BIOCATALYSIS

EDDS lyase sweetening your day

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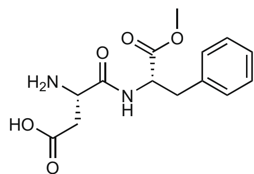
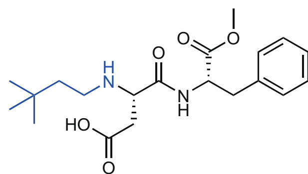
One of the most used artificial low-calorie sweeteners

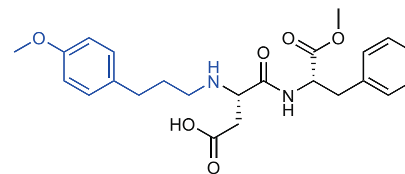


Aspartame
200 times sweeter than sucrose

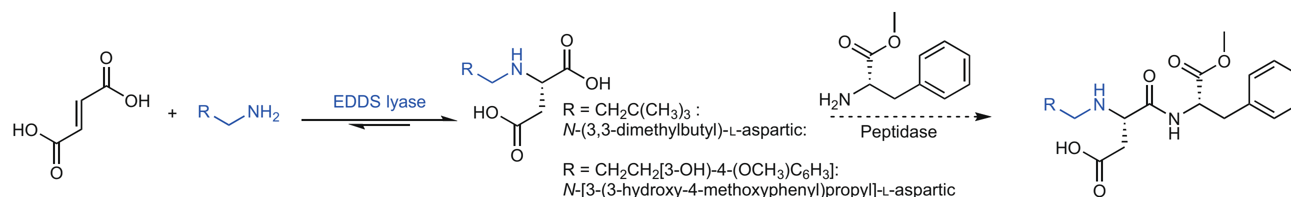
Derivatives of aspartame are up to 100-fold sweeter



Neotame
7,000–13,000 times sweeter than sucrose



Advantame
20,000 times sweeter than sucrose



Artificial low-calorie sweeteners are widely used in foodstuffs with the aim of reducing sugar consumption. One of the most used artificial sweeteners is the dipeptide aspartame. Neotame and advantame are recently approved sugar substitutes that are obtained via derivatization of aspartame with branched *N*-alkyl- or *N*-arylalkyl groups, respectively (pictured, top). These modifications increase the sweetness — with advantame being around 100 times sweeter than aspartame.

A biocatalytic asymmetric strategy for the sustainable and step-economic synthesis of these compounds was lacking. Now, Poelarends and colleagues report that EDDS lyase can convert fumaric acid to optically pure *N*-(3,3-dimethylbutyl)-L-aspartic acid and *N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L-aspartic acid, which can potentially be subsequently converted to neotame and advantame, respectively, by a peptidase-catalysed amide-bond-coupling reaction (pictured, bottom). First, the authors showed that wild-type EDDS lyase allows for the synthesis of *N*-(3,3-dimethylbutyl)-L-aspartic acid and *N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L-aspartic acid under excess of the amine substrate with high conversion and excellent enantioselectivity, but reaction times were long (7 days). Therefore, to enhance the hydroamination activity of EDDS lyase, protein engineering — based on the structure of EDDS lyase in complex with its natural substrate (*S*,*S*)-ethylenediamine-*N*,*N*′-disuccinic acid ((*S*,*S*)-EDDS) — was applied. Two residues (Asp290 and Tyr320) that were assumed to position the amine substrate in the active site were chosen for mutation. The mutant EDDS(D290M/Y320M) showed a 1,140-fold increase in activity over the wild-type enzyme. To rationalize the effect of the mutations, crystal structures of this enzyme variant were solved and the neotame precursor was docked into the wild-type and mutant crystal structure. Favourable apolar–apolar contacts of the mutated residues with the 3,3-dimethylbutyl moiety lead to a stronger binding and presumably a more productive binding of the amine compound.

Lyases are enzymes that usually catalyse the breaking of bonds. This work is another example that shows that engineering the reverse reaction of enzymes can be a powerful approach. Here, it allowed fast and easy access to optically pure precursors of valuable molecules that could find application in daily life. For example, with 0.05 mol% loading of the engineered biocatalyst, the neotame precursor *N*-(3,3-dimethylbutyl)-L-aspartic acid was produced with 96% conversion and >99% enantiomeric excess after only 2.5 hours.

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