PROTEIN EXTRACTION METHODS

BACKGROUND

[0001] Embodiments disclosed herein relate to methods for extraction of DNA compaction proteins from protein preparations.

[0002] Methods for extraction and characterization of DNA compaction proteins have been described. One such method involves exposure of a sample to a high concentration of sodium chloride, such as 2.5 M. Another method involves exposure to low pH, such as pH 1.0 or lower for durations up to 16 hours. Combinations of sodium chloride and low pH have not been used presumably because their understood mechanisms of action are antagonistic to each other. The use of surfactants with either technique has been described along with subsequent processing by size exclusion chromatography.

SUMMARY

[0003] In one aspect, the present invention refers to a method for extracting one or more histones from a preparation, the method comprising: (i) contacting the preparation with an organic cation, a neutral salt, and a nonionic surfactant, at a pH of 0.1 to 1.0; (ii) incubating the mixture over a period of 30 minutes to 150 minutes; (iii) removing solids to provide an extract; (iv) applying the extract to a particle packed column comprising (1) electropositive particles suitable for practicing anion exchange chromatography in void exclusion mode or (2) substantially uncharged size exclusion chromatography particles having an exclusion limit of 5,000 Daltons, wherein the particle packed column has an interparticle volume and wherein a volume of the applied extract is not greater than the interparticle volume; and (v) after the applying step, passing a buffer through the column to displace the sample down the column, and collecting the extracted one or more histones in a peak corresponding to the excluded volume of the column, wherein the organic cation is ethacridine or methylene blue at a concentration in a range of 0.01% to 0.1%, or guanidine at a concentration in a range of 0.5 to 1.5 M. In another aspect, the present invention refers to a method for extracting one or more histones from a preparation, the method comprising: (i) contacting the preparation with an organic cation, a neutral salt, and a nonionic surfactant, at a pH of 0.1 to.0; (ii) incubating the mixture over a period of 30 minutes to 150 minutes; (iii) removing solids to provide an extract; (iv) applying the

extract to a particle packed column comprising (1) electropositive particles suitable for practicing anion exchange chromatography in void exclusion mode or (2) substantially uncharged size exclusion chromatography particles having an exclusion limit of 5,000 Daltons, wherein the particle packed column has an interparticle volume and wherein a volume of the applied extract is not greater than the interparticle volume; and (v) after the applying step, passing a buffer through the column to displace the sample down the column, and collecting the extracted one or more histones in a peak corresponding to the excluded volume of the column, wherein the organic cation is chlorhexidine at a concentration in a range of 0.001% to 0.01%, polyethyleneimine-1200 at a concentration in a range of 0.025% to 0.05%, ethacridine or methylene blue at a concentration in a range of 0.025% to 0.200%, or guanidine at a concentration in a range of 0.5 M to 2.0 M. Also disclosed herein are examples which provide methods for extracting a DNA-compaction protein from a preparation, the method comprising contacting the preparation with an organic cation, a neutral salt, and a nonionic surfactant, at a pH of about 0.1 to about 1.0, incubating the mixture over a period of from about 30 minutes to about 150 minutes; removing solids to provide an extract, applying the extract to a particle packed column comprising (1) electropositive particles suitable for practicing anion exchange chromatography in void exclusion mode or (2) substantially uncharged size exclusion chromatography particles having an exclusion limit of about 5,000 Daltons, wherein the particle packed column has an interparticle volume, wherein a volume of the applied extract is not greater than the interparticle volume, after the applying step following with a buffer to displace the sample down the column, and collecting the extracted DNA-compaction proteins in the peak corresponding to the excluded volume of the column.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] Figure 1 shows a chromatogram showing fractionation of the initial extract by a column of electropositive particles operated in void exclusion mode. The peak highlighted in gray contains the extracted histone proteins.

CLAIMS

What is claimed is:

1. A method for extracting one or more histones from a preparation, the method comprising:

(i) contacting the preparation with an organic cation, a neutral salt, and a nonionic surfactant, at a pH of 0.1 to 1.0;

(ii) incubating the mixture over a period of 30 minutes to 150 minutes;

(iii) removing solids to provide an extract;

(iv) applying the extract to a particle packed column comprising (1) electropositive particles suitable for practicing anion exchange chromatography in void exclusion mode or (2) substantially uncharged size exclusion chromatography particles having an exclusion limit of 5,000 Daltons, wherein the particle packed column has an interparticle volume and wherein a volume of the applied extract is not greater than the interparticle volume; and

(v) after the applying step, passing a buffer through the column to displace the sample down the column, and collecting the extracted one or more histones in a peak corresponding to the excluded volume of the column,

wherein the organic cation is ethacridine or methylene blue at a concentration in a range of 0.01% to 0.1%, or guanidine at a concentration in a range of 0.5 to 1.5 M.

2. The method of claim 1, wherein the concentration of ethacridine or methylene blue is in a range selected from the group consisting of 0.02 to 0.05%, and 0.03 to 0.10%.

3. The method of claim 1, wherein the concentration of guanidine is in a range selected from the group consisting of 0.8 to 1.2 M, and 0.9 to 1.1 M.

4. The method of claim 1, wherein the neutral salt is present in a concentration range from 1 M to 3 M.

5. The method of claim 1, wherein the neutral salt is sodium chloride or potassium chloride.

6. The method of claim 1, wherein the nonionic surfactant is selected from the group consisting of Tween 20, Tween, 40, Tween 60, and Nonidet NP40.

7. The method of claim 1, wherein the nonionic surfactant is present in a concentration range from 0.05% to 0.25% w/v.

8. The method of claim 1, wherein the preparation is selected from the group consisting of a cell culture supernatant, a bodily fluid, a tissue homogenate, and a fraction from a fractionating process.

9. A method for extracting one or more histones from a preparation, the method comprising:

(i) contacting the preparation with an organic cation, a neutral salt, and a nonionic surfactant, at a pH of 0.1 to.0;

(ii) incubating the mixture over a period of 30 minutes to 150 minutes;

(iii) removing solids to provide an extract;

(iv) applying the extract to a particle packed column comprising (1) electropositive particles suitable for practicing anion exchange chromatography in void exclusion mode or (2) substantially uncharged size exclusion chromatography particles having an exclusion limit of 5,000 Daltons, wherein the particle packed column has an interparticle volume and wherein a volume of the applied extract is not greater than the interparticle volume; and

(v) after the applying step, passing a buffer through the column to displace the sample down the column, and collecting the extracted one or more histones in a peak corresponding to the excluded volume of the column,

wherein the organic cation is chlorhexidine at a concentration in a range of 0.001% to 0.01%, polyethyleneimine-1200 at a concentration in a range of 0.025% to 0.05%, ethacridine or methylene blue at a concentration in a range of 0.025% to 0.200%, or guanidine at a concentration in a range of 0.5 M to 2.0 M.