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(54) Titre : PRODUCTION DE PHOSPHOPEPTIDES A PARTIR DE LA CASEINE
(54) Title: PRODUCTION OF PHOSPHOPEPTIDES FROM CASEIN

(57) Abrégé/Abstract:
A method for the preparation of selected phosphopeptides having anticariogenic and other activities, comprising the steps of completely digesting a soluble monovalent cation salt of casein in solution with a proteolytic enzyme, adding a mineral acid to the solution to adjust the pH to about 4.7, removing any precipitate produced, adding $CaCl_2$ to a level of about 1.0 % w/v to cause aggregation of at least the selected phosphopeptides in said digested solution, separating the aggregated phosphopeptides from the solution through a filter having a molecular weight exclusion limit lying substantially within the range 10,000 to 20,000 while passing the bulk of the remaining phosphopeptides and solution, diafiltering the separated phosphopeptides with water through a filter and concentrating and drying the retentate.

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(54) Title: PRODUCTION OF PHOSPHOPEPTIDES FROM CASEIN

(57) Abstract

A method for the preparation of selected phosphopeptides having anticariogenic and other activities, comprising the steps of completely digesting a soluble monovalent cation salt of casein in solution with a proteolytic enzyme, adding a mineral acid to the solution to adjust the pH to about 4.7, removing any precipitate produced, adding $CaCl_2$ to a level of about 1.0 % w/v to cause aggregation of at least the selected phosphopeptides in said digested solution, separating the aggregated phosphopeptides from the solution through a filter having a molecular weight exclusion limit lying substantially within the range 10,000 to 20,000 while passing the bulk of the remaining phosphopeptides and solution, diafiltering the separated phosphopeptides with water through a filter and concentrating and drying the retentate.

2108678 PCT/AU / 9 2 / 0 0 1 7 5
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TITLE: PRODUCTION OF PHOSPHOPEPTIDES FROM CASEIN

Field of the Invention:

This invention relates to the production of phosphopeptides having anticariogenic and other properties from casein.

Background of the Invention:

In our Australian Patent No. 593365, we have described that four of the many phosphopeptides released by tryptic digestion of casein have anticariogenic (tooth-decay-inhibiting) activity. These peptides all contain the active sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- and correspond to α_{s1}(59-79) SEQ.ID No 2 (T_1), ß(2-25) SEQ.ID No 3 (T_2), α_{s2}(46-70) SEQ.ID No 4 (T_4) and α_{s2}(2-21) SEQ.ID No 7 (T_3). The methods described for the production of the anticariogenic phosphopeptides are selective precipitation and ion exchange chromatography. While these methods produce very pure preparations of these peptides, they have not received general acceptance in the dairy industry due to their cost and the level of technical skill required.

Recently membrane ultrafiltration has found broad acceptance in the dairy industry for milk treatment. In U.S. Patents 4,358,465, 4,361,587 and 4,495,176, Brule et al describe an ultrafiltration method for the production of casein phosphopeptides as dietetic aliments. This procedure proves unsuitable for the production of anticariogenic phosphopeptides due to the predominance of non-anticariogenic phosphopeptides

in the preparations.

In AU-B 66783/81 and 51491/85 Brule et al, a method of extracting phosphopeptides for use as nutritional complements is disclosed in which an aggregate forming bivalent cation is used in combination with ultrafiltration followed by diafiltration with water to extract the desired phosphopeptides selected for the above purpose. This procedure is similarly unsuitable for the production of anticariogenic phosphopeptides since diafiltration with water results in the de-aggregation of the anticariogenic phosphopeptides.

Summary of the Invention and Object:

It is an object of the present invention to provide a method of preparing selected phosphopeptides from casein using ultrafiltration.

In one embodiment, the present invention provides a method for the preparation of selected phosphopeptides comprising the steps of: completely digesting a soluble monovalent cation salt of casein in solution, introducing a di or trivalent metal ion to cause aggregation of at least the selected phosphopeptides in said digested solution, and diafiltering the solution containing the aggregating ion through a filter having a molecular weight exclusion limit selected to retain at least said aggregated phosphopeptides while passing the bulk of the remaining phosphopeptides in a filtrate, wherein the digested solution is diafiltrated with volumes of a solution of the di or trivalent metal ion under conditions effective to produce a composition consisting essentially of anticariogenic phosphopeptides.

