

# Lyme Toxin Chemically Similar to Botulinum Toxin, a zinc endoproteinase

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from an article on the Biochemistry of Lyme Disease, by professor Robert Bradford

## Lyme Disease Toxin

Because many of the symptoms of Lyme disease involve the nervous system, it was speculated that the spirochete produced a toxin that disrupted normal nerve function. Through the use of DNA manipulations and a database of known protein toxin DNA sequences, a match was made with a selected *Borrelia burgdorferi* (Bb) gene and a specific toxin in the database. Protein generated from this cloned Bb gene was examined biochemically and found to have characteristics similar to that of botulinum, the toxin of *Clostridium botulinum*, a zinc endoproteinase.

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The toxin from Bb belongs to a family of toxic proteins known as "zinc endoproteinases" or metalloproteases, and includes the toxin from the organism causing tetanus as well as those from many other well-known infectious diseases. The structures of this family of toxins are all very similar, as determined by x-ray crystal analysis.<sup>2</sup> They all contain zinc and perform the same proteolytic function, namely, cleaving the chemical (covalent) bond between two specific amino acids in a particular protein found in nerve cells.<sup>3</sup> The substrate for this enzyme is very large, implying that any inhibitor of enzyme activity blocking the entry of the substrate into the active site must also be very large.

One reason for learning the structure of the toxin (including the active site) is to determine the geometry of this site, the exact positions of the atoms that bind other atoms in the substrate. Knowing the arrangement of these atoms permits the development of inhibitors of the toxin, substances that compete with the normal substrate for active site occupancy.<sup>4</sup>

## Action of Toxin

The action of botulinum (as well as the toxin from the Lyme spirochete) is to prevent, through its action as a proteolytic enzyme, the release of the neurotransmitter acetylcholine. Nerve endings may

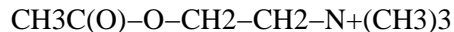
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be associated with other nerves or muscles (the neuromuscular junction). To understand this mechanism in greater detail, consider the basic principles of nerve physiology described below.

### Nerve Cells

A typical nerve cell consists of a long filament or axon, the terminal end of which lies in close proximity to another nerve cell. The space between them is known as the synaptic cleft (synapse). One nerve cell communicates with another through the release of a chemical substance known as a neurotransmitter held within small sacs (vesicles) lying near the terminal end. An electrical pulse travels the length of the axon and, when it reaches the nerve cell terminal, causes the vesicles to rupture through the presynaptic membrane and discharge the neurotransmitter into the synaptic cleft. The neurotransmitter is bound by a protein (receptor) in the postsynaptic membrane of the adjoining nerve cell causing, in turn, the transmission of an electrical pulse down the axon of the second nerve cell. By this mechanism, nerve cells communicate with one another through the action of a neurotransmitter. One such neurotransmitter is a simple organic substance known as acetylcholine. (See Chart 1.)

The structure of acetylcholine is shown by this formula:



### Mechanism of Neurotransmitter Release

Only recently has the mechanism of neurotransmitter release been understood at the molecular level. The proteins responsible for this highly detailed process have been isolated and characterized. Some parts of the puzzle are not as yet completely understood, for example, the process of membrane fusion. A study of the release of neurotransmitters from nerve endings has also revealed the mechanism of "switching," a process by which only one nerve among several in close proximity may be separately fired. This switching process is analogous to a similar process occurring in computers. Our brains work in a manner, in many ways, similar to that of computers. (See Chart 2.)

Each vesicle within a nerve ending contains only one type of neurotransmitter. The vesicle containing a specific neurotransmitter (NT) contains on its surface a specific protein designated VAMP (vesicle-associated membrane protein). This protein is a member of a family of specific proteins, differing only in the sequence of amino acids forming a chain extending from the protein. If the NT is designated NTA, the VAMP found in the membrane of the vesicle containing NTA, will always be VAMPA. In other words, a specific neurotransmitter is always associated in the vesicle with a specific type of VAMP. Finding another type of VAMP – for example, VAMPB – on the surface of a vesicle containing NTA will never occur. The difference between VAMPA and VAMPB lies only in the sequence of amino

