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Detection techniques of carboxylesterase activity: An update review

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ABSTRACT

Mammalian carboxylesterases (CESs) are essential members of serine esterase hydrolase superfamily, which are widely distributed in many tissues including liver, intestine, lung and kidney. CESs play an important role in the metabolism of various xenobiotics including ester drugs and environmental toxicants, and also participate in lipid homeostasis, so the development of CESs activity detection techniques are of great significance for drug discovery and biomedical research. With the rapid development of separated and detection technologies such as chromatography, capillary electrophoresis, fluorescent probe-based detection technology, bioluminescent sensor and colorimetric sensor in recent decade, the research of physiological functions of CESs have make huge breakthrough. This review summarizes the development and application of CESs activity detection techniques, as well as comparatively analyzes the characteristics of various detection techniques. The information and knowledge represented here will help the researchers carry out various biochemical studies for understanding activation mechanism and role of CESs in drug metabolism.

1. Introduction

Mammalian carboxylesterases (CESs) belong to the serine esterase superfamily, which are expressed ubiquitously in the endoplasmic reticulum and cytoplasm of tissues, including liver, intestine, lung and kidney [1]. The most primary physiological function of CESs identified to effectively catalyze the hydrolysis of various ester and amide-containing xenobiotics [2,3], for instance, many ester prodrugs (such as clopidogrel, irinotecan and oseltamivir) and environmental toxicants (such as pyrethroids) [4,5]. Multiple factors such as genetic polymorphism, age, drugs and disease status have been proved to alter CESs expression and function in tissues or individuals, and may ultimately cause a great impact on the therapeutic efficacy and toxicity of many drugs [6–8]. In addition, CESs also play vital role in lipid homeostasis for metabolizing endogenous esters including cholesteryl ester, triacylglycerol and chylomicron [9–11], thus dysfunction of CESs is closely related to atherosclerosis, cholesterol-induced liver injury and type 2 diabetes mellitus [12–14].

Taking into account the crucial role of CESs in both endobiotic and xenobiotic metabolism, there is great significance to establish a rapid, sensitive and efficient CESs activity detection technique for predicting drug pharmacokinetics, the rational design of prodrugs activated by a CESs, and the further studies on the lipid metabolism-disease pathogenesis. This review summarizes the development and application of CESs detection techniques in order to provide reference for the further biomedical research and activity detection techniques of CESs.

2. Classification, distribution and function of CESs

According to the homology of the amino acid sequence, CESs isozymes are classified into six main groups (CES1-CES6) and several subgroups. All CESs isozymes share 40–50% similar amino acid sequences, but exhibit differences in tissue distribution and distinct substrate [15]. The major subtypes of CESs have been identified as CES1 and CES2 in the human body, and they both locate on chromosome 16 (16q13-q22.1). CES1 gene contains 14 exons spanning of about 30 kb, whereas the CES2 gene contains 12 exons spanning of about 9 kb. CES2 is located approximately 11.1 cm downstream of CES1 and share 48% sequence homology with CES1 [16]. There are only slight nucleotide differences in the promoter and signal peptide regions of three subtypes of CES1 in humans: CES1A1, CES1A2, and CES1A3. CES1A2 gene is a variant of CES1A3 pseudogene. Both CES1A1 gene and CES1A2 gene can product mature proteins, but the transcriptional activity of CES1A2 gene is much lower than that of CES1A1 gene. On the contrary, CES2 has a relatively simple structure and only one subtype in human body [17,18].

CESs are mainly localized within the endoplasmic reticulum (ER) membranes and the cytoplasm in different mammalian tissues.

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0045-2068/

Generally, CES1 is abundantly expressed in the liver, with lesser amounts in lung, heart, kidney, testis tissue, adipocyte, monocytes and macrophages, and tends to hydrolyze compounds containing smaller alcohol groups and larger acyl groups, such as methylphenidate, oseltamivir and clopidogrel [19–21]. As one of the most abundant serine hydrolases distributed in liver and adipocytes, CES1 is prove to exhibit endogenous esters (such as cholesteryl esters, triacylglycerols and chylomicrons) hydrolase activity, thus plays pivotal roles in physiological or pathological processes, such as cholesterol homeostasis and metabolic diseases [11,22,23].

