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Model for the Transfer of Ca^{2+} from Outside the Cell to Inside the Cell with Bovine Milk Component to Justify Its Use as an Alzheimer's Treatment

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Abstract: A model of Ca^{2+} channel function rescue via treatment with a milk component is suggested. This molecule's structure is shown by ms and ms^2 evidence, from an NH_4^+ form cation exchange cartridge effluent. This model includes the participation of phospho-protein in the shuttling of Ca^{2+} through a path of anionic moieties from an N-acetamido neuraminyl group to a sulfate group. It suggests the rescue of Ca^{2+} function with bovine milk oligosaccharide dipeptide component in the treatment of Alzheimer's disease. Ca^{2+} is proposed to end at a sulfo-tyrosine then extended inside the cell internally. The rolling of the Ca^{2+} from N-acetamido neuraminyl group is thought to be due to the interaction of doubly charged calcium cation with a series of negatively charged ions, sequestered and transported via the molecule from bovine milk oligosaccharide dipeptide. Excess K^+ availability in early Alzheimer's disease is known and may cause interference in the transport of Ca^{2+} in this disease. This model predicts that K^+ can seize the Ca^{2+} channel rescue because it has no excess charge driving it forward to the end of the bovine milk component. The location of phosphate on the galactosyl group of the molecule from which the drawn structures is obtained by ms and ms^2, is described here. A pathway for the Ca^{2+} transfer along this structure is depicted here. The goal is to provide a rationale for using bovine milk as a low cost treatment for Alzheimer's disease which would allow treatment of this disease for people in the third world who cannot afford high cost treatments.

Keywords: Bovine Milk, Mass Spectrometry, ms and ms^2, Alzheimer's Disease Treatment, Model of Ca^{2+} Transport

1. Materials and Methods

Oligosaccharide di-phospho dipeptide was isolated from bovine milk (commercially obtained, 'fat free'). A sample of bovine milk (0.10 mL) was pipetted into a vial from the bovine milk. H_2O (18 mega-Ohm resistivity, 1.00 mL) was added to the vial and the mixture pushed through an NH_4^+ form cation exchange resin cartridge (ThermoFisher, Sunnyvale CA USA). The effluent was frozen before analysis. A sample was taken for ms and ms^2 on an API 2000 triple quadrapole mass spectrometer, while a small clump of ice remained in the vial. An alternate method for this component's isolation, using ethanol extraction, can be used. [1]

2. Introduction

Alzheimer's disease is thought to be caused in part by Ca^{2+} channelopathy much like Parkinson's disease. [1, 2] The etiology of the disease is thought to involve beta amyloid protein over-phosphorylation. [3, 4] Antibodies to this protein have been used to treat the disease with insufficient success. [5] Perhaps over-phosphorylation interferes with Ca^{2+} channel function by interrupting the rolling of Ca^{2+} from outside the cell to the inside of the cell or from one point in the cell to another place in the cell. A model is proposed which shuttles Ca^{2+} from an N-acetamido neuraminyl group to a sulfate anion via anionic phosphate groups linking the two groups and which could be relieved of its Ca^{2+} via a more basic carboxylate group, possibly from an amino acid

in the cytosol. The component in such a mechanism is found in bovine milk. It is suggested that this molecule can rescue Ca^{2+} channel function. The structure of this milk component is proposed using ms and ms^2 evidence, locating a phosphorous on the galactosyl group of the bovine milk material. Structures of ions generated by ms and ms^2 are depicted here. A requirement for a phosphorylated protein to maintain the traversing of Ca^{2+} is incorporated into this model. It is described below.

The primary and crystal structure of a Ca^{2+} channel has been reported. [6-9] Ca^{2+} channels' structure, physical characterization and mechanism have been reviewed. [10] Although the size of calcium channels has been reported as ~ 6 Angstroms, the size may be significantly larger, since H_2O layers could be lining the interior of the pore. [11] Of importance is that functional Ca^{2+} channel is dependent on pH, that is, its charge state. [10] Although beta amyloid protein has been shown to be glycosylated, both N-linked and O-linked substitutions, little is known about their structures. Sialic acid has been shown to be bound to an isolated proteins possibly beta-amyloid protein. [12, 13]

GM3, with sialyl lactose oligosaccharide linked to sphingosine has been shown to rescue Ca^{2+} channel function. [14] This oligosaccharide epitope is thought to be similar in structure to the bovine milk oligosaccharide dipeptide. [1, 2, 12] This establishes the possibility that milk oligosaccharide dipeptide could allow a rescue function for Alzheimer's disease, as well. [14, 15]

3. Results and Discussion

Suggested is a possible mechanism for the transfer of Ca^{2+} from the non-reducing end of the oligosaccharyl di-phospho apsaraginyl tyrosine sulfate dipeptide to the sulfo tyrosine. Roughly, a decreasing pKa is observed. This hypothesis does not take into account local protein electronic effects in the transfer of calcium along this path.

