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REVIEW

Butyrylcholinesterase as a biochemical marker

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Abstract: Butyrylcholinesterase (BChE) is an enzyme expressed in multiple organs and abundant in plasma. BChE can fluctuate in course of several reasons while both hypercholiensterasemia and hypocholinesterasemia are known. Considering evidence of BChE activity alterations, hepatocellular carcinoma, chronic liver diseases and poisoning with carbamates or organophosphates can be diagnosed by activity assay. BChE is responsible for detoxification reactions, and the compounds such as cocaine, succinylcholine, and acetylsalicylic acid are degraded in the body. The detoxification can be slowed in patients carrying the K variant of the enzyme. Summarization of literature, discussion on the meaning of BChE in the body, and the principles of BChE assay in samples are described in the review *(Tab. 2, Fig. 8, Ref. 86)*. Text in PDF *www.elis.sk.*
Key words: butyrylcholinesterase; acetylcholinesterase; liver function test; organophosphate; carbamate; poisoning; dibucaine number; Alzheimer´s disease; BChE; AChE; K variant.

Two enzymes are entitled cholinesterases, namely butyrylcholinestease (BChE; EC 3.1.1.8) and acetylcholinesterase (AChE; EC 3.1.1.7). The necessity of BChE for organism is not well understood. The fact that the enzyme is named after an artificial substrate butyrylcholine confirms this. As opposed to BChE, the role of AChE in organism is well known. The enzyme has its natural substrate, namely the neurotransmitter acetylcholine. The substrate is split by AChE into choline and acetate and the neurotransmission is terminated in this way. A part of central and peripheral nervous system based on cholinergic neurotransmission is called the cholinergic system, and AChE is an invincible part of the system. The system plays the key role in processes such as memory, immunity regulation, and vegetative organs regulation (1). Some pathological processes are linked to the cholinergic system. An effort to modulate it by drugs is therefore considered a suitable way to treat or at least suppress the diseases. E.g. Alzheimer´s disease, myasthenia gravis, schizophrenia and inflammation can be treated by drugs affecting the cholinergic system (1–3).

In some older sources, AChE is referred to as blood cholinesterase due to the fact that the bulk of its activity is localized on the surface of erythrocytes. When hemopoiesis works well, the decrease in blood AChE activity can be attributed to poisoning by some of neurotoxic compounds such as organophosphorus pesticides or nerve agents (4, 5). The disadvantage of AChE in the poisoning diagnosis is the instability of blood and necessity to homogenize samples for assay purposes. As opposed to that of AChE, the assay of BChE in plasma or serum is simpler as there is no need to pre-treat the sample. BChE can be used as another marker of neurotoxic compounds poisoning (6). On the other hand, alterations in BChE activity cannot be interpreted as simply as in case of AChE. Contrary to AChE formation *in situ*, BChE is produced in the liver and the assay of BChE activity is considered also a liver function test (7).

The present work is devoted to discussion on BChE importance in clinical diagnosis and meaning of alterations in BChE activity in the body. The work should help to interpret results from a clinical assay and understand the meaning of the enzyme.

Butyrylcholinesterase structure

The structure of BChE is very similar to that of AChE. The both enzymes belong to esterase – lipase family within α/β hydrolase-fold superfamily (8). The most ubiquitous form of BChE is a tetramer. Each subunit of the tetramer has 574 amino acids and nine carbohydrates linked to nine asparagine residues. Forty amino acids at the C-terminal are responsible for tetramerization of subunits. The total weight of one subunit is approximately 85 kDa. Scoring similarity between AChE and BChE resulted in finding that there is a 53.8 % identity between the enzymes in their sequence (9). The tertiary structure of BChE is similar to that of AChE. It contains three parts with key role in esteratic activity: a) peripheral anionic site on the enzyme surface, b) narrow aromatic gorge leading inside the enzyme and c) active site at the bottom of aromatic gorge (10). For human BChE, Asp 70 and Tyr 332 are involved in the initial binding of charged substrates such as butyrylcholine and allow them to penetrate inside the active site (11). The aromatic gorge represents the major difference between AChE and BChE. Whilst AChE has the gorge lined by 14 aromatic amino acids residues, BChE has only 8 aromatic residues in the gorge (12). The difference in the BChE´s gorge is the reason why

