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Characterization of Acetylcholinesterase from Various Sources: A Mini Review

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ABSTRACT

In this review, various aspects of acetylcholinesterase (AChE) are reviewed. Acetylcholinesterase (AChE, EC3.1.1.7) is an important neurotransmitter because it hydrolyses the neurotransmitter acetylcholine (ACh), it is also the target site of organophosphorus and carbamate insecticides. The discovery of the first neurotransmitter ACh was soon followed by the discovery of its hydrolysing enzyme, AChE. The role of AChE in terminating AChE-mediated neurotransmission made it the focus of intense research for much of the past century. The role of butyrylcholinesterase (BChE) is not known but it is assumed to participate in the growth and development and to scavenge the cholinergic toxins, it is also reported to have an auxiliary role in synaptic transmission. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are the major groups of cholinesterase differentiated by their substrate preferences. Substrate binding specificity and catalytic efficiency are tangled in enzyme catalysis. The fact that an enzyme may exist in more than one molecular form has been accepted for a number of years.

INTRODUCTION

As an important Neurotransmitter, the acetylcholinesterase (AChE, EC3.0.1.7) is also a target for phosphor and carbamate insecticides, due to their hydrolyses, the neurotransmitter acetylcholine [1]. The evidence of organophosphate toxicity and carbamate is linked to its inhibition of an important enzyme of the neurological system; acetylcholinesterase. The active site of the serine residue is carbamyl- and phosphorylated, preventing neurotransmitter acetylcholine metabolization [2].

Cholinesterase has an interesting history. Their existence was predicted by [3], some 15 years before he and Dudley showed acetylcholine (ACh) to be a natural constituent of animal tissues. Dale's prediction was based on the evaporation of the effect produced when AChE was injected into an animal [4]. [5] provided experimental support for this viewpoint by demonstrating that the effect of ACh on frogs was prolonged in the presence of eserine. They attributed this result to the inhibition of the enzyme normally responsible for destroying AChE by eserine. Cholinesterase activity was discovered in blood shortly after [6] and it was shown that there are at least two distinct enzymes, one in red cells and one in serum [7]. The kinetic features of these two enzymes have provided the foundation for classifying enzymes in other tissues. The red cell type AChE (EC 3.1.1.7) hydrolyzes ACh much faster than BuCh, but the serum type BChE (EC 3.1.1.8) hydrolyzes BuCh nearly four times faster than ACh [4]. AChE and BChE are two closely related cholinesterases that hydrolyze the neurotransmitter ACh in animals [8]. Both are evolutionarily similar, but each possesses distinct traits and properties that allow them to be separated [9]. Both enzymes are found in the brain and have been linked to neurofibrillary tangles and neuritic plaques [10]. The function of BChE is unknown, but it is thought that it scavenges tissue from ACh that has not been split by AChE [11,12]. AChE is found in nervous tissues of all species of animals [13] and besides its hydrolytic activity, there has been some suggestion that it may function as a physiological receptor [14]. Cholinesterase enzyme was reported to be an important therapeutic target for Alzheimer's disease. It is also found to be abundantly available in different fish organs, including the kidney [15].

Acetylcholinesterase (AChE)

The first neurotransmitter, ACh, was swiftly discovered and the enzyme which hydrolyzes it AChE was discovered. Most of the past century has been focused on the role of AChE in stopping ACh-mediated neurotransmission[16]. AChE is a serine enzyme (EC 3.1.1.7) playing a vital role in cholinergic synapses[17]. AChE is an enzyme necessary to halt ACh action in an after synaptic membrane inside the nervous system. The immunocompatibility of the AChE forms is high. The hydrolyzation of the neurotransmitter ACh[18] is recognised to constitute one of the most effective catalytic enzymes in the neurological system.

AChE activity is highly needed for the normal muscular function and behaviour of animals and therefore become a target for many chemical pollutants to harm the animals. Inhibition of the activity of AChE leads to the build-up of acetylcholine (Ach) which may result in the repeated, unorganised firing of the neurons and subsequently the stimulation of nerve or muscle fibres, paralysis and sometimes even death [19]. The enzyme successfully ends the chemical impulse at speeds comparable to a diffusion-controlled process, allowing for a quick and repeating reaction [20]. With a turnover time of 150 µs, this equates to the hydrolysis of 5000 ACh molecules per second. This biomarker, which prevents continuous muscle contraction, is essential for the proper functioning of both the sensory neural and neuromuscular systems [21].