CES2 expresses at relatively high levels in the small intestine but also expressed in the kidney, liver and heart. In contrast to the substrates specificity of CES1, CES2 prefers to hydrolyze compounds with larger alcohol groups and smaller acyl groups [24]. As the major CESs subtype in the intestine, CES2 mainly mediates the hydrolysis of most oral prodrugs before it enters the blood circulation through the intestinal tract, thus plays crucial roles in the metabolic activation of anticancer drugs such as irinotecan and capecitabin [25–27]. Moreover, CES2 controls a lipid network dysregulated in human obesity to reverse hepatic steatosis and glucose intolerance [10].

CES3 is mainly expressed in the liver and gastrointestinal tract. Although its level is relatively lower than CES1 and CES2, increased expression of CES3 can restore intracellular CESs hydrolytic activity [28]; CES4 and CES5 are lower expressed in humans, and CES4 (also known as CES1A3 or CES1P1) is a pseudogene of CES1 [15]; CES5 (also known as cauxin or CES7) is predominantly expressed in peripheral tissues such as brain, kidney, lung and testis. The roles of CES5 may include catalyzing lipid and cholesterol transfer processes within male reproductive and protecting nerve tissue from drugs and xenobiotics [29]; CES6 was newly discovered in the special regions of the human brain, such as the cerebellum, and it is involved in the detoxification of drugs and xenobiotics in neural, other tissues and cerebrospinal fluid [30].

3. Development of CESs activity detection techniques

With CESs getting much attention in recent year, detection of CESs real activity would be significantly helpful to biochemical researches, as well as the evaluation of therapeutic drugs, and also facilitate the further studies on the design of ester prodrug, detoxification of xenobiotics, metabolic diseases and testosterone synthesis [4,31]. In early year, Western blotting and real-time quantitative polymerase chain reaction (qRT-PCR) are commonly used to assess CESs mRNA and protein levels, but those methods are less sensitive, selective and couldn't avoid the interference of endogenous substances in complex biological samples such as cellular extracts, or serum. More importantly, these methods can only estimate CESs at mRNA or protein levels rather than the real enzyme activity [17,32]. Recently, the development and upgrade of detection techniques such as spectrophotometry, chromatography, capillary electrophoresis, fluorescent probe-based detection technology, bioluminescent sensor and colorimetric sensor have brought great breakthroughs in the file of CESs activity detection. Particularly, a wide variety of small molecule fluorescent probes with high selectivity and ultra-sensitivity for monitoring the change of CESs activity in complex biological samples have emerged. With these detection techniques, the accurate measurement of the real activity of CESs in different biological systems was achieved.

3.1. Spectrophotometry

CESs catalyze p-nitrophenyl acetate (p-NPA) into phenol. The CESs activity can be quantitatively determined by measuring the absorbance of phenol at 405 nm using a UV/VIS spectrophotometer. This method is simple, rapid, sensitive and could quantitatively determine the total CESs activity [5,33,34]. Nonetheless, the spectrophotometric analysis also has several severe shortcomings. At first, the overlapping wavelengths of absorption of substrates and products would result in interference during determination, so it is difficult to determine the relative activity of specific CESs in a complex biological sample or enzyme mixture by simple spectrophotometry. Secondly, this method requires a large sample size, which is not conducive to the detection of human biological samples [35-37].

3.2. Capillary electrophoresis

Capillary electrophoresis (CE) is a technique for separation and detection of charged particles in capillary column under high voltage electric field. In 2011, Lamego first used several substrates and inhibitors to identify and measure CES2 activity in complex biological samples by capillary electrophoresis [38]. This method had been successfully applied to the assessment of overexpression of CES2 in human cells and the quantification of CES2 activity in some mammalian serum samples. Compared to classical spectrophotometry, CE has advantage of fast, repeatable, and requiring extremely low amounts of protein (from 3 to 6 μg). Furthermore, this method proved to be applicable for biological samples even when substrates and metabolites absorb at the same wavelength [39].