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BChE can split a wide number of esters with a high turnover rate while AChE is quite specific for acetylcholine. The importance of the gorge was confirmed when mutant AChE was prepared. The mutant enzyme was very similar to BChE in its substrate specificity (13). The total number of aromatic amino acid residues plays a significant role in enzyme function. Tyr 337 in AChE is replaced by Ala 328 in human BChE resulting in differences in substrate specificity. Although side chains of both amino acid residues are hydrophobic, the presence of Tyr 337 along with the BChE´s gorge higher volume is implicated in lower penetration of pharmacologically important ligands such as huperzine A, edrophonium, and acridine derivatives into the active site (14). The compounds act as selective inhibitors for AChE with no or lower inhibitory potency toward BChE.

The active site of human BChE contains a catalytic triad of residues, Ser 198, Glu 325, and His 438, determining the esteratic activity of the enzyme (15). Active serine is a common part of serine hydrolases and it is not privileged to cholinesterases only (16). The lysis of substrate is caused by nucleophilic substitution as alcoholate anion attacks carbon in carboxylate moiety of esters.

Role of butyrylcholinesterase in the body

Some researchers can conclude their investigation by stating that BChE has no fundamental role in the body. However, it is not correct as evidence of some significant mechanisms related to BChE has been established. The understanding of the role of BChE in the body is complicated by the fact that unlike AChE, BChE has no unique physiological substrate (1). The evidence of BChE was expected to be found once butyrylcholinesterase knockout animals were prepared. However, physical examination of BChE(+/+) and BChE(-/-) did not prove any significant alterations in basal body functions (17). People with BChE deficiency, i.e. patients suffering from hypocholinesterasemia, have no or minimal BChE activity in their plasma. Nevertheless, they can live full life. Case reports provide no evidence that any detrimental consequence can be expected. E.g. mutations at codon 400 resulted in stop codon (18), substitution at codon 365 resulted in a Gly-Arg substitution (19) or mutation at codon 128 resulted in Tyr-Cys substitution (20). The mutations had no fatal consequence.

Despite the non-life-threatening state in BChE-deficient patients, the patients become more vulnerable to some drugs as discussed in chapter devoted to dibucaine number. BChE is able to split multiple drugs. Prolonged neuromuscular paralysis following narcosis with co-adminstration of myorelaxants given in tracheal intubation: succinylcholine (suxamethonium) chloride or mivacurium occurs in BChE-deficient individuals or in carriers of K variant of BChE (see later). Thus a simple surgical procedure may become complicated as evident from an example case report (21). Beside succinylcholine, BChE hydrolyzes other drugs with ester moiety, such as local anesthetics, namely procaine, dicaine, bupivacaine (22, 23) or antipyretic/analgesic drugs such as acetylsalicylic acid (24, 25). Examples of drugs´ hydrolyses are depicted in Figure 1.

The question as to what the role of BChE was, can be answered that it is an enzyme participating in the first phase of detoxification reaction. Natural toxins can be split just by BChE as seen in some examples and this task is the probable reason why BChE gained its biological meaning. It is well documented in cocaine (benzoylmethylecgonine), tropane alkaloid of coca plants (*Erythroxylum coca* and *E. novogratense*). BChE produces a non-toxic ecgonine methyl ester from cocaine. The BChE-catalyzed pathway of cocaine detoxification is a pathway competing the metabolic activation of cocaine into the more toxic norcocaine and bonzoyl ecgonine by hepatic carboxylesterases (26, 27). The pathways are summarized in Figure 2. The necessity of BChE for resolving the poisoning with cocaine was reported by Duysen et al using a model of BChE knockout mice (28). The BChE(-/-) mice had e.g. depressed respiration for 12 hours while the BChE(+/+) ones had recovered normal respiration rates in 30 minutes. The poisoning had detrimental consequences on liver tissue as well. Owing to the BChE role in the detoxification pathways, the assessment of plasmatic BChE activity should follow on from suspicion of cocaine poisoning.