acids in the peptide (protein chain) extending from the protein.<sup>5</sup>

During the random motion of vesicles in the region of a nerve ending, some encounter another protein embedded in the presynaptic membrane, designated SNAP-25 (synaptosomal-associated membrane protein). All SNAP-25 proteins belong to a family of similar proteins, differing only in the amino acid sequences of two peptides extending from the protein. A particular member of this family may, for example, be designated (SNAP-25)A. If a vesicle bearing on its surface the protein VAMP encounters the protein (SNAP-25)A lying in the presynaptic membrane, the three peptides (two from SNAP-25 and one from VAMP) rapidly intertwine and automatically form a triple helix, which twists in a manner similar to a "twist-tie" used on bread wrappers (ATP-driven). The structure of this peptide triple helix is similar to the triple helix found in collagen (a).<sup>5</sup>

The result of the twisting action is to draw the vesicle close to the surface of the presynaptic membrane. When the membrane of the vesicle contacts the presynaptic membrane, the two membranes automatically fuse, resulting in the vesicle contents (containing NTA) emptying into the synapse. The membrane flattens out and the VAMP/SNAP-25 proteins (the SNARE complex) are recycled.<sup>6</sup> (See Chart 2.)

#### NSF Protein

A third protein linked to the VAMP/SNAP-25 complex is N-ethylmaleimide-sensitive factor (NSF). N-ethylmaleimide is simply a chemical reagent used by biochemical researchers (not a normal body metabolite), capable of attaching acetyl groups [CH<sub>3</sub>C(O)-] to sulfhydryl groups (-SH) as found in the amino acid cysteine, a constituent of many proteins. The protein NSF is "sensitive" to this reagent (binds acetyl groups when exposed to the reagent), indicating that its surface is rich in sulfhydryl groups. This observation gives a hint about the activity of NSF, an agent that holds together two other proteins (VAMP and SNAP-25). Sulfhydryl groups are normally used to bind two proteins together (cross-linking) or to bind different parts of a single protein to each other. This is accomplished by the elimination of two hydrogens (-H) from two sulfhydryl groups (-SH) (usually by a single atom of oxygen, thereby forming water), resulting in a disulfide linkage (-S-S-). For this reason, NSF is believed to function as a link between VAMP and SNAP-25, forming a single rigid unit.<sup>5</sup> (See Chart 1.)

#### Specificity of Nerve Firing

If a vesicle having VAMP on its surface encounters a (SNAP-25)B (or any type other than A), no intertwining of the peptides will occur, the vesicle will not contact the presynaptic membrane and, consequently, no neurotransmitter will be released.

The NTA, released into the synapse, almost immediately contacts a receptor (RA) in the postsynaptic membrane capable of binding this

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neurotransmitter. If this receptor is found in nerve A (see Chart 2), this nerve only is fired (i.e., develops an action potential that travels down the axon). Any nerve ending in close proximity not carrying RA in its postsynaptic membrane will not be activated. If NTB is released into the synapse, only those nerve endings carrying RB will be activated. By synthesizing large amounts of vesicles containing NTA and simultaneously synthesizing an equal number of (SNAP-25)A, the corresponding type of nerve is activated.<sup>5</sup>

### Dietary Supplements in Lyme Disease

One of the known actions of the Lyme spirochete toxin is to diminish the release and availability of the neurotransmitter acetylcholine, a simple organic compound (see above for chemical structure). This substance is biosynthesized by the body as required in nerve activation and transmission. Supplementation by the precursors of acetylcholine synthesis would be of value to Lyme patients since they have a deficiency of this substance. (See Listing 1.)

#### Listing 1: Dietary Supplements Increasing Acetylcholine Synthesis Improving Neurologic Function

Phosphatidylcholine (Lecithin) Acetyl-L-Carnitine

Vitamin B5 (Pantothenic Acid)

Vitamin B6 (Pyridoxine)

Vitamin C (Ascorbic Acid)

Lysine (Amino Acid)

S-Adenosylmethionine (SAM) (Sulfur-bound Adenosyl Methionine)

If the inhibition of acetylcholine release were total, Lyme patients and those suffering from food poisoning would not be able to move; they would be completely paralyzed. Since the blockage is only partial, any increase in the amount of available neurotransmitter would benefit anyone experiencing neurotransmitter blockage. For this reason, dietary supplements increasing the amount of available acetylcholine have been shown to benefit Lyme patients.