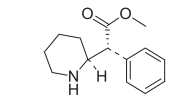
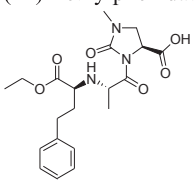
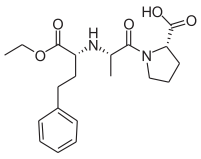
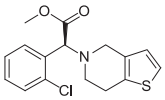
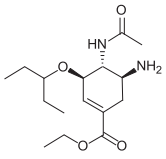
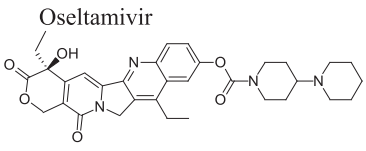
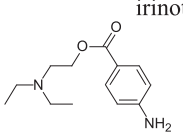
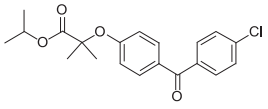
3.3. Chromatography

As the classical separation and detection techniques, high performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been widely used in the detection of CESs activity, due to their advantages of highly sensitive, accurate, short analysis time and good reproducibility. These LC-based quantitative techniques need specific substrates of CESs for complicated biological samples. The most commonly used CESs substrates and their enzymatic kinetics combined with the corresponding methods are summarized in Table 1. When in view of sensitivity, selectivity, avail, reaction efficiency and enzymatic kinetics, clopidogrel [20], oseltamivir [21] and procaine [40] *etc.* have been recognized as practical substrates for CESs. Notably, newly fluorescent substrates which are based on the liquid chromatography-fluorescence detector (LC-FD) methods for quantifying CESs activity also getting attention in recent years. For example, newly developed specific substrates of recombinant human carboxylesterase 1 (hCE1), containing small ethanol groups and large acyl groups, such as NMHN (N-(4-methyl butyrate)-4-hydroxy-1,8-naphthalimide) [41] and BMBT (2-(2-benzoyloxy-3-methoxyphenyl)benzothiazole) [42], are easily hydrolyzed by hCE1 and following ideal Michaelis–Menten kinetics. With LC separation, most polar endogenous compounds can be eluted during the column dead time, which is completely helpful to reduce the fluorescence background from endogenous compounds in complex biological samples. Compared with liquid chromatography-ultraviolet detectors (LC-UV) methods, LC-FD methods exhibit several advantages include good precision, highly sensitivity and good linearity. LC-FD methods have been successfully applied to measure the accurate activity of hCE1 in biological samples such as tissue preparations, cell preparations and human plasma [41,42]. However, those methods mentioned above also have some shortcomings including requiring expensive equipment and tedious pretreatment process of biological sample.

3.4. Fluorescent probe-based detection technology

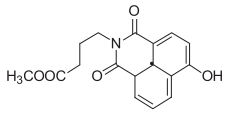
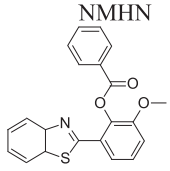
With the rapid development of optical instruments (such as microplate reader and fluorescence microscope) in the past ten years, a number of highly specific fluorescent probes for sensing the real activities of CESs in complex biological system have been designed. The fluorescent probe-based detection technology has been receiving more and more attention owing to its simplicity, high selectivity, ultra-high sensitivity, as well as applicable for high-throughput screening in the field of CESs activity quantification. The researchers have applied the

Table 1
Special substrates of CESs for LC detection.

Substrate	Enzyme source	Kinetic parameters		Method	Ref.
		K_m (μM)	V_{max} (nmol/min/mg)		
 (d−)Methylphenidate	hCE1[a]	89.9 ± 6.6	3.2 ± 0.1	LC-MS/MS	[19]
 Imidapril	HLM[c]	245	2.4	LC-MS/MS	[65]
 Enalapril	hCE1	1721	34	LC-MS/MS	[66]
 Clopidogrel	hCE1	62.7	3.56	LC-MS/MS	[20]
 Oseltamivir	hCE1 HLM	177 187	102 114	LC-MS/MS	[21]
 irinotecan	hCE2[b]	3.4	2.5	LC-FD	[26]
 Procaine	HLM	800 ± 200	3.5 ± 0.5	LC–UV	[40]
 Fenofibrate	HLM	4.1 ± 0.2	484.9 ± 3.7	LC–UV	[40]

(*continued on next page*)

Table 1 (*continued*)

Substrate	Enzyme source	Kinetic parameters		Method	Ref.
		K_m (μM)	V_{max} (nmol/min/mg)		
	hCE1	83.01	484.9	LC-FD	[41]
	HLM	53.1	684.3		
NMHN					
	hCE1	12.18	93,070	LC-FD	[42]
BMBT					