Table 1. Enzymes diffusion-controlled rate (K_{cat}/K_m) association with substrate [22].

Enzyme	Substrate	k_{cat} (sec^{-1})	K_m (M)	k_{cat}/K_m ($sec^{-1}M^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1.0×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4.0×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4.0×10^7	1.1	4.0×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2.0×10^{-5}	2.8×10^8
Fumarase	Fumarate	8.0×10^2	5.0×10^{-6}	1.6×10^8
	Malate	9.0×10^2	2.5×10^{-5}	3.6×10^7
Triosephosphate isomerase	Glyceraldehyde-3-phosphate	4.3×10^3	1.8×10^{-5}	2.4×10^8
B-Lactamase	Benzylpenicillin	2.0×10^3	2.0×10^{-5}	1.0×10^8

ACh as a chemical transmitter

The most widely accepted role of ACh in the neurological system is that of a chemical transmitter [23]. ACh is a kind of ammonium compound. In 1920, it was the first transmitter material to be isolated. According to [24], this ACh transmitter is kept in minute amounts, each corresponding to a quantum, and its true purpose is in the conduction of the nerve impulse through the axon. AChE inhibitors work indirectly by preventing ACh from being hydrolyzed (inactivated) at the receptor site [25]. This inhibition enables ACh to build and causes an intensified and prolonged receptor site stimulation. The effects of ACh activation [26-27] include the vasodilation of the blood vessels that reduces heart rate, bronchial constriction and reduced mucosal discharge into the respiratory tract, intestinal cramp, salvia secretion, suck and tears and eye constriction of pupils. This build-up of ACh produces nerve poisoning by producing restlessness, hyperactiveness, tremors, convulsions and paralysis in invertebrates (e.g., crabs).

Cholinergic synapse and AChE

ACh and noradrenaline are the two major substances transmitter in vertebrate nervous systems [13]. ACh from choline and acetyl-CoA in presynaptic neurons is catalysed in ChAT (Fig. 1). ACh is embedded by vAChT in synaptic blisters. In the absence of ACh, an AChR channel cannot be permeated to the ion passage. However, when two ACh molecules are connected to it, AChR opens and allows ions to fluctuate through the membrane of the cell: sodium within and potassium outside. This ion flow defines a plate flow throughout the muscular membrane that induces contraction when powerful enough. These action potentials allow ACh to be released into the synaptic split, where it binds to the pre and post-synaptic membranes' muscular (M) receptors. ACh discharge on the presynaptic membrane is controlled by M2 receptors through a negative reaction (**Fig. 1**).

It is believed that the M protein- 1,4,5-trisphosphate (Ins(1.4.5)P3) and Ca2+-dependent protein kinase (PKC) signals at the postsynaptic location cause action potentials through the diacylglycerol (DAG), as illustrated in Figure 1(29). As long as the ACh molecules are not digested by AChE, the biomolecular off-switch for synaptic transmission, they remain in the spindle of the neuron. AChE, which is an incredibly rapid enzyme, is a technically highly effective method for the termination of synaptic transmission for subsequent signals, and it is capable of destroying ACh molecules at rates that approach theoretical limits [30]. In a regional and temporary integration, the entire procedure is carried out [14].

AChE in Biochemistry

This enzyme has been found in several forms in a number of tissues from both vertebrates and invertebrates, and its various molecular forms can be classed as globular or asymmetric depending on their shape. Globular forms are composed of catalytic subunits in the form of monomers (G1), dimers (G2), and tetramers (G4), and they can exist as soluble or membrane-associated species, respectively. The presence of a collagenous constituent distinguishes asymmetrical structures from symmetrical ones. One, two, or three catalytic tetramers are connected to the subunit of the triple-helical collagenic tail, respectively, to generate the asymmetric forms of the enzyme. Depending on the species, these forms are designated as A4, A8, and A12, with sediment ranging from 9-10 S, 12-14 S, and 16-21 S in the sediment column. They may be incorporated into the extracellular matrix (basal lamina) or formed as a result of ionic interactions [32], among other possibilities.