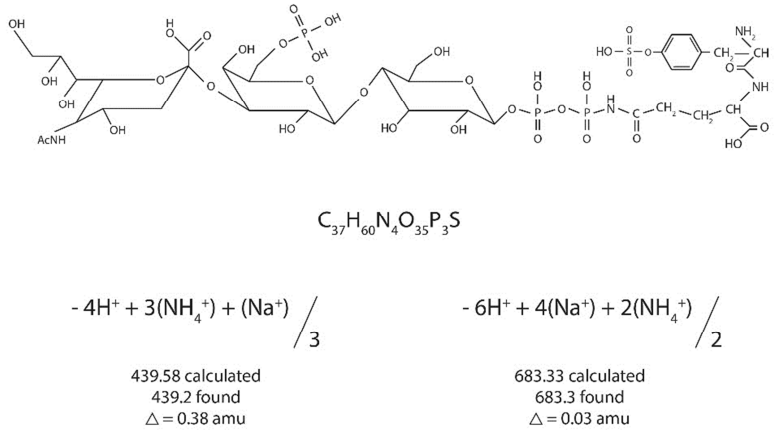

Figure 1. *Mass spectral ions form ms of isolated bovine milk oligosaccharide dipeptide.*

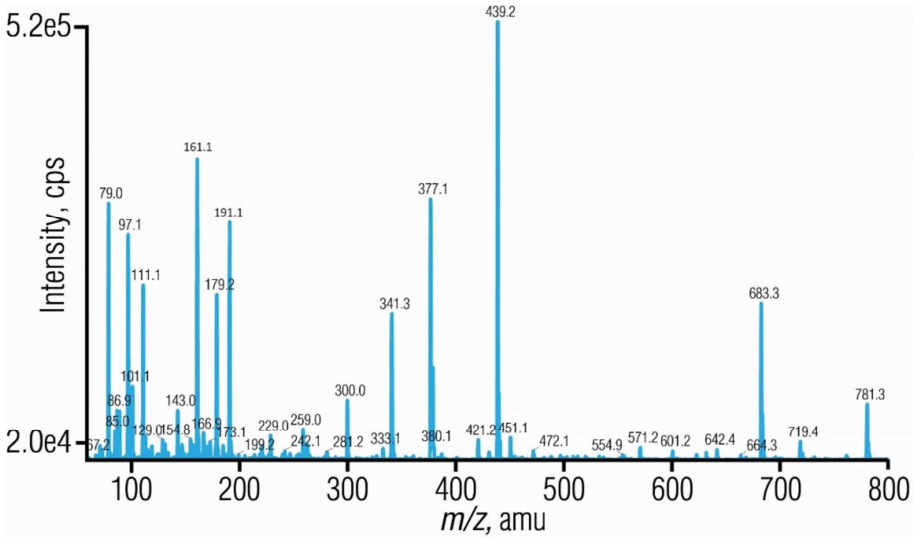

Figure 2. *Mass spectrum obtained from bovine milk NH_4^+ cation exchange resin.*

In Figure 1 from the mass spectrum found in Figure 2 there are two ions, m/z 683.3 and m/z 439.2, which depict the whole glycan dipeptide, isolated from bovine milk, with the use of cation exchange resin in the NH_4^+ form. Tyrosine is

sulfated. The galactosyl portion of the oligosaccharide is postulated to have a phosphate group on its 6' carbon OH group. The oligosaccharide is linked to sulfo tyrosinyl asparagine via a di-phospho group. Sodium ions can originate by contact of the ion with glass in the mass spectrometer.