Another role of BChE can appear after exposure to cholinergic compounds inhibiting AChE. After the exposure, BChE can substitute the missing activity of AChE. The substitution was proved on AChE-deficient mice evidently resolving hyperstimulation in hippocampus by hydrolysis of acetylcholine by BChE (29). The suitability of BChE was proven in several experimental models. Duysen et al proved that homozygotic mice bearing full function of BChE(+/+) and heterozygotes BChE(+/-) were more resistant to overdosing by drugs used in Alzheimer´s disease treatment (-)-huprezine A and donepezil (30). Comparing to animals with

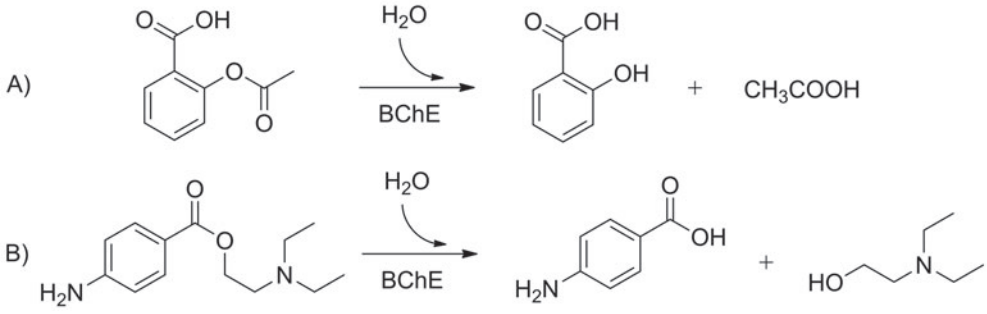

Fig. 1. Hydrolysis of acetylsalicylic acid (A) and procaine by BChE.

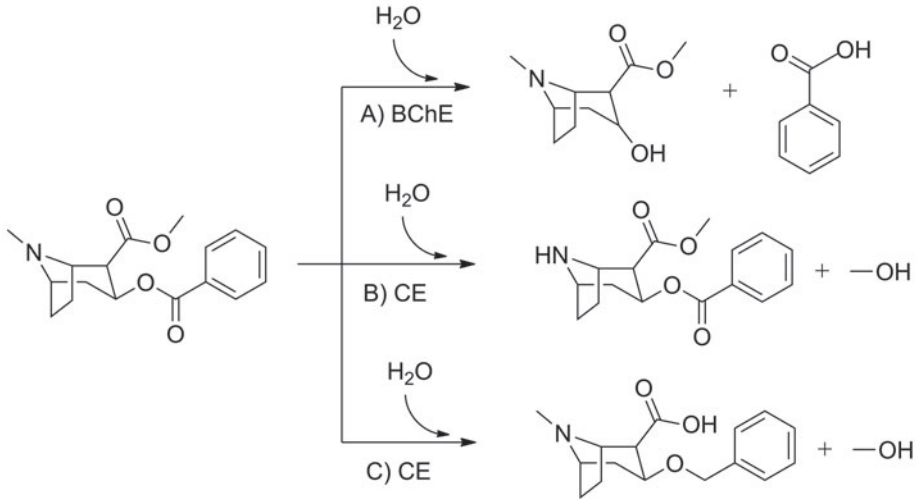

Fig. 2. Hydrolysis of cocaine by BChE (A). The competing reactions done by carboxyesterase (CE) into norcocaine (B) and benzoyl exgonine (C).

bulky BChE activity, the homozygotes BChE(-/-) are extensively sensitive to overdosing by the drugs as the inhibited AChE is not substituted by BChE. It can be concluded as an evidence of fact that patients with Alzheimer´s disease and lowered level of BChE may not tolerate drugs acting as AChE inhibitors (e.g. rivastigmine, galantamine, donepezil) and the therapy should be altered according to their higher sensitivity.

