An onion enzyme that makes the eyes water

A flavoursome, user-friendly bulb would give no cause for tears when chopped up.

The irritating lachrymatory factor that is released by onions when they are chopped up has been presumed to be produced spontaneously following the action of the enzyme alliinase, which operates in the biochemical pathway that produces the compounds responsible for the onion's characteristic flavour[1–4]. Here we show that this factor is not formed as a by-product of this reaction, but that it is specifically synthesized by a previously undiscovered enzyme, lachrymatory-factor synthase. It may be possible to develop a non-lachrymatory onion that still retains its characteristic flavour and high nutritional value by downregulating the activity of this synthase enzyme.

Previous studies[1–4] indicated that alliinase from any source was the only enzyme needed to produce lachrymatory factor (propanthial *S*-oxide) from 1-propenyl-L-cysteine sulphoxide (PRENCSO), an important substrate in onion (*Allium cepa*) (Fig. 1a). The reactions from the intermediate sulphenic acid to propanthial *S*-oxide and thiosulphinate were presumed to be spontaneous because sulphenic acid is very unstable and has never been isolated.

When we added crude extract of alliinase from garlic (*A. sativum*) to PRENCSO, however, lachrymatory factor was not produced at all, and the yield of thiosulphinate increased. Because a crude preparation of alliinase from onion added to PRENCSO could generate the factor, we investigated whether some unknown component (possibly another enzyme) in the preparation could be involved in its formation.

The fraction with lachrymatory-factor-forming activity could be completely separated from the alliinase activity by passing the crude onion alliinase preparation through a hydroxyapatite column. Further purification of this fraction gave three distinct proteins, whose amino-terminal sequences we determined.

We used the RACE (rapid amplification of complementary DNA ends) technique with degenerate gene-specific primers deduced from one of the amino-terminal sequences to obtain a complete cDNA sequence. The full-length cDNA (GenBank accession no. AB089203) consisted of 737 base pairs, with a predicted gene product of 169 amino acids.

As all of the amino-terminal sequences determined for the three proteins matched the predicted open reading frame of the gene, we assumed that these three proteins were the products of a single gene. DNA-database searches revealed that the gene encoded a new enzyme, which we named lachrymatory-factor synthase.

When we expressed the lachrymatory-factor synthase gene in *Escherichia coli*, the resulting recombinant protein exhibited the expected enzymatic activity. The enzyme showed high substrate specificity, producing lachrymatory factor from only *trans*-PRENCSO, which is the naturally occurring form in onion (Fig. 2).

Lachrymatory factor was detected only when all three components, namely purified alliinase, PRENCSO and lachrymatory-factor synthase, were present in the reaction mixture (Fig. 1b). Omission of the synthase from the reaction mixture resulted in an increased yield of thiosulphinate, the condensation product of 1-propenylsulphenic acid.

Figure 2 Don't cry for me: inhibiting the biosynthesis of lachrymatory factor could give rise to a no-more-tears formula for onions.

These results indicate that it might be possible to develop a non-lachrymatory onion by suppressing the lachrymatory-factor synthase gene while increasing the yield of thiosulphinate. Thiosulphinate is responsible for the flavour of fresh onion, and is converted to compounds reported to exert hypolipodaemic[5] and antiplatelet-aggregation effects[6,7]. Although down-regulating alliinase itself would also lead to a non-lachrymatory onion, its flavour and nutritional value might be compromised.

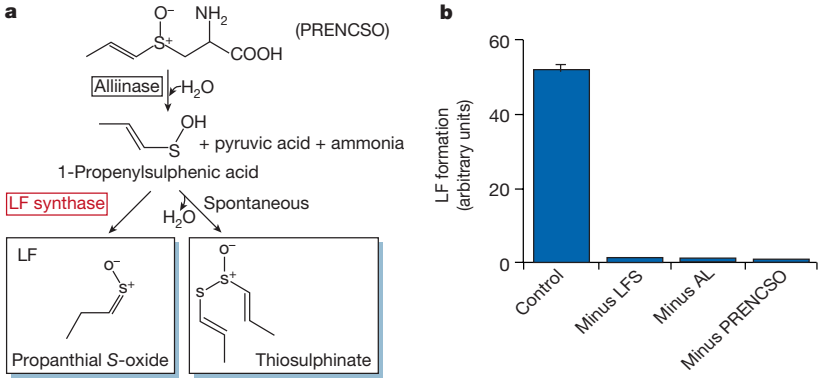

Figure 1 Obligatory involvement of the enzyme lachrymatory-factor synthase in the production of lachrymatory factor (LF) in onion. **a,** The chemical reactions that occur when onion bulbs are cut. Propanthial *S*-oxide (LF, responsible for stimulating tears) and thiosulphinate (which determines the onion's flavour and leads to the production of biologically active compounds in the onion) are generated; the step involving the newly discovered enzyme LF synthase is indicated. Previously, lachrymatory factor was believed to be formed spontaneously following the action of alliinase after cutting. **b,** LF is formed only in the presence of its synthase (LFS), alliinase (AL) and PRENCSO (control, left). If any one of the three components in the reaction mixture is omitted, no LF is detectable (right three bars). The LF produced was separated on an HPLC column, and the amount was determined from the flow-through peak area (arbitrary units).

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