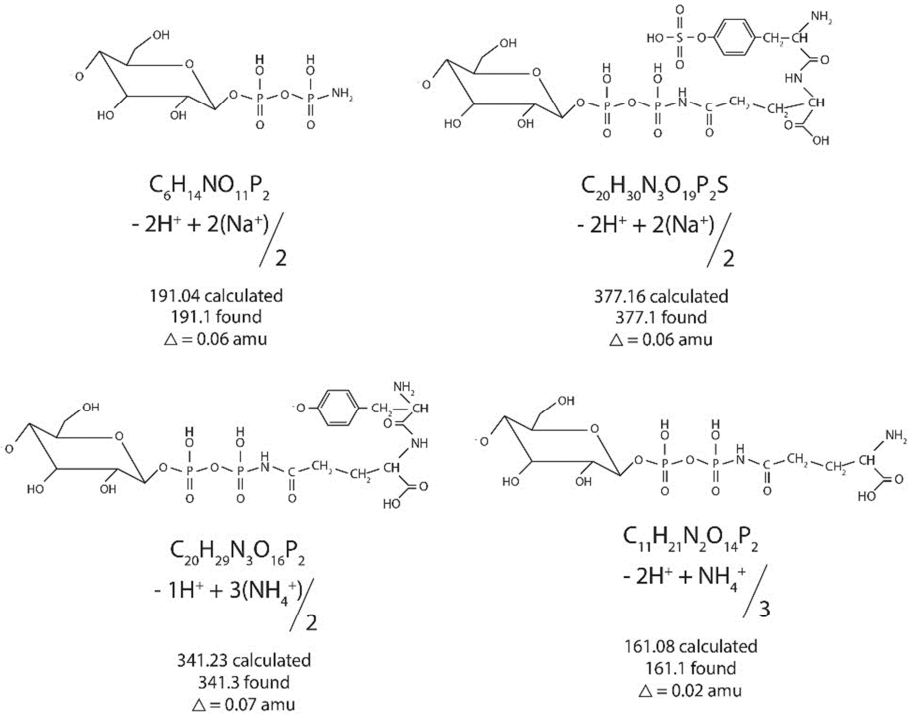

Figure 3. *Ions' structures from ms of bovine milk isolate.*

Figure 3 depicts four ions, m/z 191.1, m/z 377.1, m/z 341.3 and m/z 161.1 ions, together, they suggest the phosphate group is not found on the glucosyl group of the glycan portion of the molecule. Another way the monosaccharide upon which phosphate is substituted is suggested for the ion, m/z 191.1. It shows that the N-acetamido neuraminyl galactosyl fragment of the whole molecule is substituted on the galactosyl portion by phosphate. The ion, m/z 439.2, can be drawn to depict phosphate substitution on the glucosyl residue of the glycan. Although the assignment renders our previous conclusion, to sustain the phosphor galactosyl structure, ambiguous, we can assert the phospho galactosyl structure due to the number of supporting ions, 4 of them.