[a] hCE1 means Human recombinant carboxylesterase 1.
[b] hCE2means Human recombinant carboxylesterase 2.
[c] HLM means Human liver microsomes; (d-) dextrorotatory form.

fluorophore recognition mechanism to identify and quantify CESs, and designed a large number of fluorescent probes with good selectivity, high sensitivity and high spatial resolution for imaging intracellular processes of living cellular systems. Those probes are able to quantify CESs activity through changeable fluorescence intensity. Fluorescent probes are currently used for CESs activity detection are reactive small molecule fluorescent probes, which can be classified into "off-on" and radiometric fluorescent probes according to their fluorescence signal output modes. The fluorescent probes also can be divided into single photon and two-photon fluorescent probes on the basis of the number of photons absorbed. Table 2 lists the structures, enzyme sources, and enzymatic kinetics of various fluorescent probes currently used for CESs activity detection.

3.4.1. *"Off-on" fluorescent probe*

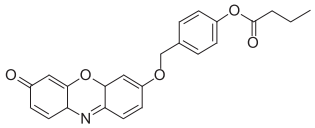
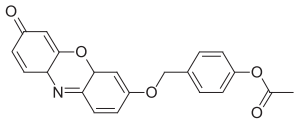
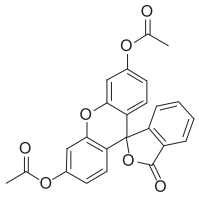
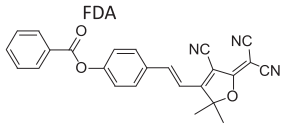
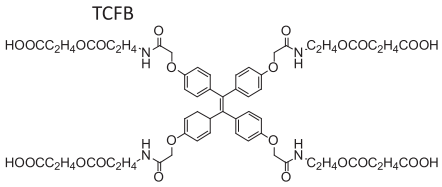
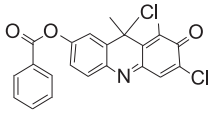
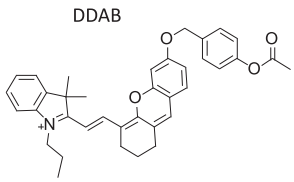
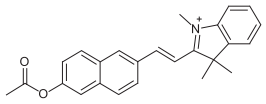
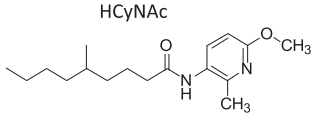
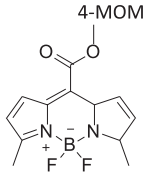
"Off-on" fluorescent probes commonly perform no fluorescence or weak fluorescence. Once these probes hydrolyzed by CESs, they immediately show strongly fluorescent. Zhang et al. constructed a resorufin-based spectroscopic off-on probe, 7-(p-acetoxyphenylmethyloxy)-3H-phenoxazin-3-one. The probe is specifically recognized for CESs and the ester bond is rapidly hydrolyzed by CESs, resulting in the releasing of resorufin, thereby the fluorescence of the reaction system could be turned on. The quenching effect of the probe provides low background signal, making the method very sensitive to detect CESs activity. In addition, the probe has good membrane permeability and has been demonstrated to monitor CESs activity in HeLa cells [43].

Based on the principle of intramolecular charge transfer (ICT), a long-wavelength fluorescent probe TCFB is designed for sensing human recombinant carboxylesterase 2 (hCE2) activity. The hCE2-mediated TCFB hydrolysis exhibited changeable color in the solution from yellow to violet, indicating that TCFB can serve as a "naked-eye" colorimetric indicator for hCE2. As a selected, sensitive probe, TCFB has ideal kinetics behavior and could eliminate matrix interference in human biological samples. TCFB has been successfully applied for real-time monitoring of hCE2 activity in complex biological samples, and bioimaging of endogenous hCE2 in living cells [44]. Unlike classical fluorophores that are easily suffer from fluorescence quenching effect at high concentration or in the solid state due to aggregation [45], the probe based on tetraphenylethylene derivative has strongly fluorescent in the aggregated form. Such unusual property attributed to it is designed follow the principle of aggregation induced emission (AIE). After hydrolyzed by CESs, the probe self-assembled into supramolecular microfibers, thus enhanced the fluorescence signals at 475 nm. The probe has good water solubility under physiological conditions and a high sensitivity with LOD as low as 29 pM, it can be used not only for the real-time monitoring of CESs activity but also for the screening of potential CESs inhibitors [46].

