SUMMARY ~~OF THE INVENTION~~

[0005] The present disclosure relates to engineered enone reductase polypeptides having altered enzyme properties relative to wildtype enone reductases. These engineered enone reductase polypeptides are capable of reducing an α,β unsaturated compound, such as an α,β unsaturated ketone, aldehyde, ester or nitrile to the corresponding saturated ketone, aldehyde, ester or nitrile.

[0005a] In one aspect, there is provided an enone reductase polypeptide comprising an amino acid sequence that is at least 95% identical to a reference sequence of SEQ ID NO: 8, which is stable to treatment conditions of 50% isopropanol at 30°C and/or 10-20% isopropanol at 40°C, and which is capable of reducing 1-cyclohex-2-enone to cyclohexanone with improved activity as compared to the enone reductase polypeptides of SEQ ID NO:4 and SEQ ID NO:6.

[0005b] In one ~~aspect~~embodiment, the enone reductases of the disclosure comprise a chimeric polypeptide of enone reductase 2 (ERED 2) of SEQ ID NO:4 and enone reductase 3 (ERED 3) of SEQ ID NO:6. In some embodiments, the chimeric polypeptide can also comprise a chimeric polypeptide of enone reductase 1 (ERED 1) of SEQ ID NO:2, enone reductase 2 (ERED 2) of SEQ ID NO:4, and enone reductase 3 (ERED 3) of SEQ ID NO:6. The chimeric polypeptides described herein are characterized by improvements in thermal and/or solvent stability. In some embodiments, the chimeric enone reductase polypeptides are stable to treatment conditions of 50% isopropanol at 30°C and/or 10-20% isopropanol at 40°C. In some embodiments, the chimeric enone reductases are capable of reducing or converting 1-cyclohex-2-enone to cyclohexanone. In some embodiments, the chimeric polypeptides are also capable of reducing or converting methyl (E)-but-2-enoate to methyl butanoate.

[0006] The present disclosure provides an enone reductase polypeptide comprising an amino acid sequence that is selected from the group consisting of: (a) an amino acid sequence that is at least 80% identical to a reference sequence selected from the group consisting of SEQ ID NOs: 8, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216 and 217; (b) an amino acid sequence encoded by a nucleic acid sequence that hybridizes under stringent conditions to a reference nucleic acid sequence selected from the group consisting of SEQ ID NOs: 7, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, and 215, or a complementary sequence thereof.

[0007] In some embodiments, the enone reductases of the present ~~invention~~disclosure can have one or more residue differences as compared to a reference enone reductase, such as the chimeric enone reductases characterized by improved thermal and/or solvent stability. In some embodiments, the residue differences can occur at one or more of the following residue positions: X5; X10; X28; X38; X40; X44; X75; X83; X117; X119; X122; X124; X147; X148; X153; X154; X179; X209; X240; X248; X251; X252; X255; X259; X294; X295; X296; X297; X302; X304; X305; X311; X315; X330; X333; X339; X358; X369; X376; X379; X384; X386; X397; X399; and X400. The presence of certain amino acids at these residue positions are associated with altered enzyme properties, including, among others,

[0020a] In one aspect, there is provided a polynucleotide encoding the enone reductase polypeptide as defined above.

[0021] In another ~~aspect~~embodiment, the present disclosure provides polynucleotides encoding the engineered enone reductases described herein, and polynucleotides that hybridize to such polynucleotides under highly stringent conditions. The polynucleotide can include promoters and other regulatory elements useful for expression of the encoded engineered enone reductases, and can utilize codons optimized for specific desired expression systems. Exemplary polynucleotides include, but are not limited to, the sequence of SEQ ID NO: 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, or 215.

[0021a] In one aspect, there is provided an expression vector comprising the polynucleotide as defined above operably linked to a control sequence.

[0021b] In another aspect, there is provided a host cell comprising the expression vector as defined above.

[0021c] In another aspect, there is provided a method for making the enone reductase polypeptide , the method comprising culturing a host cell transformed with the polynucleotide as defined above under conditions suitable for the expression of the enone reductase polypeptide.

[0022] In another ~~aspect~~embodiment, the present disclosure provides host cells comprising the polynucleotides and/or expression vectors described herein, and methods for culturing or incubating the host cells to produce the engineered enone reductase polypeptides. The host cells may be *E. coli* or they may be a different organism. The host cells can be used for the expression and isolation of the engineered enone reductase enzymes described herein, or, alternatively, they can be used directly for the conversion of an α,β unsaturated substrate to the corresponding saturated product.

[0022a] In one aspect, there is provided a method of reducing an α, β unsaturated compound, the method comprising:

(a) providing an α,β unsaturated compound selected from the group consisting of an α,β unsaturated ketone, an α,β unsaturated aldehyde, an α,β unsaturated nitrile, and an α,β unsaturated ester; and (b) contacting the α, β unsaturated compound with the enone reductase polypeptide as defined above under reaction conditions suitable for conversion of the α, β unsaturated compound to the corresponding saturated product compound selected from the group consisting of a saturated ketone, a saturated aldehyde, a saturated nitrile, and a saturated ester.

[0023] In a further ~~aspect~~embodiment, the present disclosure provides methods of using the enone reductase polypeptides described herein to reduce or convert an α,β unsaturated compound selected from the group consisting of a ketone, a nitrile, an ester, and a nitrile to the corresponding saturated ketone, nitrile, ester, or nitrile.

BRIEF DESCRIPTION OF THE FIGURES

[0024]Figure 1 illustrates a reaction carried out by enone reductases of the present invention.

[0025] Figure 2 provides an alignment of the nucleic acid sequence encoding ERED 1 (top row: SEQ ID NO:1), ERED 2 (middle row: SEQ ID NO:3), and ERED 3 (bottom row: SEQ ID NO:5), showing regions of nucleic acid sequence homology. The underlined nucleotides represent regions of differences in the sequences.

DETAILED DESCRIPTION

[0026] The present disclosure provides engineered enone reductase polypeptides, polynucleotides encoding the polypeptides and methods of using the polypeptides for the