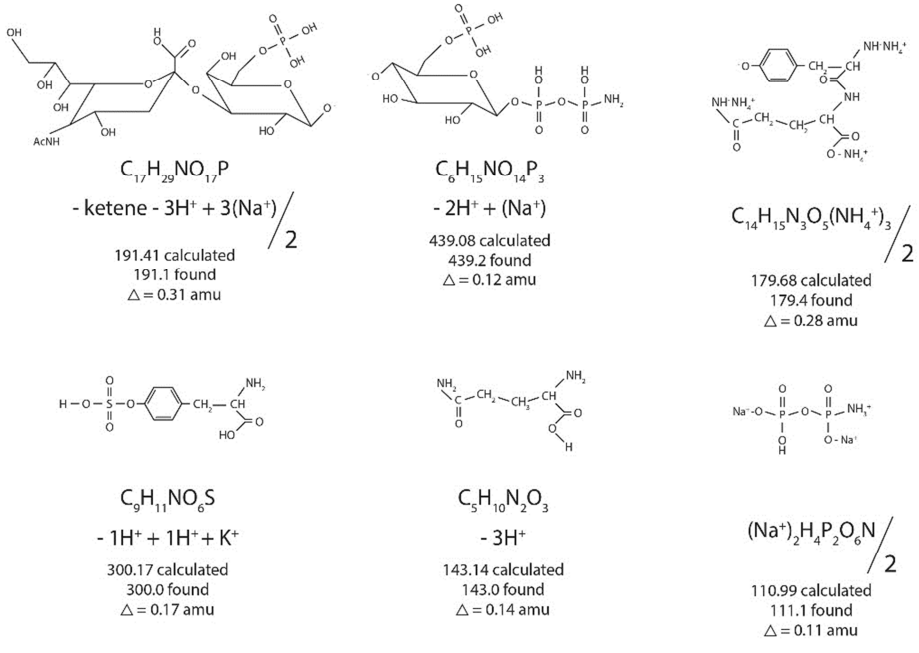

Figure 4. *Additional ions' structures from ms of bovine milk isolate.*

Ions in Figure 4, m/z 179.4, m/z 300.0, m/z 143.0 and m/z 111.1 describe the non-glycan, fragments of the whole molecule represent the oligosaccharide portion of the whole molecule. The ion, m/z 79.0, is diagnostic for phosphate. The ion, m/z 97.1, could originate from HSO_4^- or $H_2PO_4^-$ or both ions. The ms^2 data for both ions, m/z 377.1 and m/z 439.3, suggest the presence of phosphate from the ion, m/z 79.0, and sulfate and/or phosphate, m/z 97.0. There is no sulfate drawn in Figure 4 for ion, m/z 439.3, but in the apparent structure with no sulfate, phosphate alone could explain the ion, m/z 97.0.

The ms^2 spectra for ions, m/z 377.1 and m/z 439.2, are drawn in Figures 6a and 6b and indicate the phosphorylation of these ions or phosphorylation and sulfation of the respective ions.

K^+ ion would be predicted to seize the shuttling of Ca^{2+} because it has only plus one charge. There would be no excess positive charge to seek neutralization by a base in the Ca^{2+} channel pathway. Surely excess K^+ would be available in this disease state because Kv3.4 channels are upregulated in early stages of the disease and this would seize Ca^{2+} channel function. [15]

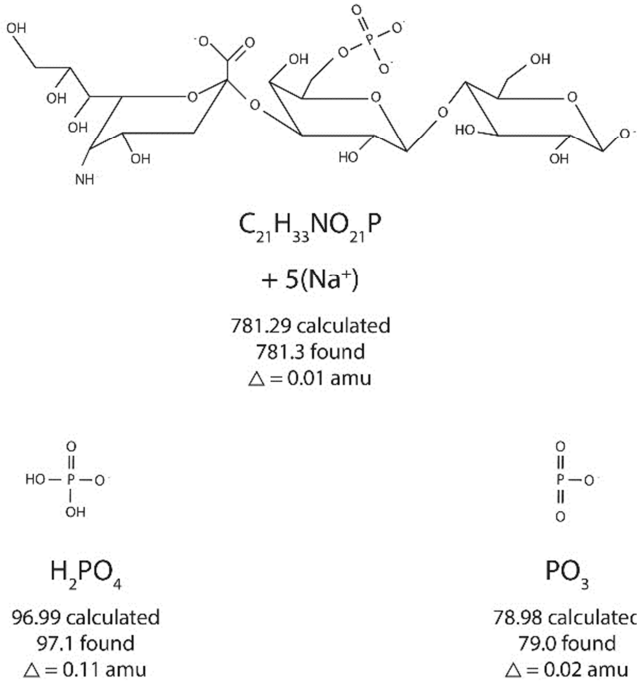

Figure 5. *Mass spectral ion structures from ms of bovine milk NH_4^+ form cation exchange resin isolate.*

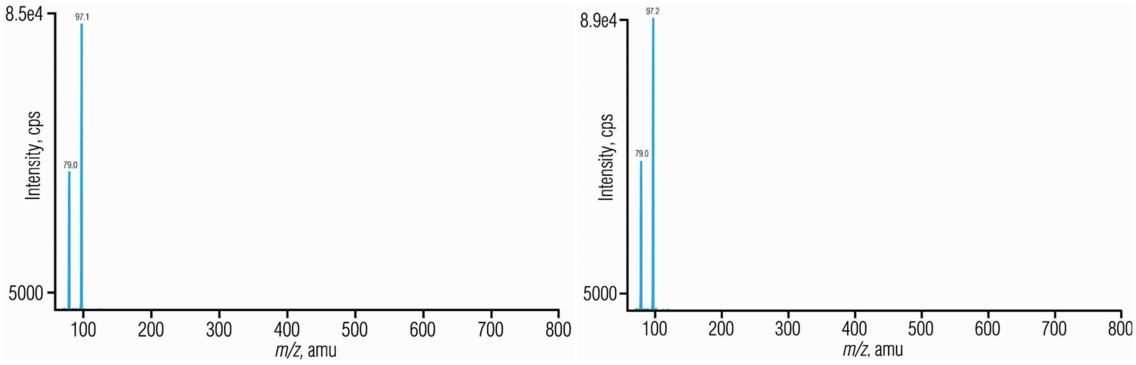

Figure 6. *a. The ms^2 of ion m/z 377.1 from bovine milk isolate ms; b. The ms^2 of ion m/z 439.2 from bovine milk isolate ms.*