In another embodiment, the present invention provides a method for the preparation of selected

phosphopeptides having anticariogenic and other activities, comprising the steps of: completely digesting a soluble monovalent cation salt of casein in solution with a proteolytic enzyme, adding a mineral acid to the solution to adjust the pH to about 4.7, removing any precipitate produced, adding $CaCl_2$ to a level of about 1.0% w/v to cause aggregation of at least the selected phosphopeptides in said digested solution, and separating the aggregated phosphopeptides from the solution by filtration including diafiltration with the $CaCl_2$ containing solution through a filter having a molecular weight exclusion limit lying substantially within the range 10,000 to 20,000 while passing the bulk of the remaining phosphopeptides and non-phosphorylated peptides and solution in a filtrate.

In the methods described by Brule et al, the object is to obtain a broad range of phosphopeptides from casein for use as a dietetic aliment. Therefore Brule et al do not teach that the hydrolysed casein compound must be diafiltrated in the presence of the aggregating ion to ensure that the selected phosphopeptides are filtered from the solution while allowing the remaining phosphopeptides and non-phosphorylated peptides to pass during the diafiltration process. This represents a significant advance in the art since it enables the use of an industry accepted method of extraction which results in a preparation which is rich (> 90% w/w) in the desired phosphopeptides.

In a preferred form of the invention, the

selected phosphopeptides are the anticariogenic phosphopeptides referred to above, and the molecular exclusion limit adopted during the filtering step of the above method preferably substantially falls within the range 10,000 to 20,000.

The soluble monovalent cation salt of casein, such as sodium caseinate or potassium caseinate, may be present in the solution in a concentration substantially falling within the range 0.1 to 50% w/w, which is preferably digested using a proteolytic enzyme, such as pancreatin, trypsin, papain or chymotrypsin, or a mixture of proteolytic enzymes such as trypsin and chymotrypsin or by chemical means, such as cyanogen bromide. The enzyme(s) to casein ratio can range from about 1:1000 to 1:10 (w:w) but this would be selected to allow complete digestion of the casein as defined above. The pH of the hydrolysis should preferably be controlled at optimum for the enzymes to allow complete casein digestion. The temperature also should be optimised for complete digestion but temperature induced degradation (deamidation, dephosphorylation and peptidolysis) should be minimised. The optimal temperature is between about 20°C and 60°C.

In a preferred form of the invention, after digestion HCl is added at room temperature to about pH 4.7 and any precipitate (this should be minimal) removed. $CaCl_2$ is then added to the supernatant to a level of about 1.0% w/v. Phosphopeptides in the presence of 1.0% w/v calcium (II) aggregate. The

anticariogenic phosphopeptides (ie. containing the sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu-) form hexamers which are separated from the smaller non-anticariogenic phosphopeptide aggregates by extensive diafiltration through a 10,000 molecular weight exclusion limit filter with a $CaCl_2$ solution preferably 1.0% w/v. The preferred molecular weight exclusion limit of the membrane filter should not be less than 10,000 or greater than about 20,000. The addition of a $CaCl_2$ solution, or some other suitable di/trivalent metal ion, such as zinc (II) or ferric (III), is essential for diafiltration in order to maintain the integrity of the anticariogenic phosphopeptide aggregates thus allowing separation of the anticariogenic from the non-anticariogenic phosphopeptides.

After several volumes of 1.0% $CaCl_2$ w/v have passed through the membrane filter to achieve greater than 90% purity of the anticariogenic phosphopeptides the ultraretentate containing the anticariogenic phosphopeptides can be diafiltered with water through a 1,000 molecular weight exclusion limit filter to remove calcium if desired. The retentate is then concentrated and spray dried.

The calcium, zinc and ferric salts of the anticariogenic phosphopeptide preparation (ACPP) can be converted to a sodium salt by acidifying a 10% w/v solution of the calcium ACPP to a low pH, circa pH 2.0, with HCl. After extensive diafiltration through a 1,000 molecular weight exclusion limit filter the

retentate is neutralised to pH 7.0 with NaOH and then diafiltered with water through the same filter to remove excess sodium chloride.