Alterations in butyrylcholinesterase level

BChE is constituted in multiple organs including the central nervous system, muscles, kidneys, heart and lungs. The bulkiest production of BChE is common in the liver which serves as its source thus releasing the enzyme into plasma (31–33). The main clinical meaning of assay of BChE in plasma is that therein BChE may alter its plasmatic level due to several reasons. Both hypocholinesterasemia and hypercholinesterasemia are known. The hypercholinesterasemia is quite rare. Two explanations for hypercholinesterasemia can be given. It can be caused by either genetic disposition or some types of hepatocellular carcinoma. The genetic disposition to hypercholinesterasemia is rare and it has been reported in sporadic case reports from Japan (34–36). Hepatocellular carcinoma followed by an increase in BChE activity in the liver and hypercholinesterasemia as assayed in plasma was reported by Tajiri et al (37). The authors infer that hypercholinesterasemia can be considered to be a marker of hepatocellular carcinoma due to good selectivity of the marker.

Like hypercholiensterasemia, hypocholinesterasemia can appear in course of several reasons. Patients with increased sensitivity to succinylcholine and mivacurium have a lowered level of BChE activity in plasma. K variant of BChE (see in chapter devoted to dibucaine number) is responsible for the sensitivity (38). Other forms of familial hypocholinesterasemia can occur as well. Activity of BChE in plasma for butyrylcholine or butyrylthiocholine is 105–240 U/l for normal health population; however, people with familial hypocholinesterasemia may keep their level of BChE at about 10 U/l as reported by Liu et al (39). The authors revealed three point mutations at positions 298 (CCA to TCA), 1 410 (CGT to CGG), and 1 615 (GCA to ACA). The first and the last mutations caused alterations of proline to serine and alanine to threonine, respectively. Lower (~ 30 %) activity of BChE is in people with K variant (see later). In rare cases, hypocholinesterasemia can be acquired by transplantation of the liver from a donor with the K variant (40). The donation has, however, no effect on transplantation efficacy or any pertinent adverse effects.

As BChE presented in plasma is constituted in the liver, its ability to constitute BChE can be used as a liver function test. Once familial dysfunction of BChE is excluded, the meaning of diagnosed hypocholinesterasemia can be interpreted as a chronic liver disease, acute hepatitis or liver cirrhosis (41). The alterations in BChE level are similar to albumine. Hypocholinesterasemia is expected to be diagnosed concurrently with hypoalbuminemia and the concurrent diagnosis of hypoalbuminemia can exclude poisoning with cholinesterase neurotoxins. Both hypocholinesterasemia and hypoalbuminemia correlate well in e.g. virus hepatitis (42). The other proteins produced in the liver such as coagulation factors and complement components may also correlate with BChE level (43). Simultaneously with hypocholinesterasemia, hypertransaminasemia as a more common marker is diagnosed in patients with chronic liver diseases (44).

Cholinesterases can be inhibited by several chemical compounds. It was extensively reviewed previously (1). From the toxicological point of view, organophosphorus and carbamate insecticides (desmedipham, fenoxycarb, methiocarb, phenmedipham and pirimicarb propamocarb currently used in European Union) and nerve agents (e.g. sarin, soman, tabun, VX) used in chemical warfare are the most common examples of compounds inhibiting the activities of cholinesterases. Nevertheless, the current insecticides are allowed to be used only after metabolic activation and not directly. The drug currently used for Alzheimer's disease rivastig-

Tab. 1. Summarization of hypercholineasterasemia nd hypocholinesterasemia etiology.