Reactive small molecule fluorescent probes have plenty of superiorities over other detection techniques such as spectroscopy and chromatography in detecting CESs activity, but these probes have a severe limitation on application for deep tissue imaging in living systems, since they have a short excitation wavelength (usually < 500 nm), which may result in severe fluorescence intensity reduction, shallow tissue penetration and biggish photodamage when applied for biological samples [44,47–49]. Accordingly, researchers have make much effort to discover the near-infrared (NIR) fluorescent probes with excitation and emission wavelength between 600 and 900 nm region [50–52]. Such excellent properties make NIR probes holding great potential for the detection of endogenous CESs activity in living cells, tissues and animals. The reported NIR probes DDAB (6,8-dichloro-9,9-dimethyl-7-oxo-7,9-dihydroacridin-2-yl benzoate) [50] and (E)-2-(2-(6-((4-acetoxybenzyl)oxy)-2,3-dihydro-1H-xanthen-4-yl)vinyl)-3,3-dimethyl-1-propyl-3H-indolium [51] are capable of quantifying CESs activity in living mice and zebrafish, as well as visualizing endogenous CESs in deep tissue. In addition, the two-photon excitation probe has a longer excitation wavelength than single photons, which can provide low interference from biological matrix and autofluorescence, so the background is low and the signal:noise ratio is high [52–55]. A fast responsive and two-photon fluorescent probe HCyNAc which based on naphthalene and hemicyanine derivatives is developed in 2018 by Wang et al. [52]. In view of its simplicity, high specificity, good light stability and high fluorescence quantum yield, HCyNAc display unique advantages in two-photon imaging of endogenous CESs on living cells.

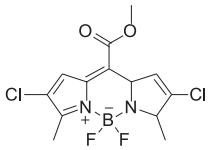
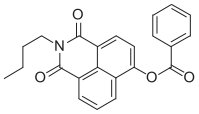
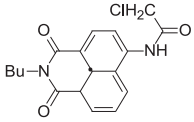
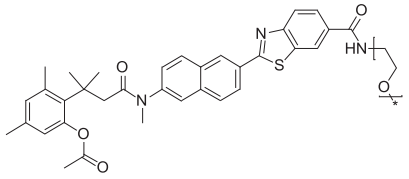
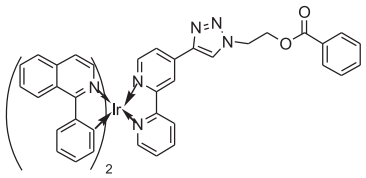
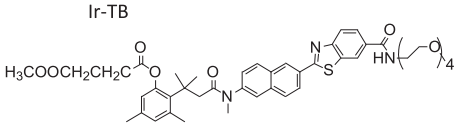
Over the past decade, many researchers have successfully developed specific fluorescent probe substrates for CES2. On the contrary, practical fluorescent probes for the detection of CES1 activity in complex biological samples are seldom reported, as most of fluorophores are polycyclic phenol compounds, whose corresponding ester derivatives prefer to be metabolized by CES2 [56,57]. Based on its excellent spectroscopic properties and catalytic features of CES1, *meso*-carboxyl-BODIPY was used as the basic fluorophore and designed as two novel fluorescence probes. The probes are successfully used for specifically monitoring CES1 activity in various biological systems, as well as to imaging endogenous CES1 in a range of biological systems, including living cells, fresh tissue slices, whole organs and zebrafish. Furthermore, this probe has also been successfully used for high-throughput

Table 2
Fluorescent probe used to detect the activity of CESs.

Type	Structure	Enzyme source	Kinetic parameters		LOD	$\lambda_{ex}/\lambda_{em}$ (nm)	Ref.
			Km (μM)	Vmax (nmol/min/mg)			
"Off-on"		CESs	ND	ND	ND	571/585	[67]
"Off-on"		CESs	ND	ND	8.6×10^{-5} U/mL	550/585	[43]
"Off-on"		CES2 HLM HIM	4.82 4.87 4.04	14,600 18,500 39,050	ND	483/525	[47]
"Off-on"	FDA 	hCE2 HLM	3.06 2.19	5.84 14.7	0.46 mg/mL	560/612	[44]
"Off-on"	TCFB 	CESs	55.3	150	29 pM	375/475	[46]
"Off-on"		CESs HLM	1.83 1.92	20.4 16.5	ND	600/662	[50]
"Off-on"	DDAB 	CESs	ND	ND	4.5×10^{-3} U/mL	670/705	[51]
"Off-on"		CESs	4.4	ND	1.8×10^{-3} U/mL	820/521	[52]
"Off-on"	HCyNAc 	hCE1 hCE2	28 8.9	480 4	3.9 ng	304/394	[68]
"Off-on"	4-MOMMP 	hCE1 HLM	2.9 2.3	ND	ND	505/560	[58]

