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Research Article

A STUDY ON CHANGE IN CONFORMATION OF TUBULIN ON INTERACTING WITH PROPOFOL VIA TIME OF FLIGHT MASS SPECTROSCOPY

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ABSTRACT

The present study revels to determine the change in secondary structure of protein when an anesthetic drug is added to it. The protein used was tubulin (20µg), which is the building block of MT. The change in conformation was observed in the presence and absence of 1ml of solution of tubulin glycerol buffer and propofol along with 0.1% TFA at 4°C via MALDI TOF MS (ABScix 4800). The result the mass spectrograph showed base peak at 53329.5 (m/z) in the absence of propofol which is approximately near the mass of tubulin. The other base peak, was observed at 72526.9 (m/z) indicating change in tubulin in presence of propofol. Based on the present study, we conclude that propofol hinders the polymerization of tubulin by changing its secondary structure and thus effect the consciousness in brain.

Keywords:

Mictotubule (MT), Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectroscopy.

INTRODUCTION

Microtubule is a part of the cytoskeleton of the cell. It helps to maintain the structure of the cell by providing support. It is especially important for transport of the organelles and vesicles in the cells as motor proteins use them as tracks to move from one part of the cell to another. As microtubules are radially aligned in the cell they play an important role in pigment translocation. The flagella in many bacterial cells are made of the microtubules. Microtubules also play an important role in cell division. Microtubules are essential cytoskeletal elements that provide architectural support for the growth and maintenance of the axons, and also provide a substrate along which organelles are transported both the direction within the axons. (Bass et.al., 1995)[2]

The interior of MT consist of water molecule which creates a dipole moment and an electrc filed. The plus and minus of each tubulin produce a dipole. The MT produces a fast growth at the plus end towards the cell periphery and a slow growth at the minus end. The charge separation in MT is wide enough to store information. The favorable condition to store and information processing is found to be near the human body temperature (J.Faber et.al., 2006)[11].

Consciousness is a major unsolved problem of the modern science. However, Sir Roger Penrose and Hameroff postulated a theory known as Orch-OR (objective reduction) theory of consciousness. According to this theory, Sir Penrose and Hameroff postulated that **MT** produce quantum level manipulation in matter. They also envisage that conformational states of the **MT** subunits (i.e.tubulin) are coupled to interact at quantum events, and computed with other tubulin. They further assumed that the coherent superposition of quantum-coupled tubulin conformational states occur throughout brain volume and provide significant information about the brain's condition (Hameroff.S et al., 1998)[8]. Researcher has stated that consciousness is generally thought of being comprised to two critical components – arousal and awareness. This is mainly due to certain conformational changes in the MAPs. The Penrose-Hameroff theory of OR reduction also subscribes to this view (Hameroff.S et al., 1998)[8].

Earlier experiment on anesthetic binding with tubulin suggested that Propofol (the drug used in this experiment) strongly affects polymerization of tubulin or self-organization of microtubules. The past experiments via Circular dichroism(CD) evidently showed that, in presence of Propofol major changes take place in its overall conformation of tubulin (Sahni.P et.al. 2017)[20,21]. In continuation of the above study, an attempt has been made to study the change in secondary structure of tubulin heterodimer in the presence as well as in the absence of Propofol using Matrix-Assisted Laser Desorption Ionization- Time of Flight Mass Spectroscopy (MALDI-TOF MS). MALDI-TOF MS is a tool used for the detection and localization of drugs, proteins, lipids i.e. identification of molecules having molecular mass approximately 50kDa or greater (Villanueva et al., 2002)[28].

There are about 100 billion neurons in the human brain. Each neuron has synapses which are of the order 1000 which operates at 100 operations per second per synapses. So the capability of the human brain is 10^{16} operations per second. However, neurological studies have shown that there is a sub-neuronal structure, known as microtubule. They are as many as 10^8**MT** inside each neuron and operate at 10^7 operations per second. So it brings this speed into the realm of quantum theory. It becomes something of the order of 10^{26} operations per second in the human brain, which goes beyond the achievements via Artificial Neural Networks. Thus, this raise the intriguing question as to how many conformation state is present in the tubulin associated protein and the change in conformation of protein while incorporated with a drug.

