maturation step is part of a rVWF production process for the treatment of von Willebrand Disease Type B and part of the manufacturing process for recombinant Factor VIII - half life (rFVIII-HL). Furin belongs to the family of the pro-protein convertases and is dependent on calcium ($Ca^{2+}$). Furin specifically cleaves the C-terminal peptide bond of arginine within a specific sequence, containing arginine at positions —1 and —4. This sequence can be found in numerous human proteins, showing that furin plays a major role in the maturation of a number of human pro-proteins.

[0009] The production of activated proteins is of high clinical and diagnostic importance. For example, active or mature proteins, like mature VWF, may be used to control blood coagulation. The present invention provides improved recombinant furin (rFurin) which is substantially animal protein-free rFurin for the subsequent production of activated proteins. More specifically, the present invention provides substantially animal protein-free rFurin for transforming pro-VWF into mature VWF.

## SUMMARY

[0009a] In one aspect, the present disclosure refers to a composition comprising substantially animal protein-free recombinant furin (rFurin) having a specific activity of at least 300 U/µg protein, wherein the composition comprises host cell DNA in a concentration between about 0 to 0.4 pg DNA/U furin activity, wherein the rFurin activity is measured on a short synthetic peptide containing the dibasic recognition sequence attached to a fluorescent amino-methyl coumarin (AMC) group, that is released after cleavage (BOC-RVRR-AMC), whereby one activity unit is defined as the release of 1 pMol of AMC per minute at 30°C.

[0009b] In another aspect, the present disclosure refers to a method of making the composition as disclosed herein comprising the step of growing a host cell transformed or transfected with a polynucleotide encoding furin in serum-free medium in a batch refeed culture process under conditions that permit secretion of the furin into the medium, binding the secreted furin on a multimodal cation exchange resin; and recovering the secreted furin from the multimodal cation exchange resin.

[0009c] In yet another aspect, the present disclosure refers to a method of using the composition as disclosed herein comprising the step of contacting a pro-protein comprising a furin cleavage site with the composition under conditions to cleave a pro-peptide from the pro-protein to form a mature protein.

[0010] The present disclosure provides recombinant furin (rFurin), which is substantially animal protein-free recombinant furin (rFurin), and methods for producing same. Such rFurin is substantially free of other proteins which may normally be associated with the production of rFurin, such as serum proteins and host cell proteins. This rFurin allows for the subsequent production of mature proteins with high specific activity and high purity without side effects associated with protein contaminant in the rFurin preparation. More specifically, this rFurin allows for the production of mature VWF with high specific activity and high purity. Accordingly, the disclosure provides methods for selection and adaptation of recombinant host cells to chemically-defined medium, expression of rFurin which is secreted into cell culture supernatant, and purification of rFurin after cell removal.

[0011] The substantially animal protein-free rFurin of the disclosure includes preparations or compositions of rFurin comprising host cell protein in a concentration which ranges between about 0.1 to 0.6 ng protein or less/Unit furin activity or between about 2 and 11 µg protein or less/mL and essentially lacking contaminating proteins from serum in the culture medium. In one example, the substantially animal

protein-free rFurin encompasses preparations of rFurin comprising contaminating host cell DNA in a concentration between about 0 to 0.4 pg DNA or less/Unit furin activity or between about 0 and 24 ng DNA or less/mL and essentially lacking contaminating proteins from serum in the culture medium.

[0012] The disclosure includes compositions comprising substantially animal protein free recombinant furin at an activity of at least 10000 U furin/mL and host cell protein at a concentration less than about 11 µg protein/mL. Such compositions may also comprise host cell protein at a concentration less than about 1.0 ng protein/U furin activity. In one example, the host cell protein is from a CHO cell.

[0013] In another example, the disclosure includes compositions comprising substantially animal protein free recombinant furin at an activity of at least 10000 U furin/mL and host cell DNA at a concentration less than about 14 ng DNA/mL. In various examples, such compositions also comprise host cell DNA at a concentration less than about 0.5 pg DNA/U furin activity. In one example, the host cell DNA is from a CHO cell.

[0014] The disclosure also includes compositions comprising substantially animal protein free recombinant furin at a specific furin activity of at least about 100 U/µg and host cell protein at a concentration less than about 11 µg protein/mL. Such compositions may also comprise host cell protein at a concentration less than about 1.0 ng protein/U furin activity. In one example, the host cell protein is from a CHO cell.

[0015] The disclosure further includes compositions comprising substantially animal protein free recombinant furin at a specific furin activity of at least about 100 U/µg and host cell DNA at a concentration less than about 14 ng DNA/mL. Such compositions may also comprise host cell DNA at a concentration less than about 0.5 pg DNA/U furin activity. In one example, the host cell DNA is from a CHO cell.

