METHODS

Rapid Method for Diagnosis of Abnormal Activity of Butyrylcholinesterase

D. N. Vlaskin, E. T. Gainullina, S. B. Ryzhikov, and V. F. Taranchenko

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We present the results of spectral studies of the interactions between butyrylcholinesterase and ethidium bromide fluorophore inhibitor. The ethidium bromide fluorescence selectively increased in the presence of butyrylcholinesterase. A rapid method for evaluation of serum butyrylcholinesterase activity is developed.

Key Words: *fluorescence; butyrylcholinesterase; reversible inhibitor*

Ellman's kinetic method [4] is most widely used for measurements of butyrylcholinesterase (BCE) activity. This method is based on evaluation of the rate of enzymatic hydrolysis of acetylcholine substrate or butyrylcholine with 5,5'-dithiobis-(2-nitrobenzoic) acid as the thiol group indicator (interaction of this acid with thiocholine results in the formation of 5-mercapto-2-nitrobenzoic acid with maximum absorption at 410 nm) [4]. The use of Ellman's method does not show the linear relationship between optical density of the reaction mixture and BCE activity, because of high BCE activity in the blood (the rate of reverse reaction of 5-mercapto-2-nitrobenzoic acid with thiocholine increases with increasing BCE activity in the analyzed sample) [4].

Another known method for the diagnosis of abnormal BCE activity in the blood is based on quenching of acridine and quinoline derivatives (fluorophore inhibitors) fluorescence in the presence of BCE [2,3]. Patient's blood can contain substances suppressing the fluorophore inhibitor (for example, iodine), and hence, it is preferable to evaluate BCE activity by an increase in the intensity of fluorophore inhibitor fluorescence in the presence of this enzyme.

Ethidium bromide (ED), a potent inhibitor of BCE (the dissociation constant of the enzyme-inhibitor complex during competitive inhibition of this enzyme is 50 μM) was selected as the optimum fluorophore; it is water-soluble, characterized by high fluorescence intensity beyond the BCE spectra, high photo- and thermostability, stable fluorescence at pH 7.4-8.5 (optimum pH range for BCE).

The aim of our study was to develop a rapid method for the diagnosis of abnormal activity of serum BCE on the basis of evaluation of BCE effect on EB fluorescence spectra.

MATERIALS AND METHODS

The following reagents were used in the study: 2,7-diamino-10-ethyl-9-phenyl-phenantridiniumbromide (EB), indole, BSA, ovalbumin, equine serum cholinesterase (EC 3.1.1.8), 5,5'-dithiobis-(2-nitrobenzoic) acid, and butyrylthiocholine iodide (all reagents were from Sigma).

The fluorescence and absorption spectra were recorded on a Hitachi MPF-4 spectrofluorometer and Specord spectrophotometer.

Physical Faculty, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** sbr@genphys.phys.msu.ru. S. B. Ryzhikov

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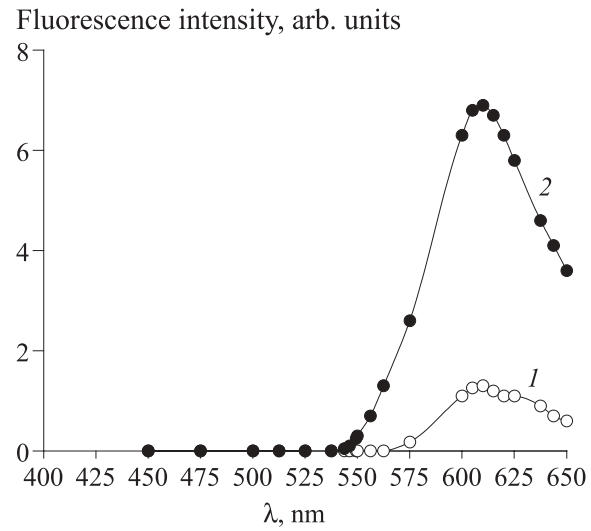

Fig. 1. Ethidium bromide fluorescence spectra (*1*, *2*) in the absence and presence of butyrylcholinesterase (BCE) in phosphate buffer (pH 8.0). *1*) ethidium bromide (6.0 μM); *2*) ethidium bromide (6.0 μM) in the presence of BCE (30 U/ml).