The calcium ACPP can be converted to calcium phosphate ACPP by addition of $CaCl_2$ and Na_2HPO_4 where the Ca/P final ratio is 1.67. The peptide α_{s1}(59-79) can bind 21 Ca and 13 PO_4. The filtrate of the above process is suited for the purification of other bioactive casein peptides by size and charge-based separation technologies and can be used as microbiological growth media, as dietary supplements after debittering or as a nitrogen fertilizer.

A presently preferred embodiment of the invention will now be described with reference to the following example.

EXAMPLE

Sodium caseinate was prepared by acidifying milk with 0.1 M HCl to pH 4.7 and neutralising the precipitate with NaOH to pH 7.0. A 10% w/v solution of sodium caseinate was prepared and adjusted to pH 8.0. Trypsin (Novo) was added to 0.2% w/v and the hydrolysis allowed to proceed to completion at 50°C with adjustment to pH 8.0 by constant addition of NaOH. The pH of the solution was then adjusted to pH 4.7 with 5 M HCl and the precipitate removed at room temperature by centrifugation. The supernatant was microfiltered through an 0 micron filter, and then adjusted to pH 7.0 with NaOH and $CaCl_2$ added to a level of 1.0% w/v. This solution was then diafiltered through an Amicon™ YM10 (10,000 molecular weight

exclusion limit) with 3 to 5 volumes of 1.0% w/v $CaCl_2$. The retentate was then washed with 1 volume of distilled/deionised water through an Amicon YM1 filter (1,000 molecular weight exclusion limit). The individual peptides of this preparation were separated by ion exchange FPLC and reverse phase HPLC as described in the aforementioned patent and identified by amino acid composition and sequence analyses after conversion of the Ser(P) residues to S-ethyl cysteine. An analysis of the preparation is shown in Table 1.

Table 1. Composition of an Anticariogenic Phosphopeptide Preparation

Peptide%	w/w
α_{s2}(1-21) (SEQ.ID No 8)	0.8
ß(1-25) (SEQ.ID No 1)	22.3
α_{s2}(2-21) (SEQ.ID No 7) (T_3)	5.7
ß(2-25) (SEQ.ID No 3) (T_2)	17.9
α_{s1}(59-79) (SEQ.ID No 2) (T_1)	21.4
Desamido$^{74}\alpha_{s1}$(59-79) (SEQ.ID No 5)	6.3
α_{s2}(46-70) (SEQ.ID No 4) (T_4)	6.8
Desamido$^{74,78}\alpha_{s1}$(59-79) (SEQ.ID No 6)	6.4
α_{s1}(43-79) (SEQ.ID No 9)	3.3
NAP*	9.1

* NAP = non-anticariogenic peptides

This preparation contains the four anticariogenic phosphopeptides described in the aforementioned patent, [ß(2-25), T_2, α_{s1}(59-79), T_1, α_{s2}(2-21), T_3 and α_{s2}(46-70), T_4], those related peptides incompletely hydrolysed by trypsin [α_{s2}(1-21), ß(1-25) and α_{s1}(43-

79)] and also minor levels of the two deamidated forms of α_{s1}(59-79), desamido^{74} and desamido^{74,78} which result from temperature induced deamidation, this occurs in an even greater extent in commercial production of sodium caseinate due to higher temperatures and extremes of pH, although the presence of the deamidated forms has no effect on anticariogenic activity. The anticariogenic phosphopeptides were 90.9% w/w of the peptides produced.

If pure α_{s1}-casein is used in place of casein then α_{s1}(59-79) will be obtained by this process with minor amounts of the deamidated forms of this peptide depending on hydrolysis conditions. If pure ß-casein is used then only ß(1-25) and ß(2-25) will be obtained using this process.

When crude enzymes are used (such as pancreatin), slight truncation (both N- and C- terminally) of the nine listed phosphopeptides can occur. As long as this truncation is only slight, there is no loss of activity. In fact, pancreatin produces a preparation with slightly greater specific activity on a weight basis when compared with purified trypsin.