METHODOLOGY AND MATERIALS

The present piece of work was carried out using standard protocol and procedure reported in literature.

Sinapic acid as matrix (30% ACN, 70% H2O and 0.3% TFA). Tubulin (99.98% pure) porcine brain, tubulin glycerol buffer and propofol were supplied by Cytoskeleton Inc. Denver, CO. USA. MilliQ water.

Procedure

Proposed plan of work can be depicted as follows

Preparation of matrix

1. In an Eppendorf tube 10 mg of Sinapic acid was weighed.
2. To the above 70% deionized water, 0.3% TFA and 30% acetonitrile/ACN was added.
3. The matrix was Vortex for 1 minute to dissolve the contents.
4. Further centrifuged for 1 minute to precipitate out any undissolved Sinapic acid.
5. The supernatant (900µl) formed was further used in the experiment.

Preparation of sample for MALDI-TOF MS

1. Sample A consisted of 20µg tubulin dissolved in 1ml of solution of water and 0.1% TFA at 4°C.
2. Sample B consisted of 20µg tubulin dissolved in 1ml of solution of tubulin glycerol buffer and propofol along with 0.1% TFA at 4°C. The samples were kept in Vortexfor 30 seconds to dissolve the contents. Both the sample was incubated for 15 minutes.

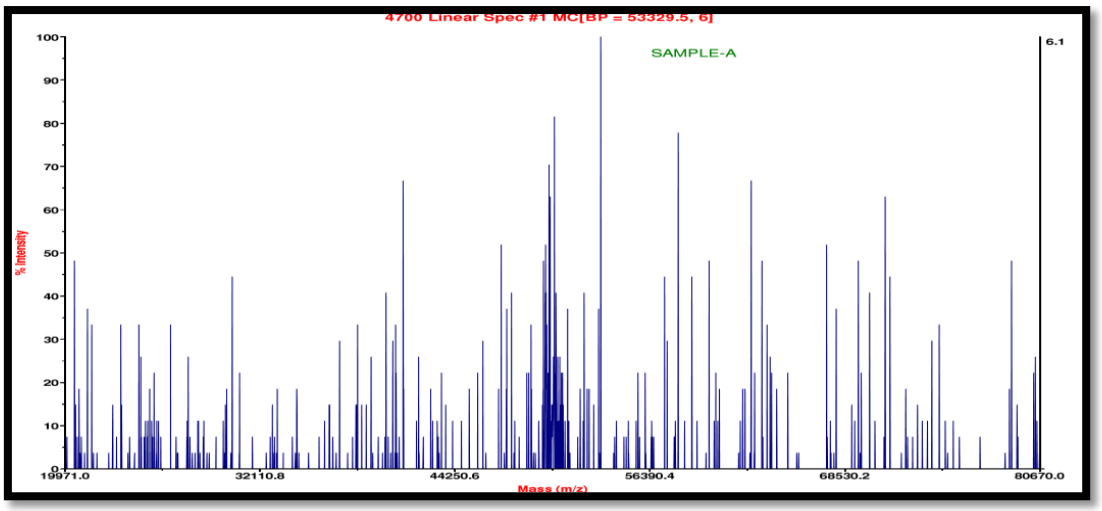
Spotting

Pre-spotting was done on MALDI-MS sample plate with 0.5µL of MALDI matrix stock solution.