Effect	Etiology	Reference
None – normal BChE activity	activity 105 – 240 U/l for butyrylthiocholine is expected in normal plasma	39
Hypercholinesterasemia – ↑ of plasmatic BChE activity	genetic disposition	34–36
	hepatocellular carcinoma	37
Hypocholinesterasemia – ↓ of plasmatic BChE activity	mutations at positions 298 (CCA to TCA), 1 410 (CGT to CGG), and 1 615 (GCA to ACA), activity in plasma ~ 10 U/l	39
	30 % lower activity of BChE in the K variant carrying individuals	63
	transplantation of liver from the K variant carrying donor	40
	virus hepatitis	42
	chronic liver diseases	44
	poisoning with organophosphates, carbamates or tacrine	1, 48

mine and the currently withdrawn drugs metrifonate and tacrine are inhibitors of both AChE and BChE. On the other hand, donepezil, galantamine and huperzine are inhibitors of AChE whilst only with no or minimal inhibitory potency to BChE (1, 45). Alterations in BChE activity correlate well with poisoning symptoms (46). The poisoning with pesticides can be revealed not only by using assay of BChE, but some other enzyme markers presented in serum and plasma correlate with the BChE. In an example, Araoud and coworkers (47) proved a contemporary decrease in BChE and paraoxonase 1 activity in serum of Tunisian agricultural workers accidentally poisoned with cholinesterase inhibitors. Moreover, gamma glutamyl transferase correlates with the two mentioned markers. It can be inferred that assay of paraoxonase 1 can confirm diagnosis based on assay of BChE. The suitability of BChE for assay purposes was demonstrated e.g. on patient self-poisoned with chlorpyrifos (48). The alterations in plasmatic level of BChE in course of several reasons are summarized in Table 1.

Dibucaine number

In majority of population worldwide, BChE is present in A variant. A minority of people have BChE in K variant. The K variant is not able to split some drugs with ester moiety such as succinylcholine or mivacurium. The main difference between A and K variants is in substitution of Ala in position 539 to Thr (49, 50). Once the patient bears the K variant of BChE, myorelaxants given in tracheal intubation and some other surgical procedures, such as succinylcholine and mivacurium, can cause complications and postpone recovery from anesthesia (21). The K variant of BChE is in a minority of people who then can be AA homozygotes, AK heterozygotes or KK homozygotes. In an extensive research in Germany based on 24,830 patients examined, Pestel et al revealed 1.23 % of AK heterozygotes and 0.07% of KK homozygotes (51). The distribution of K variant can, however, differ among individual nations. In a comparing study based on 400 patients, Hosseini et al proved a significantly higher distribution of K variant in Iranian population when compared to the Irish (52).

In order to examine the form of BChE, an assay of either dibucaine number (commonly abbreviated as DN) or fluoride number (commonly abbreviated as FN) is suitable for their distinguishing (53, 54). The assay is based either on inhibition of BChE by dibucaine (cinchocaine) (Fig. 3) or fluoride anion. With increased DN or FN, the probability of BChE in K variant is elevated (55).

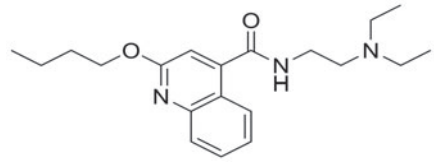

Fig. 3. Dibucaine (cinchocaine).

An AA homozygote has DN ≥75. On the other hand, a KK homozygote has DN <20. AK heterozygotes have DN typically in an interval of 40–70 (56).

In addition to assay of DN as a marker of risk for anesthesia, there is evidence that K variant can interfere in Alzheimer´s disease therapy due to late response to cholinesterase inhibitors application (57). Although etiology of Alzheimer´s disease is not understood, there is evidence of link between BChE and disease development (58, 59). The commonly accepted risk factor for Alzheimer´s disease is apolipoprotein E ε4 (60). It seems that BChE correlates with the apolipoprotein E ε4 and the K variant of BChE may be another risk factor (61). Epidemiologic investigation confirmed a lower probability to develop Alzheimer´s disease in the K variant of BChE-carrying people (62, 63). On the other hand, the K variant is a risk factor for fronto-temporal dementia (63). Owing to the implication of BChE in neurodegenerative disorders and different probability to receive the disorder when BChE is in the K variant, assay of DN is a helpful tool for anamnesis of the disorders.