The sequences of the nine peptides, which include the peptides T_1 to T_4 of the aforementioned patent, and the other peptides referred to above are detailed in the following sequence listing

2108678 PCT/AU 92/00175
RECEIVED 03 MAR 1993

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: REYNOLDS, ERIC CHARLES

(ii) TITLE OF INVENTION: PRODUCTION OF PHOSPHOPEPTIDES FROM CASEIN

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE:
(B) STREET:
(C) CITY:
(D) STATE:
(E) COUNTRY:
(F) ZIP:

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: FLOPPY DISK
(B) COMPUTER: IBM PC COMPATIBLE
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WORD PERFECT

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A)
(B)
(C)

(ix) TELECOMMUNICATION INFORMATION:
(A)
(B)
(C)

(2) INFORMATION FOR SEQ.ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: Amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Protein
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 15
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 17
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 18
(D) OTHER INFORMATION:

Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 19
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO:1:

Arg Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu
1 5 10 15

Ser Ser Ser Glu Glu Ser Ile Thr Arg
20 25

(2) INFORMATION FOR SEQ.ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Protein
(ix) FEATURE:
(A) NAME/KEY: Pyroglutamate
(B) LOCATION: 1
(D) OTHER INFORMATION:
A certain amount will exist in this form
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 6
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 8
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 9
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 10
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 17
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO:2:

Gln Met Glu Ala Glu Ser Ile Ser Ser Ser Glu Glu Ile Val Pro Asn
1 5 10 15

Ser Val Glu Gln Lys
20

(2) INFORMATION FOR SEQ.ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Protein
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 14
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 16
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 17
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 18
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO:3:

```
Glu Leu Glu Glu Leu Asn Val Pro Gly Glu Ile Val Glu Ser Leu Ser
1               5                  10                  15

Ser Ser Glu Glu Ser Ile Thr Arg
            20
```

(2) INFORMATION FOR SEQ.ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: Amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Protein
(ix) FEATURE
(A) NAME/KEY: Phosphnoserine
(B) LOCATION: 11
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 12
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 13
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 16
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine

(xi) SEQUENCE DESCRIPTION: SEQ. ID NO:4:

```
Asn Ala Asn Glu Glu Glu Tyr Ser Ile Gly Ser Ser Ser Glu Glu Ser
1               5                  10                  15

Ala Glu Val Ala Thr Glu Glu Val Lys
            20                  25
```

(2) INFORMATION FOR SEQ.ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Protein
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 6
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 8
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 9
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 10
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 17
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine

(xi) SEQUENCE DESCRIPTION: SEQ. ID NO:5:

Gln Met Glu Ala Glu Ser Ile Ser Ser Ser Glu Glu Ile Val Pro Asp
1 5 10 15

Ser Val Glu Gln Lys
20

(2) INFORMATION FOR SEQ.ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Protein
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 6
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(a) Name/Key: Phosphoserine
(b) Location: 8
(d) Other information:
Post-translationally phosphorylated serine
(ix) Feature:
(a) Name/Key: Phosphoserine
(b) Location: 9
(d) Other information:
Post-translationally phosphorylated serine
(ix) Feature:
(a) Name/Key: Phosphoserine
(b) Location: 10
(d) Other information:

Post-translationally phosphorylated serine
(ix) Feature:
(a) Name/Key: Phosphoserine
(b) Location: 17
(d) Other information:
Post-translationally phosphorylated serine

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO:6:

Gln Met Glu Ala Glu Ser Ile Ser Ser Ser Glu Glu Ile Val Pro Asp
1 5 10 15

Ser Val Glu Glu Lys
20

(2) INFORMATION FOR SEQ.ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Protein
(ix) Feature:
(a) Name/Key: Phosphoserine
(b) Location: 7
(d) Other information:
Post-translationally phosphorylated serine
(ix) Feature:
(a) Name/Key: Phosphoserine
(b) Location: 8
(d) Other information:
Post-translationally phosphorylated serine
(ix) Feature:
(a) Name/Key: Phosphoserine
(b) Location: 9
(d) Other information:
Post-translationally phosphorylated serine
(ix) Feature:
(a) Name/Key: Phosphoserine
(b) Location: 15
(d) Other information:
Post-translationally phosphorylated serine

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO:7:

