A Software Package to Streamline the Titrimetric Determination of Lipase Activity

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ABSTRACT: A computer software method, the Lipase Titrimetric Analysis Program, has been developed which considerably improves the utility of the Radiometer TitraLab/pH Stat system for the determination of lipolytic activity. The program features increased user interaction in the calculation of maximal lipolytic activity, allows additional titrations to be performed while previously collected data are being analyzed, and permits the permanent storage of titration data.
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Lipases (triacylglycerol acylhydrolase, E.C. 3.1.1.3) catalyze the hydrolysis of triacylglycerols to yield glycerol and free fatty acids. Typically, these enzymes are assayed either by monitoring the decrease in the amount of triacylglycerol present or the increase in the liberated acyl moiety (1). The amount of free fatty acid released is commonly measured by titrating the reaction mixture with an alkaline solution, usually sodium hydroxide, of known concentration. Titrimetric methods for the determination of lipolytic activity are either endpoint in nature, with liberated free fatty acid levels being determined after some predetermined length of incubation, or continuous (pH stat), with fatty acid release constantly being titrated during incubation. The latter method is preferable to the former because the pH of the reaction mixture remains constant during incubation, thus avoiding acidification and concomitant enzyme inactivation. We (2) and others (3,4) have used a Radiometer TitraLab/pH Stat system (Copenhagen, Denmark and Westlake, OH) to make these measurements.

However, we have experienced some limitations with this system and have devised ways to overcome them. Three major limitations are: (i) a lack of operator control over some aspects of the calculation of the maximum slope of a typical titration curve; (ii) a new titration cannot start until the previous data have been analyzed; and (iii) once a new titration has started, the previously collected data cannot be reaccessed or reevaluated. This paper presents a simple method of interfacing the TitraLab system to a computer, collecting and storing the data in an ASCII file, and using LTAP (Lipase Titrimetric Analysis Program), a Lotus 123 macro file that we have developed, to analyze the data.

EXPERIMENTAL PROCEDURES

Lipase assay. Lipase and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Lipolytic activity was quantitated by a continuous titrimetric assay with emulsified olive oil as substrate. The reaction was started by adding 0.20 mL of *Candida cylindracea* lipase, L 1754 (1 mg/mL in deionized water), and 0.40 mL of deionized water to 5.0 mL of an emulsion that contained 18% (wt/vol) olive oil, 42% gum arabic, and 15 mM $CaCl_2$, all adjusted to pH 7.5 with 0.1 N NaOH. The incubation was carried out at 25°C with 0.1 N NaOH added by the TitraLab system as required to maintain the pH at our chosen setpoint of 7.5. Enzyme activity was calculated from the maximal rate of base addition, assuming molar equivalence between fatty acid release and alkali consumption.

Titrator. The titrator was a Radiometer TitraLab system consisting of a VIT90 Video Titrator with a VIA 104 pH Stat Cartridge, an ABU91 Autoburette (5 mL), and a SAM90 Sample Station.

Computer/software. The software was designed to run on any IBM PC-compatible computer with a free serial port, and capable of running Lotus 123 (Cambridge, MA) or Microsoft Excel (Redmond, WA). A communication program, such as PROCOMM (Datastorm Technologies, Inc., Columbia, MO), that can download an ASCII file, is also required. We have tested LTAP with Lotus 123 v2.0, v2.4, and v3.0, and Microsoft Excel v4.0, and have not experienced any problems.

VIT90/computer interface. The VIT90 and computer are connected *via* a straight-through serial cable by using pins 1, 2, 3, 4, 5, 6, 7, and 20 because the VIT90 is DCE and the computer is generally DTE. The VIT90 must be configured in the following manner: The "Extended Data Exchange (external computer)" and the "External ENTER Key" options on the Peripherals screen must be set to YES. All other options on the Peripherals screen should be set to NO. The Extended

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Data Exchange section must be set as follows: Hardware handshake, YES; CTS (output), YES; RTS (input), YES; DTR (input), NO; software handshake, NO; XON/XOFF, NO; block protocol, NO; baud rate, 9600; and parity, NONE. The "Amount of Data Exchange" screen in the configuration section must be set as: Method parameters, NO; setup parameters, YES; all measuring points, YES; results, NO; and statistics, NO. Finally, the communication program should be set for 9600 baud, no parity, 8 data bits, 1 stop bit.