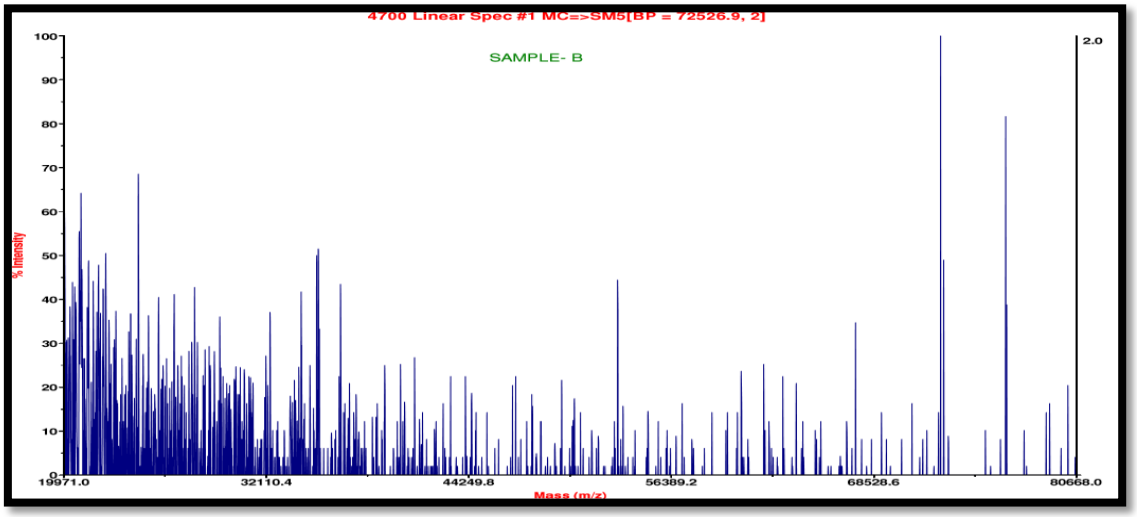
1. Spot were allowed to evaporate to dryness, under laminar flow.
2. Dispensed 0.5µL of protein Sample Solution A and Sample Solution B.
3. Matrix: sample (1:1) crystals was placed on the steel plate and allowed to dry.
4. Placed the MALDI plate in MALDI-TOF MS ion source and analyze.

RESULTS AND DISCUSSION

Matrix Assisted Laser Disorption/Ionization Time Of Flight Mass Spectrometry (MALDI TOF-MS) for the same reaction sets were carried out to validate the results of circular dichroism measurements.



Sample A: MALDI TOF MS spectra and data of secondary structures content for Tubulin without propofol



Sample B: MALDI TOF MS spectra and data of secondary structures content for tubulin in with propofol

Mass spectrometry is the most accurate method for determining the molecular mass of the compound and its elemental composition. The molecules are ionized and broken up into many fragments, some of which are positive ions. For most ions, the charge is one and thus, the m/z ratio is simply the molecular mass of the ion.

Sample A data shows the MS spectra consisting of tubulin without propofol. The base line is found at 53329.5 (m/z), which is near to the molecular weight of tubulin i.e 50-55 KDa. Thus, the presence of base peak at 53329.5 confirms the presence of tubulin. Sample B data shows the MS spectra consisting of tubulin with propofol and glycerol buffer. The base line is found to shift beyond 56390 and lines at 72526.9 which confirm the binding of propofol and tubulin.

The formation of excess of fragments ions from 19971.0 (m/z) to 32110.4 (m/z) in the MS of sample B conveys that in presence of propofol destabilizes the tubulin, thus are in capable of forming the base peak. However, the rate of formation of fragments decreases as the propofol is consumed and the concentration decreases. Thus, via this data, it is further interfered that propofol binds with the hydrophobic pockets of the tubulin heterodimer and changes the secondary structure of tubulin protein. It is also inferred that the polymerisation of tubulin is also affected in the presence of propofol.

Thus, inhibits the assembly of tubulin into microtubules, which perhaps affect the consciousness in the human brain under anesthesia.

CONCLUSION

MALDI TOF-MS measurements show that propofol affects the secondary structures of tubulin, which was already confirmed by Circular dichoirism measurements .Studies show that there is not a single mechanism for anesthetic action. Propofol hinders the propagation of conformational changes along protofilaments by affecting tubulin polymerization. Thus it can be concluded that, Microtubule of human brain in presence of propofol is incapable of carrying out collective action. Anaesthetics seem to cause physical unconsciousness or clinical unresponsiveness when they block the brain's ability to integrate information.

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