There is an anionic gap between the phosphorylated galactosyl residue and the di-anionic di-phospho linked to Asn-Tyr-SO_4^- Researchers have discovered a reaction in which there is a phosphorylated protein kinase which may serve as a Ca^{2+} receptor of the voltage sensitive calcium channel. It could span this gap if properly positioned. [3, 4] This anionic bridge could shuttle Ca^{2+} from the 6' phosphoryl group to the di-phosphoryl group linking the reducing sugar glucose and asparagine. The anionic phospho-protein could also shuttle calcium off to another site other than the terminal sulfo tyrosine if the phospho protein moved away or another more basic anion is within the negative field of the anionic phosphoryl group on the protein. Such a bridge phospho-protein has been found in brain Ca^{2+} channel in an omega conotoxin- sensitive brain channel. [3] It is phosphorylated by a c-AMP dependent kinase and protein kinase. [4]

In Figure 7 is drawn a possible mechanism for Ca^{2+} transfer with the oligosaccharide dipeptide found in bovine milk. The lower structure shows the molecule folded with the Ca^{2+} ion bound to the N-acetamido neuraminyl substituent. The remainder of the molecule is drawn completely ionized. The lower drawing shows the molecule with an anionic gap between the di-phospho group di-anion and the galactosyl phosphate group. With rotation of the Ca^{2+} substituted sulfo tyrosine as noted by the arrow, in this manner, Ca^{2+} is delivered. Following the arrows to the presumed galactosyl phosphate and to the phospho-protein (which could fill the anionic gap), then to di-phospho group and then to the sulfo-tyrosine residue.

In Figure 7 the presumed direction of the Ca^{2+} channel mimic is shown, along with the postulated phospho-protein intervention position. They are arranged left to right, with increasing base strength, except the sulfo-tyrosine group. Ionization of the sulfo-tyrosine group could occur by

participation of a channel amino group, possibly lysine, to remove the sulfo proton with subsequent binding of the Ca^{2+}. The molecule could extend to the cytosol. A weaker acid, stronger base, such as an acid carboxylate group could receive the Ca^{2+} ion from the putative sulfo-tyrosine.