Artificial substrates of butyrylcholinesterase

Beside esterase and thioesterase activity, both AChE and BChE exert aryl acylamidase activity. Currently, there are a number of cholinesterase substrates useful for colorimetric, fluorimetric or electrochemical assay of their activity (64). The aryl acylamidase activity of BChE allows to use o-nitroacetanilide, m-nitroacetanilide, o-nitrophenyltrifluoroacetamide and 3-(acetamido) N,N,N-trimethylanilinium as chromogenic substrates (65). The o-nitroacetanilide is probably the most frequently used chromogenic substrate from acylamides. The mechanism of o-nitroacetanilide is depicted in Figure 4. The main disadvantage of aryl acylamidase activity assay lies in the fact that the specific activity of monomer of BChE is higher than that of tetramer (66, 67). Chaotropic reagents and non-standard manipulation with samples

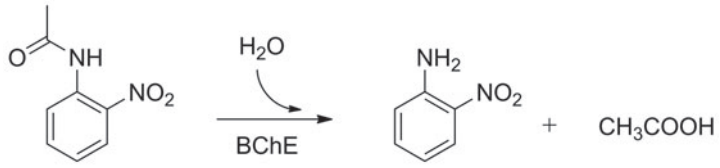

Fig. 4. Aryl acylamidase activity of BChE on o-nitroacetanilide.

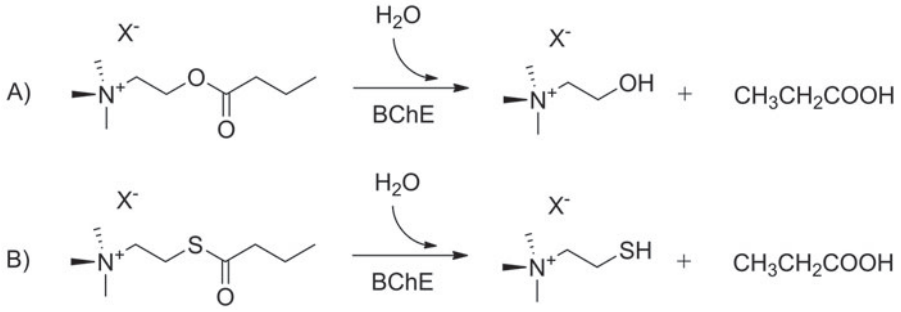

Fig. 5. Esterase and thioesterase activity of BChE on conversion of butyrylcholine (A) into choline and butyrylthiocholine (B) into thiocholine with simultanous releasing of butyric acid.

can cause an increase in assayed activity and misinterpretation of the results.

Natural substrate of BChE is not known and the enzyme is named after an artificial substrate, butyrylcholine. This is unlike AChE whose substrate is acetylcholine and the Michaelis constant is the lowest just for acetylcholine. BChE is able to split a wide group of esters and thioesters including the endogenous acetylcholine. Although the name of BChE could be considered a proof that BChE splits it in the body, butyrylcholine is not a physiologically relevant compound. Evidence of butyrylcholine as a neurotransmitter remains unconfirmed despite the fact that some authors reported its physiological role in the nervous system (68). The splitting of butyrylcholine and butyrylthiocholine as an example of esterase and thioesterase activity is depicted in Figure 5.

Owing to substrate specificity, the Michaelis constant of BChE is quite low not only for butyrylcholine and butyrylthiocholine but also for other esters such as benzoylcholine, benzoylthiocholine, acetylcholine, acetylthiocholine, propionylcholine, propionylthiocholine, succinylcholine, succinylthiocholine and the above-reported cocaine (69–76). Michaelis constants for artificial substrates are summarized in Table 2. As seen from the latter table, BChE is able to split both aryl and acyl (thio)esters. The Michaelis constant of BChE is even lower for benzoyl(thio)choline and succinythiocholine than for butyrylthiocholine. Considering acetylthiocholine and butyrylthiocholine to be common substrates in cholinesterase activity assay, butyrylthiocholine is split by BChE with nearly a four-time greater turnover rate when compared to acetylthiocholine.