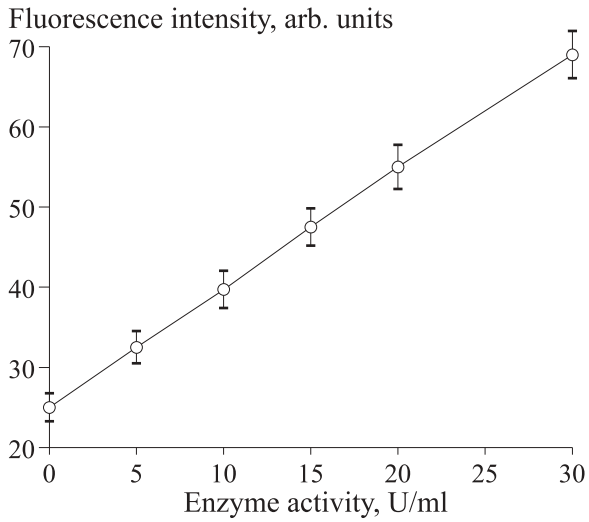

Fig. 2. Calibration curve of relationship between fluorescence intensity of ethidium bromide (6.0 μM) and BCE activity in phosphate buffer pH 8.0.

BCE solution in 0.08 M phosphate buffer (pH 8.0, 50 U/ml) and EB solution (80 μM) were used.

BCE solution (1 ml) of the needed concentration in phosphate buffer was added to 3 ml water solution of EB, the mixture was poured into a quartz cuvette (10×10×40 mm), and the fluorescence or absorption spectra were recorded. All measurements were carried out in 0.02 M phosphate buffer (pH 8.0) at 20°C.

RESULTS

EB absorption can be recorded starting from 475 nm. The fluorescence spectra and intensity were recorded at excitation wavelength λ_{ex}=475 in all experiments.

EB had a fluorescence band at λ_{max}=610 nm in the visible spectrum band (Fig. 1, *1*). Concentration quenching was not observed at EB concentrations ≤100 μM. EB in concentration of 6 μM was used in further studies.

BCE appreciably modified EB fluorescence. Addition of BCE into EB solution increased fluorescence intensity without shift in its maximum at λ=610 nm (Fig. 1, 2), the relationship between the increase of the fluorescence intensity and increase in enzyme activity was linear (Fig. 2). The choice of the interval for measuring the effect of BCE on EB fluorescence was explained by serum cholinesterase activity in human blood (3-14 U/ml normally and higher in disease). In our experiments EB fluorescence intensity in the presence of BCE changed within less than 5 sec and this intensity remained stable for more than 30 min.

The selective effect of BCE on EB fluorescence was confirmed by the results of evaluation of its fluorescence spectra in the presence of BSA and ovalbumin. Addition of these proteins, not fluorescing at λ=400-700 nm, did not modify the EB fluorescence spectrum.

TABLE 1. BCE Activity (U/ml) Measured by Ellman's Method and Fluorescent Method (*M*±*m*)

BCE activity in reference solution*	BCE activity (Ellman's method)	BCE activity (fluorescent method)
4	3.90±0.30	4.10±0.25
8	8.20±0.50	7.90±0.40
10	9.80±0.60	10.20±0.40
12	11.30±0.90	12.30±0.55

Note. *evaluated by potentiometric titration of butyric acid released as a result of enzymatic hydrolysis of butyrylcholine.

The increase in EB fluorescence intensity at λ=610 nm in the presence of BCE can be explained by the increase of its relative quantum yield as a result of increased fluorescence quenching time during reversible reaction between EB with BCE, as was shown for energy transfer to EB molecule from nucleotides of phage DNA [1].

The results of this study can be used as a rapid method for evaluation of BCE activity.

The error in evaluation of human serum BCE activity by the proposed fluorescent method (Table 1) did not exceed the error of measurement by Ellman's method, but the fluorescent method is more rapid and simple.

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REFERENCES

1. N. L. Vekshin, *Zh. Priklad. Spektroskopii*, **65**, 261-268 (1998).
2. E. T. Gainullina, S. A. Eremin, and S. B. Ryzhikov, *Byull. Rosagentstva po Patentam i Tovarnym Znakam*, No. 11, 24-35 (2001).
3. E. T. Gainullina, S. A. Eremin, and S. B. Ryzhikov, *Ibid.*, No. 31, 12-29.
4. W. R. Christenson, D. L. Van Goethem, and R. S. Schroeder, *Toxicol. Lett.*, **71**, 139-150 (1994).