[0009] Furthermore, Hytonen et al., (2005), J. Biol. Chem., 280: 10228-10233 reported heat stabilization of avidin without the formation of disulfide bonding. A chimera of avidin and AVR4 (protein encoded by Avidin-related gene 4) having higher heat resistance than avidin was formed to create an avidin mutant ChiAVD (I117Y), which exhibited increased residual biotin-binding activity of up to 98% after a treatment at 99.9°C for 32 min (residual activity: 4% for avidin, 72% for AVR4).

[0010] Almost all of the heat-resistant avidin mutants are prepared using an insect cell expression system using baculovirusm, while streptavidin mutants are prepared using an *E. coli* expression system, which requires a step of solubilization from insoluble inclusion bodies during the process. Thus, such proteins as described above, any of which require considerable costs and efforts for manufacturing them, have not yet been put into practical application.

CITATION LIST

PATENT LITERATURE

[0011] Patent Literature 1: International Publication No. WO02/072817

Patent Literature 2: International Publication No. WO2010/018859

NON-PATENT LITERATURE

[0012] Non-Patent Literature 1: Takakura, et al., (2009), FEBS J., 276: 1383-1397

Non-Patent Literature 2: Reznik et al., (1996), Nat. Biotechnol., 14: 1007-1011

Non-Patent Literature 3: Nordlund et al., (2003), J. Biol. Chem., 278: 2479-2483

Non-Patent Literature 4: Hytonen et al., (2005), J. Biol. Chem., 280: 10228-10233

SUMMARY OF INVENTION

TECHNICAL PROBLEM

[0013] The problem to be solved by the present ~~invention~~disclosure is to provide a modified tamavidin 2 which can be expressed highly in a soluble form in *E. coli* and has increased heat resistance which can maintain its protein structure even after being heated at a high temperature.

SOLUTION TO PROBLEM

[0014] The present inventors, who have diligently studied in order to solve the foregoing problems, have successfully obtained a modified tamavidin 2 which is highly expressed in a soluble form in *E. coli* and has increased heat resistance which can maintain its protein structure even after being heated at a high temperature and have arrived at the present ~~invention~~disclosure.

[0015] Specifically, in the present ~~invention~~disclosure, a modified biotin-binding protein, which has increased heat resistance strong enough to maintain the biotin-binding activity after a treatment at 99.9°C for 32 min, is obtained by replacing the 115th asparagine residue in the amino acid sequence (SEQ ID NO: 2) of native tamavidin 2 (hereinafter may be referred to as "TM2" in this specification) with cysteine (SEQ ID NO: 4) This modified tamavidin 2 had also improved resistance to aprotic polar organic solvent.

[0015a] In an aspect, there is provided a method for separation, concentration, capture, purification and/or detection of a biotin-linked substance, which comprises the following steps:

1) contacting a support, to which a modified biotin-binding protein is immobilized, with a biotin-linked substance, whereby the biotin-linked substance is bound to the support;

2) washing off contaminants which are not bound to the support with a solution containing 60% to 80% of aprotic polar organic solvent; and

3) collecting the biotin-linked substance which is bound to the support to thereby separate, concentrate, capture or purify the substance and/or detect the substance; wherein

the modified biotin-binding protein comprises an amino acid sequence represented by SEQ ID NO: 2 or an amino acid sequence having an identity of not less than 95% to the amino acid sequence of SEQ ID NO: 2, and has biotin-binding activity, provided that the modified biotin-binding protein has an asparagine residue at position 115 of SEQ ID NO: 2 replaced with cysteine, wherein the modified biotin-binding protein maintains biotin-binding activity of not less than 75% after a heat treatment at 99.9°C for 30 min, compared with that of before the treatment.

[0016] Preferred Embodiments of Present ~~Invention~~Disclosure

The present ~~invention~~disclosure includes the following preferred embodiments.

[Embodiment 1]

A modified biotin-binding protein comprising an amino acid sequence represented by SEQ ID NO: 2, an amino acid sequence having one or more amino acid mutations in the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having an identity of not less than 80% to the amino acid sequence of SEQ ID NO: 2 and having biotin-binding activity, wherein an asparagine residue at position 115 of SEQ ID NO: 2 is replaced with cysteine.

[Embodiment 2]

The modified biotin-binding protein according to embodiment 1, wherein the modified biotin-binding protein maintains the biotin-binding activity of not less than 75% after a heat treatment of 99.9°C for 30 min, compared with that of before the treatment.

[Embodiment 3]

The modified biotin-binding protein according to embodiment 1 or embodiment 2, wherein the modified biotin-binding protein maintains not less than 50% of the biotin-