

can be replaced without any significant effect on structure and function.

Recent work shows that changes can also be made to protein A (SpA) to effect similar properties. US patent application publication US 2005/0143566, which is hereby incorporated by reference in its entirety, discloses that when at least one asparagine residue is mutated to an amino acid other than glutamine or aspartic acid, the mutation confers an increased chemical stability at pH-values of up to about 13-14 compared to the parental SpA, such as the B-domain of SpA, or Protein Z, a synthetic construct derived from the B-domain of SpA (US 5,143,844, incorporated by reference in its entirety). The authors show that when these mutated proteins are used as affinity ligands, the separation media as expected can better withstand cleaning procedures using alkaline agents. Further mutations of protein A domains with the purpose of increasing the alkali stability have also been published in WO 2008/039141, JP 2006304633A, EP 1992692A1, EP 2202310A2, WO 2010/110288, WO 2012/086660, WO 2012/083425, WO 2012/087230 and WO 2014/146350, all of which are hereby incorporated by reference in their entireties. However, the currently available mutants are still sensitive to alkaline pH and the NaOH concentration during cleaning is usually limited to 0.1 M, which means that complete cleaning is difficult to achieve. Higher NaOH concentrations, which would improve the cleaning, lead to unacceptable capacity losses.

There is thus still a need in this field to obtain a separation matrix containing protein ligands having a further improved stability towards alkaline cleaning procedures.

Summary

There is provided a polypeptide with improved alkaline stability. This is achieved with a polypeptide as defined herein.

In an aspect, there is provided an Fe-binding polypeptide comprising a mutant of an Fe-binding domain of Staphylococcus Protein A (SpA), as defined by, or having at least 90% such as at least 95% or 98% identity to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:22, SEQ ID NO 51 or SEQ ID NO 52, wherein at least the asparagine or serine residue at the position corresponding to position 11 in SEQ ID NO:4-7 has been mutated to an amino acid selected from the group consisting of glutamic acid, lysine, tyrosine, threonine, phenylalanine, leucine, isoleucine, tryptophan, methionine, valine, alanine, histidine and arginine.

One advantage is that the alkaline stability is improved over the parental polypeptides, with a maintained highly selective binding towards immunoglobulins and other Fc-containing proteins.

In another aspect, there is provided an Fc-binding polypeptide which comprises a sequence as

5 defined by, or having at least 90% or at least 95% or 98% identity to SEQ ID NO 53.

KEX₁Q X₂AFYEILX₃LP NLTEEQRX₄X₅F IX₆X₇LKDX₈PSX₉ SX₁₀X₁₁X₁₂LAEAKX₁₃
X₁₄NDAQAPK (SEQ ID NO 53)

wherein individually of each other:

10 X₁=A or Q

X₂=E,K,Y,T,F,L,W,I,M,V,A,H or R

X₃=H or K

X₄=A or N

X₅=A or G

15 X₆=Q or E

X₇=S or K

X₈=E or D

X₉=Q or V

X₁₀=K,R or A

20 X₁₁=A,E or N

X₁₂=I or L

X₁₃=K or R

X₁₄=L or Y

25 There is also provide a multimer with improved alkaline stability, comprising a plurality of polypeptides. This is achieved with a multimer as defined herein.

In another aspect, there is provided a multimer comprising or consisting essentially of a plurality of polypeptides as defined above.

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There is also provided a nucleic acid or a vector encoding a polypeptide or multimer with improved alkaline stability. This is achieved with a nucleic acid or vector as defined herein.

5 In an aspect, there is provided a nucleic acid or a vector encoding a polypeptide or multimer as defined above.

There is also provided an expression system capable of expressing a polypeptide or multimer with improved alkaline stability. This is achieved with an expression system as defined herein.

10 In an aspect, there is provided an expression system, which comprises a nucleic acid or vector as defined above.

15 There is also provided a separation matrix capable of selectively binding immunoglobulins and other Fc-containing proteins and exhibiting an improved alkaline stability. This is achieved with a separation matrix as defined herein.

In another aspect, there is provided a separation matrix, wherein a plurality of polypeptides or multimers as defined above have been coupled to a solid support.

20 There is also provided an efficient and economical method of isolating an immunoglobulin or other Fc-containing protein. This is achieved with a method as defined herein.

In another aspect, there is provided a method of isolating an immunoglobulin, wherein a separation matrix as defined above is used.

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Further suitable embodiments of the disclosure are described herein.

Definitions

The terms “antibody” and “immunoglobulin” are used interchangeably herein, and are understood to include also fragments of antibodies, fusion proteins comprising antibodies or
5 antibody fragments and conjugates comprising antibodies or antibody fragments.

The terms an “Fc-binding polypeptide” and “Fc-binding protein” mean a polypeptide or protein respectively, capable of binding to the crystallisable part (Fc) of an antibody and includes e.g. Protein A and Protein G, or any fragment or fusion protein thereof that has maintained said
10 binding property.

The term “linker” herein means an element linking two polypeptide units, monomers or domains to each other in a multimer.

15 The term “spacer” herein means an element connecting a polypeptide or a polypeptide multimer to a support.