Секция «Тезисы на английском языке»

Enzyme activity determination using clinical diagnostic kits: computing procedure optimization case study assessment of amylase activity in whole mixed saliva

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Unification of enzymatic diagnostics using automated analyzers relies on procedures implying standard time periods for tracing changes in substrate or product levels to calculate average value of the reaction rate for fixed time intervals. However, this approach may result in uncontrolled underestimation biases.

The objective of the present work was to optimize the procedure of kinetic determination of amylase activity in whole mixed human saliva using diagnostic kits where 2-chloro-4-nitrophenol conjugated with maltotriose is used as a substrate [1, 4]. Continuous changes in 405-nm optical density at 37°C at constant stirring of incubation medium were traced with PerkinElmer Lambda 35 spectrophotometer. The primary data were differentiated numerically to estimate reaction rate [2]. Unlike the standard procedure, not the difference between the initial and final optical density values in the first three minutes of tracing was used as the measure of the average amylase activity, but the only maximum value of the reaction rate which was found in 10 to 40 sec interval (the initial reaction rate) (Fig.). Data treatment was carried out using our scripts in R programming language [3].

Our results show that the estimates of amylase activity obtained by data averaging can be several times lower than the real initial reaction rate, which is more appropriate as a measure of enzyme activity. Approaches similar to ours can be used to assess the activities of other diagnostically significant enzymes and the levels of metabolites measured with enzymatic kits. Biochemical diagnostic kits and associated protocols are usually developed for application in blood or urine testing and may be not appropriate for testing other human biological fluids, such as saliva or tear fluid, biopsy material and biological samples of other origin (pets, wild and laboratory animals, plants, microorganisms, etc). Adapting clinical kits to other objects requires laborious preliminary studies, which may be reduced by using the approach that we suggest.

The study was carried out using equipment available at Resource Center "Observatory of Ecological Safety" of the Research Park of St. Petersburg State University.

Источники и литература

1) Foo Y. A., Bais R. Amylase measurement with 2-chloro-4-nitrophenyl maltrotrioside as substrate // Clin. Chim. Acta. 1998. V. 272. P. 137-147.

2) Lyangusov A. Yu., Petrova T. A., Stefanov V. E. A new approach to computation of parameters in enzymatic kinetics // Doklady Biochemistry and Biophysics. 2009. V. 424. No 1. P. 49-51.

3) R Project: https://www.r-project.org/

4) SpinReact: http://www.spinreact.com/files/Inserts/Bioquimica/BEIS27_AMILASA_LQ_02-2016.pdf

Иллюстрации

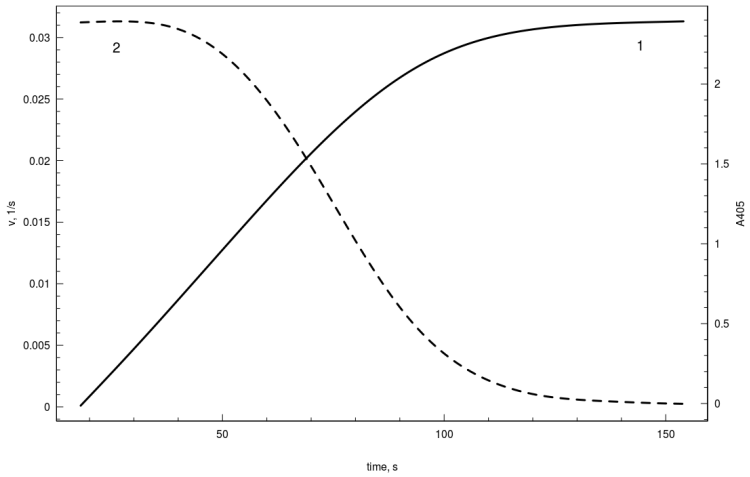

Рис. 1. Kinetic (1) and enzyme rate (2) curves of amylase reaction