Cloning and sequencing of a cDNA encoding a taste-modifying protein, miraculin

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A cDNA clone encoding a taste-modifying protein, miraculin (MIR), was isolated and sequenced. The encoded precursor to MIR was composed of 220 amino acid (aa) residues, including a possible signal sequence of 29 aa. Northern blot analysis showed that the mRNA encoding MIR was already expressed in fruits of *Richadella dulcifica* at 3 weeks after pollination and was present specifically in the pulp.

INTRODUCTION

*Richadella dulcifica* (*Rd*) is a native shrub of tropical West Africa. It yields red berries, called ‘miracle fruit’, that have the unusual property in modifying a sour taste into a sweet taste. For example, lemons elicit sweet taste after the pulp of the berries has been chewed. In previous studies, the active principle of miracle fruit, which is called miraculin (MIR), was completely purified (Theerasilp and Kurihara, 1988) and its aa sequence was determined (Theerasilp et al., 1989). MIR is a 191-aa glycoprotein. The structures of *N*-linked oligosaccharides (Takahashi et al., 1990) and the sites of disulfide bridges (Igeta et al., 1991) were also determined. In the present study, we report the cloning and nt sequence of the MIR-encoding cDNA. The cDNA can now be used not only for large-scale production of MIR but also for analysis by site-directed mutagenesis of variant proteins.

EXPERIMENTAL AND DISCUSSION

(a) MIR cDNA cloning and sequencing

Using a probe encoding part (Glu98 Thr217) of MIR (Fig. 1), we screened a cDNA library derived from the pulp of *Rd* berries and finally obtained 13 clones out of 3.0 x 10^5 plaques. One clone with a 801-bp cDNA insert was subjected to nt sequencing (Sanger and Coulson, 1975). The clone contained a single ORF of 660 bp. The aa sequence determined in a previous study (Theerasilp et al., 1989) matched the deduced sequence from aa 30 to 220, except for one residue. The N-terminal extension of 29 aa (Met-Ala) is rich in hydrophobic aa and appears to be a signal sequence. Thus, the encoded precursor to MIR seems to be processed post-translationally at the N terminus.

The aa sequence deduced from the nt sequence of *MIR* cDNA differed by only one aa from that determined by...
the direct analysis of the aa sequence of the purified protein (Theerasilp et al., 1989). The aa 129 was deduced to be Trp from the nt sequence analysis but was determined to be Ser by automatic Edman degradation of MIR (Theerasilp et al., 1989). The difference might be due to microheterogeneity and/or allelic polymorphism of the gene for MIR or, alternatively, to an error in sequencing of the protein.

(b) Northern blot analysis

Northern blot analysis (Lehrach et al., 1977) was performed to examine the expression of MIR mRNA. As shown in Fig. 2A, a single band of approx. 1.1 kb was detected at a nearly constant intensity throughout maturation from 3 weeks after pollination. As reported previously, the MIR protein was detected 8 weeks after pollination by an immunologic method with an antibody raised against MIR (Nakajo et al., 1988). A possible explanation for the discrepancy between the timing of synthesis of the MIR protein and the expression of the MIR mRNA is that the translation and/or posttranslational modification of the product of the MIR gene is strictly regulated. As shown in Fig. 2B, the band of MIR mRNA was detected only in the analysis of the pulp. It is, therefore, apparent that the mRNA is expressed specifically in pulp and not in any other tissues.

(c) Conclusions

(1) A 0.8-kb cDNA from the pulp of Rd berries has been sequenced and shown to contain the MIR gene.

(2) The deduced aa sequence shows that the precursor of MIR is composed of 220 aa, including a signal sequence of 29 aa.

(3) The aa 129 is deduced to be Trp from the nt sequence analysis but was determined to be Ser by the direct analysis of the aa sequence of MIR (Theerasilp et al., 1989).

(4) Northern blot analysis showed that the MIR mRNA was already expressed in Rd fruits at 3 weeks after pollination and was present specifically in the pulp.
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REFERENCES