The solubilization and hydrodynamic properties of the enzyme are heterogeneous, reflecting differences in the mechanism of attachment of the enzyme to cellular structures as well as differences in the quaternary structure of the enzyme itself. Amphiphilic forms, such as those seen in the mammalian central nervous system and chicken [33], can commonly be solubilized at least partially without the use of a detergent [34]. Although all molecular forms of AChE and BuChE are glycosylated, changes in the oligosaccharide between AChE monomers and oligomers, and even between different AChE monomers in muscle, and differences in the oligosaccharide between BuChE tetramers in brain and plasma have been discovered [34]. It appears that proper glycosylation of the AChE forms is necessary for proper secretion as well as for protecting the enzyme against proteolysis [35].

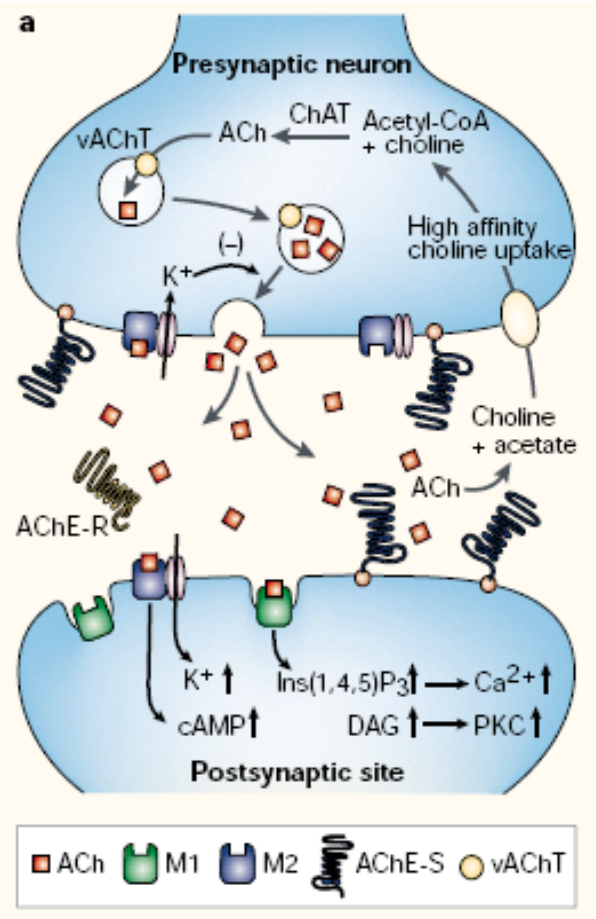

Fig. 1. Cholinergic synapse mechanism [31].

AChE is a particularly relevant subject for thorough structure-function research because of its distinct biochemical features and physiological relevance. An array of evolutionary distinct vertebrate and invertebrate taxa have so far been cloned, with AChE-coding sequences having been identified in insects, nematodes, fish and reptiles, birds and various mammals, including humans. The first crystal model for AChE from Torpedo californica, which has historically been one of the most important sources of AChE for research, was created shortly after the sequence data were published. In later research, crystal structures of the mouse, Drosophila, and human were discovered to be structurally and functionally comparable [31].

X-ray crystallography identified 3 previously unknown functional areas of AChE: (a) a catalytic triad that operates the active site mechanics, (b) a gorge that connects the active site region to the protein surface, and (c) a peripheral anionic site at the protein surface [36-37]. These functional zones were previously unknown. [38] revealed the three-dimensional structure of *Torpedo californica* AChE, and it was determined that AChE contains a catalytic triad similar to that observed in other serine hydrolyses. It also revealed that this triad is near the bottom of a deep and narrow hollow 20 (Fig. 2), which has been termed the "aromatic gorge" because it is lined by the rings of 14 aromatic residues that have been determined to be well preserved [38]. Certain conserved aromatic residues play key roles in both the esteratic and aromatic subsites [34], indicating that they are important in both.

