibre LTP. Given these combined results, we believe that GluR5 is unlikely to play a role in mossy-fibre synaptic transmission or plasticity; although LY382884 may antagonize GluR5-containing kainate receptors, its effect on mossy-fibre LTP is more likely to involve some other interaction of this drug with synaptic transmission.

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Bortolotto et al. reply — Nicoll et al. challenge our finding that kainate receptors are involved in mossy-fibre LTP in the hippocampus. Their argument is based on a discrepancy of our results with earlier work, as we show (our Fig. 3) that two kainate-receptor antagonists, kynurenate and CNQX, can also block mossy-fibre LTP. We do not dispute that it may be possible to induce mossy-fibre LTP in the presence of kainate-receptor antagonists under certain conditions. For this reason, we neither state nor implied that activation of kainate receptors is always necessary for mossy-fibre LTP. Concerning the resolving power of our experiments, it is important to consider three factors. First, although the effect of CNQX on the induction of LTP had to be assessed three hours after the tetanus owing to the slow washout of CNQX, we often included a non-tetanized input as a control, which also invariably returned to the same, pre-CNQX, level. Second, in our experiments with the selective AMPA-receptor antagonist GKY153655, we also had to measure LTP three hours after washout of the antagonist, and LTP was always observed in the tetanized input. Finally, the kynurenate experiments required only one hour for complete washout.

The relatively low expression of GluR5 messenger RNA in principal cells within the dentate gyrus and area CA3 was not a major concern for us because the relation between GluR5 gene expression and protein levels at this synapse is not known. In addition, as discussed previously, it is possible that the LY382884 class of compounds can also act on GluR5-lacking heteromeric assemblies of kainate receptors, such as GluR6-KA1, which have not been tested with these antagonists.

The fact that others have observed mossy-fibre LTP in the presence of kynurenate and CNQX has several possible explanations, the most likely being, we suspect, that involvement of GluR5-containing kainate receptors in mossy-fibre LTP can be bypassed in certain circumstances. To determine conditions under which LY382884 might fail to block the induction of mossy-fibre LTP, we have applied multiple trains at test intensity and find that LTP is always blocked by the antagonist; however, when we greatly increase the stimulus strength during the tetanus, some LTP remains.

Multiple routes for the induction of LTP at CA1 synapses, involving both NMDA and mGlu5 receptors, have already been reported. Crosstalk between receptors and their signalling cascades may make LTP more difficult to understand, but it provides synapses with a much richer array of mechanisms with which to build memories.

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