LTAP. The Lipase Titrimetric Analysis Program is a Lotus 123 macro that imports the ASCII data file (must have a .prn suffix) produced by the VIT90. It automatically determines the number of data points and displays a graph of the volume of titrant consumed as a function of time. The user then selects the time interval of the curve that is to be analyzed, and LTAP calculates the maximum slope by performing a linear regression on the first 10 data points of the selected interval, moving 5 points and repeating the 10-point regression analysis. This procedure is continued until all data points selected have been analyzed. The maximum slope detected is then used to calculate the enzyme activity, which is reported in units of microequivalents of free fatty acid released per min per mL enzyme.

RESULTS AND DISCUSSION

A typical titration curve generated during a lipase assay is shown in Figure 1. The curve can be divided into four sections. Section 1 indicates rapid alkali consumption in response to the addition, at time zero, of the lipase sample whose pH was less than 7.5. Section 2 encompasses a lag phase, where lipase activity is below maximal activity. Section 3 represents the maximal activity of the lipase, and section 4 is a period of less than maximal activity. Ideally, analysis of this titration curve should focus on section 3, where the slope of the curve is highest. However, the pH Stat module, as provided by the manufacturer, calculates the maximum slope by dividing the entire curve into ten equal regions, based on the preselected titration end time, and then employing a linear regression within each region. The maximum slope is then used to calculate the maximal lipase activity based on the concentration of the titrant, the sample volume, a calculation factor, and a blank value.

If section 3 contains relatively few points compared to the entire curve, it is possible that the TitraLab analysis will underestimate the maximum slope. The accuracy of the calculation can be improved by using a "time offset" feature of the VIT90 Video Titrator to eliminate sections 1 and 2, during calculation of the maximum activity, making the ten regions slightly smaller. However, section 4 cannot be eliminated in this manner by the TitraLab/pH Stat system. Furthermore, the exact choice of the offset time can substantially affect the calculated maximum slope. Valuable titration time is lost while reanalyzing the data to find the largest maximum slope value.

LTAP analyzes the ASCII data file produced by the TitraLab/pH Stat system and thus frees the VIT90 to begin

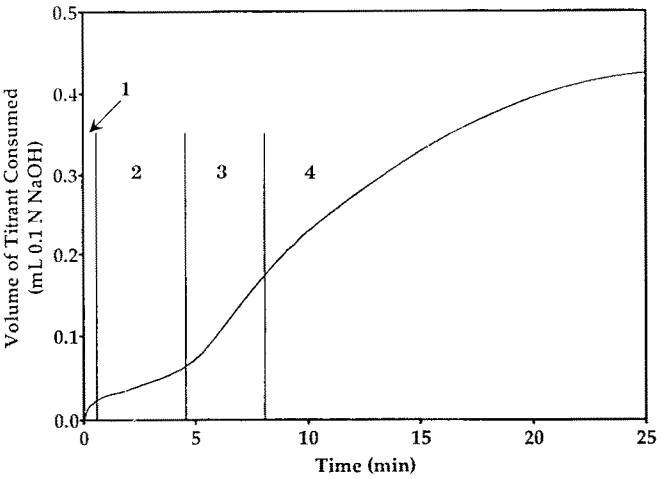

FIG. 1. Titration curve depicting the consumption of 0.1 N NaOH as a function of time during the hydrolysis of an olive oil emulsion by *Candida cylindracea* lipase. Details of the composition of the reaction mixture are described in the text. The vertical lines divide the curve into four distinct, numbered sections (see the Results and Discussion section).

the next titration. LTAP also calculates the maximum slope of the titration curve in any time interval selected by the operator (i.e., sections 1, 2, and 4 in Fig. 1 can be eliminated), and within this interval the curve will be evaluated by using ten data points at a time. Data analyzed by both the TitraLab/pH stat and LTAP methods gave comparable results. LTAP also permits reanalysis of the titration curve by using other time intervals, alteration of the original enzyme volume value, printing of a summary report, and saving of the titration curve graph on a floppy disk.

We have further automated this lipase assay by using a communication package that allows the use of command files. These command files add the .prn suffix to all data files and automate the transfer of control between communication and spreadsheet packages.

Copies of the LTAP macro are available free of charge and will be sent upon request *via* either printed or electronic means. Send electronic requests to cichowic@lasalle.edu and specify the Lotus 123 or Excel version.

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