(*continued on next page*)

Table 2 (*continued*)

Type	Structure	Enzyme source	Kinetic parameters		LOD	$\lambda_{ex}/\lambda_{em}$ (nm)	Ref.
			Km (μM)	Vmax (nmol/min/mg)			
"Off-on"		hCE1 HLM	2.1 3.2	ND	ND	530/595	[56]
Ratiometric	MMB 	hCE2	ND	ND	0.5×10^{-3} mg/mL	346/528	[49]
Ratiometric	EBHF 	hCE2	ND	ND	1×10^{-3} mg/mL	452/564	[48]
Ratiometric	 NCEN	hCE2 HLM HIM[b]	8.58 6.13 8.12	34.87 4.52 5.08	1.2×10^{-2} mg/mL	430/542	[53]
Ratiometric	 SE1	PLE[a]	4.33	280.2	0.5 nM	373/455–540	[54]
Ratiometric	 Ir-TB	hCE2	2.21	43.9	ND	445/639	[59]
Ratiometric		hCE1 hCE2	15.27 8.75	28.5 32.8	ND	373/455	[55]

[a] PLE means Pig liver esterase.
[b] HIM means Human intestine microsomes; ND means not determined.

screening of CES1 inhibitors using living cells as enzyme sources [56,58].

3.4.2. Ratiometric fluorescent probe

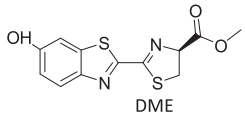
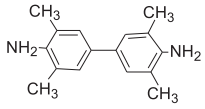
Ratiometric fluorescent probes are most widely used in CESs activity detection. Compared with "off-on" fluorescent probes, ratiometric fluorescent probes are not susceptible to probe concentration, instrument efficiency and environment factors (pH, polarity, temperature, *etc.*), so their sensitivity and selectivity are greatly improved [59]. According to their principle, they can be divided into intramolecular charge transfer (ICT) [54], excited-state intramolecular proton transfer (ESIPT) [49], fluorescence resonance energy transfer (FRET) [60] and so on.

Based on the ESIPT mechanism, a ratiometric fluorescent probe derived from 3-hydroxyflavone has been developed for selective detection of CES2 activity. Particularly, CES2 mediate the probe hydrolysis with the remarkable changes in fluorescence spectrum and desirable Stokes shift, resulting of low interference from biological matrix, deep penetration and minimize photodamage to biological samples. The probe is able to screen and evaluate the effects of CES2 modulators by using tissue or cell preparations as the enzymes sources [49]. Another probe BMBT for detecting hCE1 is also designed based on ESPI mechanism. Upon reaction with hCE1, the probe displays a remarkable red-shifted emission in fluorescence spectrum (120 nm large emission shift), which provides the basis for the sensitive detection of hCE1 activity. The probe could be used to detect and imaging the activity of hCE1 in living cells [57].

The first two-photon fluorescence probe for detection of hCE2 activity is reported in 2015 by Jin et al. [53]. The probe termed NCEN shows excellent sensing properties including highly selective, low LOD (12 ng/mL). Apart from that, the probe owns great advantages for bioimaging endogenous hCE2 in living cells, relying on its properties of high ratio imaging resolution and deep-tissue imaging depth.

In order to improve the photostability of CESs probes, researchers

Table 3
Substrates of sensor for detecting the activity of CESs.