[0016] The disclosure also includes methods of making a composition comprising substantially animal protein-free recombinant furin described herein. Such methods comprise the step of adapting the host cells to growth in medium with increasingly lower concentrations of serum until all serum is removed from the medium. In another example, the methods comprise the step of transferring the host cell from growth in medium comprising serum to growth in serum-free medium. In an exemplary example, the host cell is a CHO cell.

[0017] The disclosure includes methods of using a composition comprising substantially animal protein-free recombinant furin described herein. Such uses comprise the step of contacting a pro-protein with the composition under conditions to cleave a pro-peptide from the pro-protein to form a mature protein. The rFurin can be used in the formation of any mature protein from a pro-protein that is cleaved by furin. In one example, the mature protein is von Willebrand Factor. In another example, the mature protein is Factor VIII. In addition, the disclosure contemplates that the rFurin of the disclosure is useful for both *in vitro* and *in vivo* processing of any pro-protein that it cleaves.

## BRIEF DESCRIPTION OF THE DRAWING

[0018] A further illustration of the invention is given with reference to the accompanying drawings, which are set out below in Figures 1-18.

[0019] Figure 1 depicts the expressed active rFurin protease construct in one embodiment of the invention. The rFurin construct is truncated at the C-terminal end at AA 577 to remove the Cys-rich transmembrane and cytosol domains.

[0020] Figure 2 depicts a pedigree of the generation of the CHO/rFurin clone #488-3.

[0021] Figure 3 shows a pedigree of the generation of the CHO/rFurin clone #289-20.

[0022] Figure 4 sets out a comparison of the graphical distribution of the rFurin producers in the cell populations of PMCB#01 and PMCB#04. 80.74% of the cells in PMCB#04 express rFurin; 74.06% of the cells in PMCB#01 express rFurin.

[0023] Figure 5 shows a "Doehlert Matrix" where five temperatures were combined with three pH values, resulting in seven combinations of temperature and pH.

[0024] Figure 6 shows a surface plot analysis of the data in reference to the volumetric productivity. The coordinates of the data in Figure 6 are marked as points. The surface shows the assumed correlation of the single data.

[0025] Figure 7 shows a contour plot which illustrates the influence of temperature and pH on the volumetric productivity. The dots indicate the conditions (pH / temp.) which had been tested experimentally.

WHAT IS CLAIMED IS:

1. A composition comprising substantially animal protein-free recombinant furin (rFurin) having a specific activity of at least 300 U/µg protein, wherein the composition comprises host cell DNA in a concentration between about 0 to 0.4 pg DNA/U furin activity, wherein the rFurin activity is measured on a short synthetic peptide containing the dibasic recognition sequence attached to a fluorescent amino-methyl coumarin (AMC) group, that is released after cleavage (BOC-RVRR-AMC), whereby one activity unit is defined as the release of 1 pMol of AMC per minute at 30°C.

2. The composition of claim 1 comprising recombinant furin at specific activity of at least 350 U/µg protein.

3. The composition of claim 1 comprising recombinant furin at a specific activity of at least 400 U/µg protein.

4. The composition of claim 1 comprising recombinant furin at a specific activity of at least 450 U/µg protein.

5. The composition of claim 1 comprising recombinant furin at a specific activity of at least 500 U/µg protein.

6. The composition of claim 1 comprising recombinant furin at a specific activity of at least 600 U/µg protein.

7. A method of making the composition of claim 1 comprising the step of growing a host cell transformed or transfected with a polynucleotide encoding furin in serum-free medium in a batch refeed culture process under conditions that permit secretion of the furin into the medium, binding the secreted furin on a multimodal cation exchange resin; and recovering the secreted furin from the multimodal cation exchange resin.

8. The method of claim 7 comprising the step of adapting the host cell to grow in medium with increasingly lower concentrations of serum until all serum is removed from the medium.

9. The method of claim 7 comprising transferring the host cell from growth in medium comprising serum to growth in serum-free medium.

10. The method of claim 8 or 9 wherein the host cell is a CHO cell.

11. A method of using the composition of claim 1 comprising the step of contacting a pro-protein comprising a furin cleavage site with the composition under conditions to cleave a pro-peptide from the pro-protein to form a mature protein.

12. The method of claim 11 wherein the mature protein is von Willebrand Factor.

13. The method of claim 11 wherein the mature protein is Factor VIII.

14. The composition of claim 1, wherein the host cell is a Chinese Hamster Ovary (CHO) cell.