Butyrylcholinesterase activity assay

Although there are a large number of substrates suitable for cholinesterases activity assay, the protocol based on Ellman´s reagent has become an unofficial standard. The protocol uses 5,5'-dithio-bis-2-nitrobenzoic acid as a chromogenic reagent and acetyl or butyrylthiocholine as a substrate. It was established by Ellman and coworkers in the 1960s (77) and it remains usable today (78). The principle of BChE activity assay using the Ellman´s reagent is depicted in Figure 6. In the assay, butyrylchiocholine is split to thiocholine and butyric acid by BChE. In the second step, thiocholine spontaneously reacts with the Ellman´s reagent providing 5-thio-2-nitrobenzoic acid. An isomer of the 5-thio-2-nitrobenzoic acid

Tab. 2. Michaelis constants (Km) for selected substrates of BChE.

Substrate	Km (mmol/l)	BChE	Reference
Acetylcholine	0.080	An isolate from Trichinella spiralis	69
Acetylthiocholine	0.080	Human, normal type	70
Benzoylcholine	0.0080	Both normal human and K variant of BChE	71
Benzoylthiocholine	0.0084	Human, normal type	72
Cocaine (− isomer)	0.065		73
Cocaine (+ isomer)	107		
Butyrylthiocholine	0.021		74
Propionylthiocholine	0.76 / 0.57	Human, normal type/atypical resistant to pesticides	75
Succinylthiocholine	0.003 / 0.011	Human, normal type/human, mutant in peripheral anionic site	76

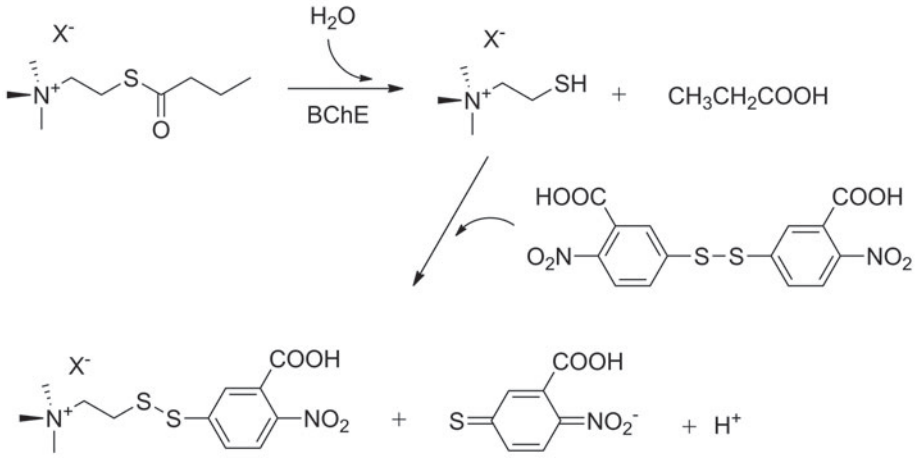

Fig. 6. Principle of BChE activity assay using butyrylthiocholine and Ellman´s reagent.

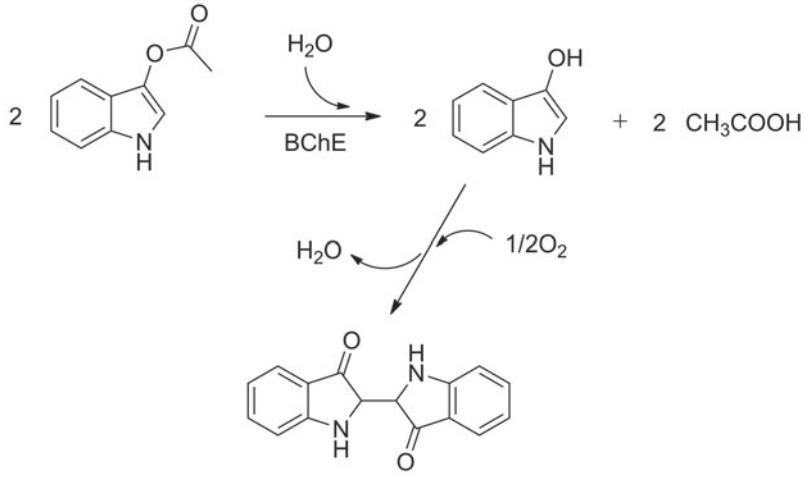

Fig. 7. Principle of BChE activity assay using indoxylacetate.

which is stable in neutral and alkaline conditions provides yellow coloration strongly absorbed at 412 nm. It should be emphasized that the assay is suitable for AChE as well when acetylthiocholine is used as a substrate. BChE is able to split acetylcholine, too. To distinguish whether the sample contains BChE or AChE, a specific inhibitor for BChE, tetraisopropyl pyrophosphoramide known also as iso-OMPA, can be added into the sample (79).