### Acetylcholine Formation

In Chart 3, we can see phosphatidylcholine is a constituent of lecithin, a well-

known dietary supplement. Acetylcholine is simply choline to which an acetyl group (CH<sub>3</sub>CO-) has been attached. Lecithin is the source of choline, and acetyl-L-carnitine (ALC) is the source of the acetyl group. Carnitine is synthesized by the body and requires several factors, including the amino acid lysine and vitamin C (ascorbic acid). The supplement known as SAM (S-adenosylmethionine) supplies methyl groups (CH<sub>3</sub>-) to lysine, forming trimethyllysine. This compound is further processed, requiring additional vitamin C, resulting in carnitine that supplies the necessary acetyl group.<sup>8,9</sup>

### History of Lyme and Related Spirochetal Diseases

The discovery by Burgdorfer that Lyme disease was caused by a

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spirochete placed it in a category of other diseases known to be caused by spirochetes. An example of such a disease is syphilis, the scourge of Europe for hundreds of years. Arsenic and some of its compounds had been known for quite some time as a highly successful and popular means of fatally poisoning someone (remember the King in Shakespeare's Hamlet). Following the discovery of the Germ Theory of Disease by Louis Pasteur (1822–1895), it was theorized that, if arsenic was toxic enough to kill, it may also be effective in killing the organisms that cause disease. In the early 1900s, the German chemist–physician Paul Ehrlich (1854–1915) developed a chemical treatment for syphilis. By using a "shotgun" approach of trying hundreds of compounds in an effort to find one that worked, Ehrlich discovered what became known as Salvarsan or "606" after 606 compounds had been tested. Salvarsan is an organic compound of arsenic and may be highly toxic if not properly used. For his monumental discovery, Ehrlich was awarded the Nobel Prize in 1908. Salvarsan may be considered the first man–made antibiotic.<sup>26</sup> Arsenic belongs to that column in the periodic table of chemical elements known as the "Group V elements," which also include phosphorus, antimony and bismuth. (See Chart 4.)

Following the success of Salvarsan as a treatment for syphilis, other compounds of antimony and bismuth were also prepared and tried against spirochetes. Examples of these compounds include bismuth subcitrate, bismuth subsalicylate (Pepto–Bismol), bismuth subgallate, and many others. An example of an antimony–containing antibiotic is Pentostam (an antimonial, antimony sodium gluconate).<sup>27,28</sup>

A biological molecule known as ATP (adenosine triphosphate) supplies energy to biological systems through the high energy bonds found in a chain of three terminal phosphate groups. One of the mechanisms by which arsenic exerts its toxic effect is the substitution of phosphorus by arsenic in ATP, since both arsenic and phosphorus lie in the same column of the periodic table of chemical elements and have similar chemistry. (See Chart 5.)

When this substitution occurs, the molecule experiences immediate hydrolysis, breaks down, and no longer functions as a source of energy for the cell. Both antimony and bismuth are also found in this column of the periodic table (Group V).<sup>29,30</sup> (See Chart 6.)

What may be the first case of Lyme disease was noted about 1974 in a 14–year old boy, taken to the hospital with extreme pains in the muscles of his legs and unable to walk. This case, coupled with other pertinent facts related to the boy and a highly classified US government laboratory conducting research on contagious animal diseases in this same area, is suggestive of a link between these two events. The government laboratory alluded to is found on Plum Island, just north of Long Island, NY, and south of Lyme, Connecticut. Because of its secret nature, access to the island was only by ferry boat and restricted to the government workers employed there. The 14–year old

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boy lived near the ferry boat dock. Although not providing proof, these considerations are highly indicative of a possible link between this research laboratory and the subsequent outbreak in 1975 of an unknown disease involving juveniles in the same area of Lyme, Connecticut.<sup>32</sup>