Asn Thr Met Glu His Val Ser Ser Ser Glu Glu Ser Ile Ile Ser Gln
1 5 10 15

Glu Thr Tyr Lys
20

(2) INFORMATION FOR SEQ.ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Protein
(ix) FEATURE:
(a) Name/Key: Phosphoserine
(b) Location: 8
(d) Other information:
Post-translationally phosphorylated serine
(ix) FEATURE:
(a) Name/Key: Phosphoserine
(b) Location: 9
(d) Other information:

Post-translationally phosphorylated serine
(ix) FEATURE:
(a) Name/Key: Phosphoserine
(b) Location: 10
(d) Other information:
Post-translationally phosphorylated serine
(ix) FEATURE:
(a) Name/Key: Phosphoserine
(b) Location: 16
(d) Other information:
Post-translationally phosphorylated serine

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO:8:

Lys Asn Thr Met Glu His Val Ser Ser Ser Glu Glu Ser Ile Ile Ser
1 5 10 15

Gln Glu Thr Tyr Lys
20

(2) INFORMATION FOR SEQ.ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37
(B) TYPE: Amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Protein
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 4
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 6
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 22
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 24
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 25
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 26
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine
(ix) FEATURE:
(A) NAME/KEY: Phosphoserine
(B) LOCATION: 33
(D) OTHER INFORMATION:
Post-translationally phosphorylated serine

(xi) SEQUENCE DESCRIPTION: SEQ.ID NO:9:

Asp Ile Gly Ser Glu Ser Thr Glu Asp Gln Ala Met Glu Asp Ile Lys
1 5 10 15

Gln Met Glu Ala Glu Ser Ile Ser Ser Ser Glu Glu Ile Val Pro Asn
20 25 30

Ser Val Glu Gln Lys
35

CLAIMS:

1. A method for the preparation of selected phosphopeptides comprising the steps of:

completely digesting a soluble monovalent cation salt of casein in solution,

introducing a di or trivalent metal ion to cause aggregation of at least the selected phosphopeptides in said digested solution, and

diafiltering the solution containing the aggregating ion through a filter having a molecular weight exclusion limit selected to retain at least said aggregated phosphopeptides while passing the bulk of the remaining phosphopeptides in a filtrate, wherein the digested solution is diafiltrated with volumes of a solution of the di or trivalent metal ion under conditions effective to produce a composition consisting essentially of anticariogenic phosphopeptides.

2. The method of claim 1, wherein the molecular weight exclusion limit adopted during the filtering step substantially falls within the range 10,000 to 20,000.

3. The method of claim 1 or 2, wherein the soluble monovalent cation salt of casein is present in the solution in a concentration substantially falling within the range 0.1 to 50% w/w.

4. The method of any one of claims 1 to 3, wherein the digestion step is performed using a proteolytic enzyme and the ratio of proteolytic enzyme to soluble monovalent cation salt of casein in the solution falls substantially within the range 1:1000 to 1:10 (w:w) selected to allow complete digestion of the casein salt.

5. The method of any one of claims 1 to 4, wherein the pH and the temperature of the solution is controlled to allow complete digestion of the casein salt.

6. The method of claim 5, wherein the temperature of the solution lies substantially within the range 20°C to 60°C.

7. A method for the preparation of selected phosphopeptides having anticariogenic and other activities, comprising the steps of:

completely digesting a soluble monovalent cation salt of casein in solution with a proteclytic enzyme,

adding a mineral acid to the solution to adjust the pH to about 4.7,

removing any precipitate produced,

adding $CaCl_2$ to a level of about 1.0% w/v to cause aggregation of at least the selected phosphopeptides in said digested solution, and

separating the aggregated phosphopeptides from the solution by filtration including diafiltration with the $CaCl_2$ containing solution through a filter having a molecular weight exclusion limit lying substantially within the range 10,000 to 20,000 while passing the bulk of the remaining phosphopeptides and non-phosphorylated peptides and solution in a filtrate.

8. The method of claim 1 or 7, wherein the soluble monovalent cation salt of casein is selected from sodium caseinate and potassium caseinate.

9. The method of claim 7 or 8, wherein the proteolytic enzyme is selected from pancreatin, trypsin, papain, chymotrypsin and mixtures thereof.