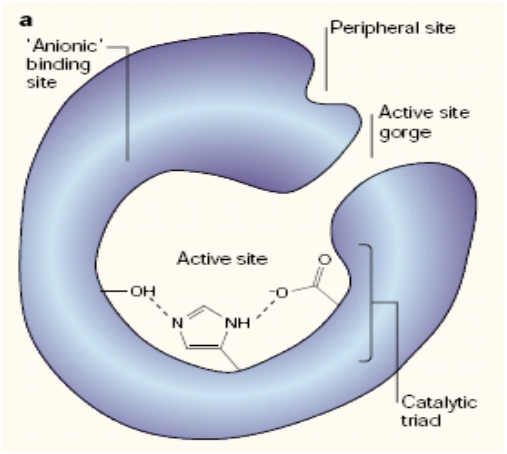

Fig. 2. Structural features of AChE enzyme. X-ray crystallography has identified an active site at the bottom of a narrow gorge, lined with hydrophobic amino-acid side chains [31].

AChE catalytic mechanism

AChE can be categorised in a variety of ways, but its mechanism of action is that of a serine hydrolase. The active site of AChE is composed of two subsites, one of which is anionic and the other of which is esteratic. It is the anionic site that is responsible for specificity with regard to the alcohol moiety, whereas the esteratic site is responsible for the actual catalytic activity. It is clear that the structure around this subsite is what defines specificity in terms of the acid function of the substrate's acid function. The anionic site, as its name indicates, is the location of an electrically negative potential that draws the quaternary ammonium head of ACh to it. The esteratic site, like the catalytic sites of serine proteases such as trypsin, many blood clotting factors, and others [31], includes a catalytic triad of serine, histidine, and an acidic residue (Fig. 3).

The enzyme-substrate complex is one of the most widely proposed processes, in which the hydroxyl group of serine acts as a nucleophile to displace choline and create an acetyl enzyme [39]. The hydroxyl group of serine acts as a nucleophile to displace choline and generate an acetyl enzyme. In this case, the imidazole of histidine acts as a general base, and a proton is shown at the esteratic site to consider the potential that a proton may be involved in the catalysis, possibly via transfer to make choline (rather than the choline dipolar ion) the leaving group [40].

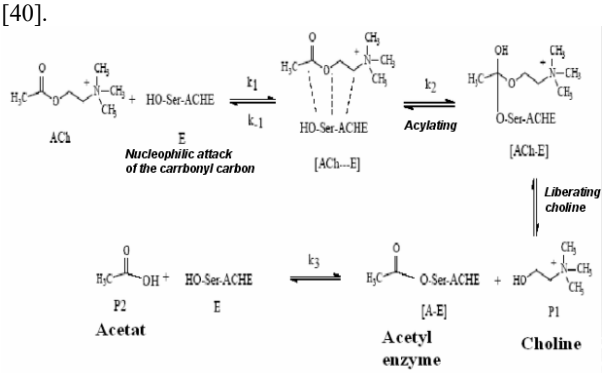

Fig. 3. AChE catalytic mechanism scheme. It is the nucleophilic attack on the carbonyl carbon that initiates the hydrolysis reaction and causes the enzyme to acylate, releasing choline from the enzyme. Acute hydrolysis results in the formation of acetic acid, which is then hydrolyzed to restore the esteratic site to its original state [41].

AChE allosteric effects

The only binding sites that have been considered in the context of the AChE-ACh reaction are the anionic and esteratic subsites of the active centre. In recent years, it has become increasingly evident that the ability of an enzyme to bind substances (or ligands) is not entirely limited to the active site of the enzyme as previously thought. [42] According to current thinking, these regulatory proteins have two, or at the very least two stereospecifically distinct non-overlapping receptor sites [43]. It is one of them, the active site, that is responsible for the biological activity of the protein since it is responsible for binding the substrate. Aside from that, the allosteric site is a structural complement to another molecule known as the allosteric effector, to which it binds selectively and reversibly [42, 43]. A discrete, reversible change in the protein's molecular structure, known as an allosteric transition, is assumed to occur when the complex forms, resulting in changes in the properties of the active site and, as a result, changes in one or more of the kinetic parameters that characterise the enzyme's biological activity. However, this is not always the case.

AChE molecular weight

Over two decades ago, solubilized acetylcholinesterase was discovered by irradiation inactivation as a non-spherical unit with a molecular weight of 105,000, which was the first time this enzyme had been discovered. Following that, several attempts to separate acetylcholinesterase by solubilisation, gel filtration, and column chromatography have yielded a variety of findings from a variety of enzyme sources and isolation techniques. There is widespread agreement that solubilized acetylcholinesterase may be found in numerous forms, with molecular weights ranging from 60,000 to 400,000 [45]. This is due to the large number of biochemical studies that have been published.