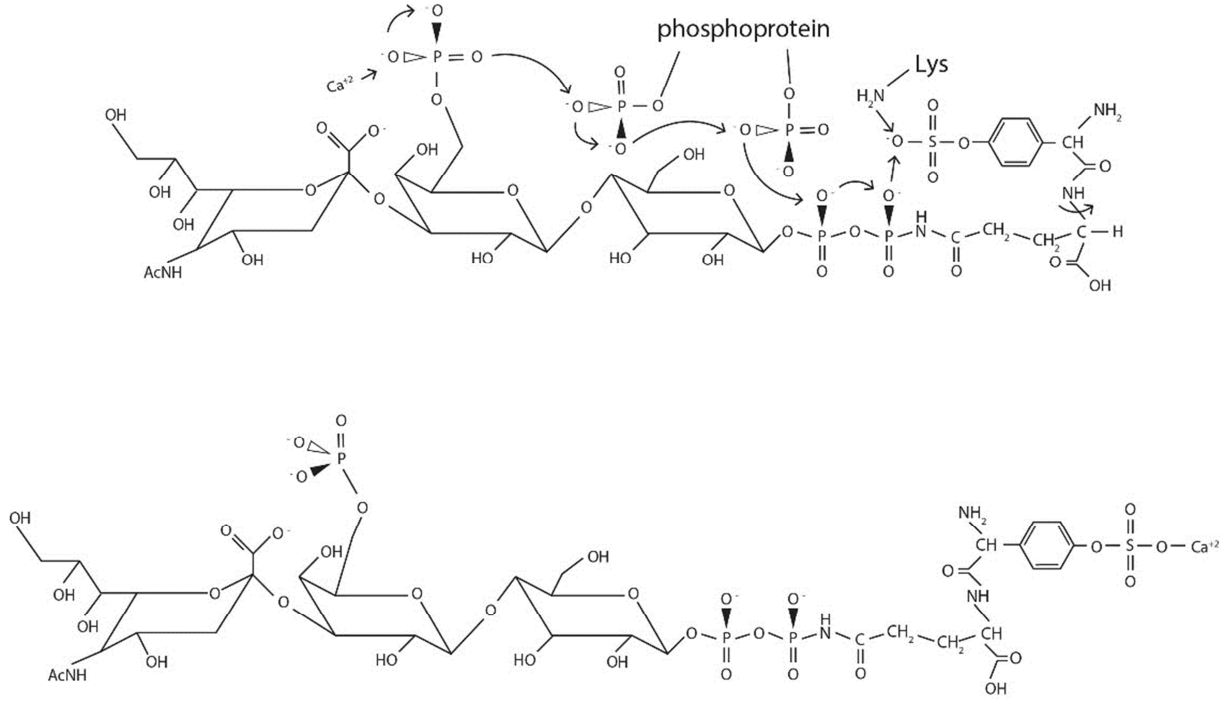

Figure 7. *Mechanism of Ca^{2+} sequestration and transfer with bovine milk trisaccharide dipeptide.*

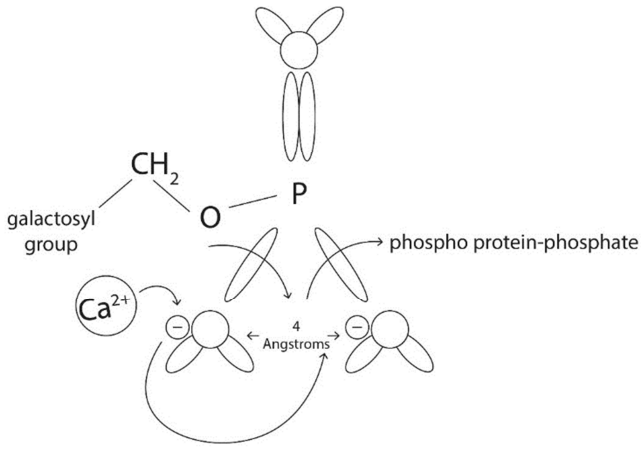

Figure 8. *Transfer of Ca^{2+} ion between phospho O^- atoms.*

The Ca^{2+} ion is partially neutralized by the negative charge on the N-acetamido neuraminyl carboxylate anion. The phospho group on the galactosyl group has one negative phospho group with one negative charge facing toward the un-neutralized plus one positive charge from the plus two Ca^{2+} ion. The P-O^- Ca^{2+} bond may rotate toward the second negative charge on the oxygen of the same P atom, The Ca^{2+} is transferred to the O^- atom and it rotates the P-O bond by the force of the negative charge to the not realized Ca^{2+}. This is how the calcium $(II)^+$ is transferred to the phospho group linked to the phospho-protein and to the phospho group linked to the anomeric carbon of the oligosaccharide and then to the phosphoramide negative charge. The Ca^{2+} is taken by the sulfo anion, made anionic, possibly, by channel lysine amino group.

Presumably Ca^{2+} ion is followed by another Ca^{2+} behind. There would be no backward attraction by the previous O^- ion. For Ca^{2+} transfer through the proposed mechanism, increasing anion base strength, could be thought to be required. This would predict no transfer from the anionic phosphoramide to the sulfo anion. A suggested explanation is, as noted above, that the micro-environment near the sulfo-tyrosine is more basic than the O^- phosphoramide.

The Ca^{2+} ion is postulated to be transferred away from the N-acetamido neuraminyl containing substituent by rotation of the tyrosine sulfate amino group out of the noted micro-environment, as in Figure 7, with full extension of the oligosaccharide di-phospho Asn-Tyr-SO_4^- dipeptide.

4. Conclusions

Proposed model of Ca^{2+} ion channel function by bovine milk oligosaccharide di-phospho dipeptide sulfate is supported.

Proposed model predicts Ca^{2+} channel dysfunction with up-regulation of K^+ Kv3.4 found in early stage Alzheimer's disease.

Proposed model suggests possible Alzheimer's disease treatment with bovine milk component.

The proposed mechanism for Ca^{2+} transfer to the inside of the cell from the outside or to one place in the cell from another makes treatment of Alzheimer's disease with bovine milk a reasonable endeavor and should be investigated

because of its ready access to most people in the world who may suffer from this terrible disease.

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