BChE activity can be simply assayed by a titrimetric or potentiometric device as butyric acid is formed from a pH-neutral compound (64). Fluorogenic substrates are available for BChE activity assay beside the chromogenic ones. The substrate indoxylacetate provides blue-colored indigo that is to be simply measured by a colorimetric, spectrofotometric or fluorimetric assay. The principle of the assay is shown in Figure 7. Accumulated indigo can be measured spectrophotometrically at 670 nm (80) or fluorimetrically with maximal absorption at 610 nm, and emission at 653 nm (81). Indoxylacetate is suitable for both AChE and BChE assay but the turnover number is 2.65 times higher for BChE than for AChE (82). Indoxylacetate was proven to be suitable for construction of disposable sensors for a fast assay of cholinesterase´s inhibitors using cholinesterase as a biorecognition element (83, 84)

2,6-dichloroindophenyl acetate is another substrate providing a compound measurable spectrophotometrically and fluorimetrically (85, 86). The formed 2,6-dichloroindole is red in acidic conditions. Once pH is buffered into basic, the red 2,6-dichloroindole turns into blue 2,6-dichloroindolate. No et al tested 2,6-dichloroindophenyl acetate as a substrate for cholinesterase-based disposable sensor; nevertheless, the authors stated that indoxylacetate should be preferred as a colorimetric substrate (83). The reaction based on 2,6-dichloroindophenyl acetate is depicted in Figure 8.

Conclusion

BChE is an enzyme associated in multiple pathologies. Assay of the enzyme can be suitable for simple proving of pathologies or it can be used as a marker in addition to most common markers such as transaminases in liver function test. Contrary to most of biochemical markers, BChE activity in biological samples such as

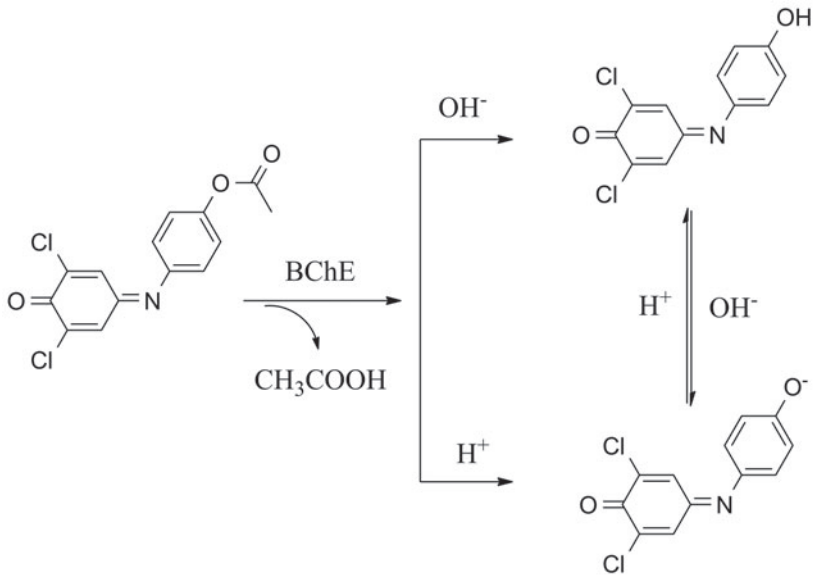

Fig. 8. Assay of BChE activity using 2,6-dichloroindophenyl acetate.

plasma can be easily measured using chromogenic or fluorogenic substrates without any pretreatment of samples.

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