Torpedo californica AChE was first thought to have a molecular weight in the millions, according to early estimations [46, 47]. From viscosity and light scattering measurements, [47] obtained an AChE polymer with a specific activity of 425 mmoles ACh/mg protein/hr, and then estimated molecular weights of 25 000,000 and 31 300,000 based on the viscosity and light scattering data, respectively. A smaller molecule (330 000) was discovered in one of the experiments. Because of the decreased specific activity, [47] speculated that it may be the monomer. [48] As purification technologies have become more refined, estimates of molecular weight have dropped while estimates of specific activity have grown (**Table 2**). According to the results of experiments conducted to establish the molecular weight of AChE, the molecule can be broken down into subunits, although it is unclear what these subunits represent in terms of enzyme activity. Even though some early characterization of the AChE crystals from the organ tissue of *Electrophorus electricus* had been published [36], it was the first time that they were purified.

According to [48], insect AChE is most likely exclusively found in globular forms. This agrees with globular and subunit values from *Coturnis japonica, Murex brandaris,* and *Galleria mellonella* [49], which were 246 kDa, 260 kDa, and 240 kDa (native form) and 63 kDa, 66 kDa, and 60 kDa (subunit form) correspondingly (**Fig. 2**). The tetramer form, which has an apparent molecular weight of 420 kDa, corresponds well with isolated AChE from the chicken brain and is composed of two polypeptide chains with apparent molecular weights of 105 kDa and 100 kDa [50]. The presence of a quaternary structure has been reported to be common for AChEs from vertebrates and invertebrates [11].

Contrary most of the invertebrates showed dimeric form of AChE, occurrence from *Schizaphis graminum* [51]*, Nephotettix cincticeps* [52]*, Leptinotarsa decemlineata* [53] and *Lygus hesperus Knight* [54] with 129 kDa, 130 kDa, 130 kDa and 199 kDa (native form) respectively and 72 kDa, 75 kDa, 65 kDa and 94 kDa (subunit form) respectively (**Fig.** 2). The molecular weight of globular AChE in tetramer form was often in the range of 200-400 kDa, while in dimeric form it was in the range of 100-200 kDa. AChE subunits with molecular weights in the range of 60-90 kDa have been identified from both invertebrate and vertebrate sources (**Fig. 2**). The small variation in AChE molecular weight can be ascribed to a variety of glycosylated processes involving oligosaccharides in the enzyme's structure. In order to ensure proper secretion and to protect the enzyme from proteolysis [55], it will be required.

Isozymes of AChE

The fact that an enzyme may exist in more than one molecular form has been accepted for a number of years. The existence of multiple molecular forms of enzymes was first detected by electrophoresis. When run on SDS-PAGE, preparation of enzyme separated into a number of bands with different electrophoretic mobilities but which were catalytically equipotent. The various molecular forms of enzyme thus revealed were termed isozymes [56]. Isozymes were considered to be identical in all respects except electrophoretic behaviour. Subsequently, however, heterogeneity was revealed by many other tests and gradually the definition of an isozyme has become broader [51]. The functional significance of the different isozymes forms of AChE is unclear. AChE molecules are influenced by pH and ionic strength, and it is suggested very tentatively that this phenomenon could be of relevance in the control of the permeability cycles in membranes [57].

AChE substrate specificity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are major groups of cholinesterase differentiated by their substrate preferences. AChE is always specific to its substrate acetylcholine (Ach), due to its rapidly catalysing the hydrolysis of ACh the enzyme partakes in the termination of cholinergic neurotransmission in the neuromuscular synapses [58]. In enzyme catalysis, the specificity of the substrate binding and the efficiency of the catalytic reaction are intertwined. Because of the binding energy between the enzyme and the substrate, the activation energy of k_{cat}/K_M is depressed, and there is an interconversion between the binding and chemical reaction activation energies. For a low barrier reaction to occur, both the substrate and the protein must be in their usual positions. In addition to this, it is expected that the orientation of the bound substrate will have a major influence on enzyme catalysis [59].

Although its precise role is uncertain, it is assumed to be involved in growth and development as well as scavenging cholinergic poisons. It has also been demonstrated to have a supporting role in synaptic transmission [60]. On the basis of their kinetic and pharmacological properties, AChE and BChE may be distinguished. AChE hydrolyzes Ach and is less active on BCh, whereas BChE hydrolyzes both substrates and is hence less selective. The susceptibility of enzymes to diagnostic inhibitors can also be used to distinguish between them [61].