Structure	Enzyme source	Kinetic parameters		LOD	Detection conditions (nm)	Ref.
		Km (μM)	Vmax (nmol/min/mg)			
 DME	hCE1 HLM	4.51 3.60	1,208,000 1,441,000	1×10^{-5} mg/mL	λ_{em} = 560	[63]
 TMB	CESs	ND	ND	0.013 U/L	λ_{ab} = 370/650	[64]

established a FRET-based ratiometric fluorescent system by using tetraphenylethene derivative nanoaggregate (TPE-N + dots) and fluorescein diacetate (FDA). The system can avoid the limitations of fluorescence quenching caused by fluorophores aggregation. Therefore, this probe gets better photostability and will be benefit for application during a longer period (up to 40 min). Furthermore, the detection process is relatively facile and could serve as a one-step straightforward assay for the detection of endogenous CESs activity in human serum [60].

In general, the fluorescence probes are considered as efficient tools for detection and real-time monitoring CESs activity in living cells and tissues, due to their excellent properties like high selectivity, ultrasensitivity and easy management. In case of these probes is commercially available, they maybe become the most useful tool for CESs activity detection in the future.

3.5. Bioluminescent sensor

Bioluminescent sensors are special device producing visible light signal by a chemical reaction between bioluminescent enzyme (luciferase) and its specific substrates (luciferin or amino luciferin) [61,62]. Wang et al. designed a practical bioluminescent sensor (D-luciferin methyl ester, DME) upon the evaluation of human recombinant carboxylesterase 1 (hCE1) activity for the first time. DME (Table 3) could be quickly hydrolysed by hCE1 and release D-luciferin, which initiated the firefly luciferase–luciferin system to generate bioluminescence signals. Compared with the fluorescent probe, bioluminescent sensors don't need excitation light to produce emission light, hence the detection method can vastly restrain the interference by absorption of biological matrix, as well as avoid phototoxicity of the excitation light to live cells. Furthermore, the bioluminescent sensor could serve as a reliable, selectivity and sensitive tool to explore hCE1 related biological processes in living cells co-expressing luciferase, and could be applied for the high-throughput screening of hCE1 regulators [63].

3.6. Colorimetric sensor

Traditional colorimetric method generally has low sensitivity and poor anti-interference ability. In order to overcome the shortcomings above, a colorimetric signal amplification sensing platform through enzymatic cascade reaction as a signal amplification technique for highly sensitive and selective CESs activity detection was conducted [64]. FDA could be readily hydrolyzed by CESs and convert to active fluorescein, then fluorescein possessed excellent photocatalytic capability to transform colorless 3,3′,5,5′-tetramethylbenzidine (TMB) (Table 3) into blue oxidized TMB (oxTMB) under the induction of visible light. The analysis method greatly improves the detection sensitivity by chromogenic reaction as a signal amplifier, with LOD of 0.013 U/L. That colorimetric biosensor is facile, economical, green, as well as attain high catalytic activity through 10 min visible light irradiation [64].

4. Summary

CESs are widely distributed in the human body, and play a key role in physiological processes such as fatty acid metabolism and cholesterol hydrolysis. Moreover, CESs are responsible for metabolism and detoxication of ester- or amide-containing compounds, while dysfunction of CESs would lead to physiological and pathological changes. Therefore, it is of great significance to establish a simple, efficient, sensitive and selective CESs activity detection method for drug discovery and biomedical research. In the past decade, researchers made significant breakthrough on the development of several methods for detecting the real activity of CESs in biological samples. In general, detection techniques such as spectrophotometry, capillary electrophoresis, and chromatography perform higher selectivity and sensitivity in CESs activity quantification, but with several disadvantages such as tedious operation, strict pretreatment of samples, as well as susceptible to interference of biological matrix.

Fluorescent probe-based detection technology has developed rapidly in recent years, and it possess of excellent properties, such as speediness, high sensitivity, low background, as well as the capability of bio-imaging of CESs in living cells, tissues or organs. However, the small-molecule fluorescent probes still have certain limitations in practical applications: firstly, the sample may be contaminated when probes are dissolved in the sample solution, thus the sample cannot be reused; secondly, the toxicity, complex synthesis and low yield of probes would limit their wide application in life sciences and biomedicine. Compared with large amount of attention to fluorescent probes, only a handful of studies have focused on the bioluminescent sensors in the field of CESs activity detection. Due to bioluminescence is a common and naturally occurring phenomenon and the substrate being derived from biological sources, bioluminescent sensor has particular advantage in low toxicity, non-destructive, non-invasive detection. The method has great application potential in the monitoring of special CESs activity in complex biological system.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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