Cholinesterases, which are found in abundance across the animal kingdom, were divided into three groups based on their substrate specificity: AChE, PChE, and BuChE. AChE differs from BuChE in that it hydrolyzes ACh or, more specifically, its homologue ATC, more efficiently and more quickly than it does any other substrate. It is thought that the natural substrate for

vertebrate AChE is ACh, which is the most abundant choline ester present in the tissues. In the laboratory preparations of AChE generally exhibit maximal activity towards ACh but with the same species, propionylcholine and another choline ester present in a certain animal is hydrolyzed as fast or even faster [58]. This is particularly true of the enzyme in avian brain tissue. AChE activity towards BuCh and proprionylcholine is very low compared with that towards ACh; nevertheless the enzyme in certain species will split BuCh to a significant extent [61].

Table 2. AChE substrate specificity.

Cholinesterase source	K_m / µM			V_{max} U/mg			Reference
Substrate	ATC	PTC	BTC	ATC	PTC	BTC	
Nephotettix cincticeps	51.1	39.1	41.6	70.2	30.5	4.6	[52]
Schizaphis graminum	57.6	31.3	33.4	78	37.4	2.3	[51]
Leptinotarsa decemlineata	14.9	8.2	9.4	177.7	65.1	5.7	[53]
Hornfly (*Haematobia irritans*)	24.2	20.4	8.4	18.7	9.03	0.6	[67]
Eurytemora affinis	32	Nil	Nil	100	10	10	[2]
Murex brandaris	78	46	45	Nil	Nil	Nil	[64]
Hiruda medicinalis	484	292	85	361	725	386	[65]

Effect of excessive substrate, pH and temperature on AChE

AChE is sensitive to inhibition when an excess of substrate is available. This removal of AChE through the surplus substrate is one of the features of pseudocholinesterase (BChE). AChE is highly active at low Ach levels and is inhibited by high Ach levels[68]. Several causes for the changes in AChE behaviour in the presence of additional substrates were presented. The inhibition of the substratum is according to the [34] a decrease in enzyme-catalytic effectiveness induced by conformational changes caused by the presence of excess substrate, rather than a creation of a stable complex of acylenzymes-substrate. Others hypothesised that adding additional molecules of substrates to alosteric locations might induce significant conformative modifications to prevent normal catalysis [52].

The active region of the crystal structure of Torpedo calinifornica (Tc) AChE is concealed beneath the narrow gorge, parallel to the conserved aromatic residues [38]. When the substrate concentration is high, AChE is inhibited. All available AChE models predict the presence of two substrate-binding sites (the active and peripheral anionic sites) [69].

Because of the presence of an electrolyte in the sub-phase at partially neutral pH, acetylcholinesterase (AChE) forms an extremely stable layer (pH 6.5). The mixed polar and non-polar state of AChE, as well as its high molecular weight, contribute to its stability. The ionic character of the sub-phase had no effect on the creation of the AChE monolayer, while pH had a substantial impact on the reduced molecular area and surface pressure of the monolayer [70]. Larger molecular areas and higher surface pressures were obtained in an acidic pH (3-4) than in a basic pH (7-8), indicating that pH has a significant role in determining enzyme adsorption and stability, and pH of the reaction medium is one of the major aspects of enzyme catalysis [71].

Temperature is an essential environmental factor that influences the physiological performance of organisms; high temperatures impair growth, enzyme activity, oxygen usage, and, in some cases, mortality [72]. Many abiotic variables, particularly temperature, have been observed to influence the activity of the acetylcholinesterase enzyme. Maximum AChE activity was observed in estuary blue mussels (*Mytilus* sp.) during in the summer and lowest activity during the winter [73].

CONCLUSION

The purification and characterization of acetylcholinesterase (AChE) from a variety of sources has been discussed in this article. AChE is a neurotransmitter present in the cholinergic synapse of the nervous system in both insects and all vertebrates. It is found in both insects and all vertebrates. The activity of acetylcholinesterase is influenced by a variety of factors, including the substrate, pH, and temperature. As a result, AChE is considered to be a reliable marker for detecting environmental contamination.

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