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A Simple and Modified Photometric Method for Measuring Lipase Activity

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리파제 활성측정을 위한 간편한 비색정량법

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Abstract

A simple and modified method is developed to determine a lipase activity. A linear relationship exists between the color intensity and the free fatty acid liberated by enzyme action. The range of determination is from 0.05 to 1.5 μ*moles* of long chain fatty acid (oleic acid) and 0.2 to 2.0 μ*moles* of short chain fatty acid (caproic acid). The cumbersome procedure of the removal of the upper aqueous phase which was required in the previous copper soap extraction method was eliminated by the movement of solvent phase to upper phase in the respective biphasic system with a mixture solvent (chloroform: *n*-hexane:ethanol = 49:49:2) and copper reagent saturated with sodium chloride.

Introduction

Lipase (triacylglycerol acylhydrolase, E.C. 3.1.1.3) hydrolyzes emulsified triglycerides and diglycerides, and in some cases monoglycerides of the long chain fatty acid. In each step catalyzed by lipase one fatty acid is liberated. Rate of lipase reaction can, therefore, be measured by determining either the rate of disappearance of the substrates or the rate of production of the fatty acids.

Recently, a number of procedure based on manometry,[1] and titrimetry[2] or photometry[3,4] have been developed to determine quantitatively the fatty acid liberated by lipolytic enzymes. The first two procedures are more time-consuming and less sensitive than photometric method. Recently, Hirayama[5] have reported a sensitive procedure for a lipolytic enzyme assay by microdetermination of fatty acid using rhodamine 6G reagent. But the color developed by interaction of reagent with the fatty acid extracted from enzyme mixture was interfered by bile salts which are used for stabilization of emulsion. Rhodamine 6G method was effective only for the fatty acids which have carbon number higher than that of lauric acid, because the recovery of fatty acid was decreased suddenly if the carbon number is lower than 12. Schmidt[4] described a procedure for photometric determination of fatty acids in the form of copper soap extracted into chloroform layer and then detected the copper photometrically by addition of diethyldithiocarbamate. However, the efficiency of copper soap procedure is limited by using a cumbursome separation procedure of the chloroform phase from respective biphasic system. Consequently, recovery of the fatty acids is

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Table 1. Effect of solvent composition and salt concentration of copper reagent on color intensity

Solvent composition	Oleic acid (1 μeq).		Caproic acid (1 μeq).	
	No salt	Sat. salt	No salt	Sat. salt
Chloroform	1.37±0.032*	1.37±0.043	0.68±0.040	0.84±0.052
Chloroform: hexane: ethanol (49 : 49 : 2)	1.21±0.031	1.24±0.061	0.69±0.031	0.76±0.033
Hexane: etchanol (48 : 2)	1.00±0.061	1.10±0.073	0.51±0.048	0.63±0.061

* Mean ±SD of 10 measurement

somewhat incomplete.

In this paper, we describe an improved copper soap extraction method for a rapid lipase assay. The present method involves the steps of sample treatment, incubation, fatty acid-copper complex extraction and photometric determination.

Materials and Methods

It was found that extractability of free fatty acid-copper complex to solvent phase from enzyme mixture was varied by solvent composition and affected by a saturated sodium chloride-copper reagent as shown in Table 1. Although the highest extractability was found in case of using chloroform alone, the mixture solvent was rather desirable. In this case, the separation procedure was very simple because the solvent mixture phase moves to upper layer with stable biphasic system. From the results described above, the standard assay was performed as follows.

Preparation of Reagents

The preparation of copper reagent was modified from that of Schmidt *et al.*[4] 18.6g of triethanolamine HCl was dissolved in 50*ml* of distilled water and 6.45*g* $Cu(NO_3)_2 \cdot 3H_2O$ in 50*ml* distilled water was added and adjusted pH to 8.0 with 5*N* NaOH solution. The mixture was diluted to 100*ml* with saturated sodium chloride solution. The mixture was stored in the dark at room temperature. Chloroform:*n*-hexane:

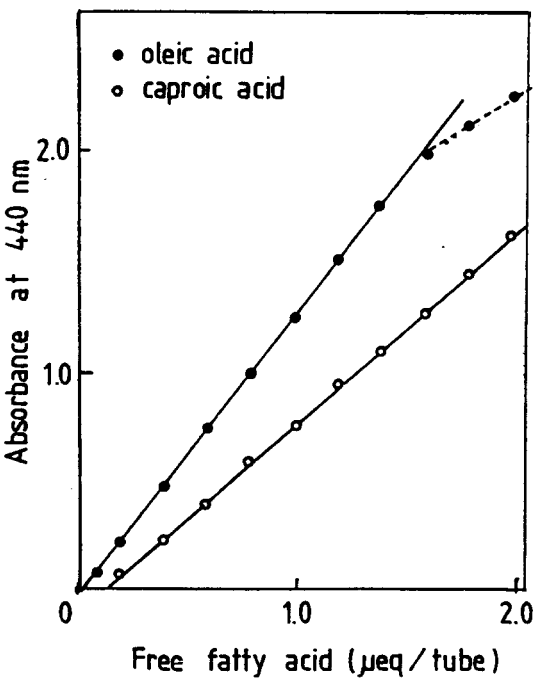

Fig 1. Standard assay curve for oleic and caproic acid

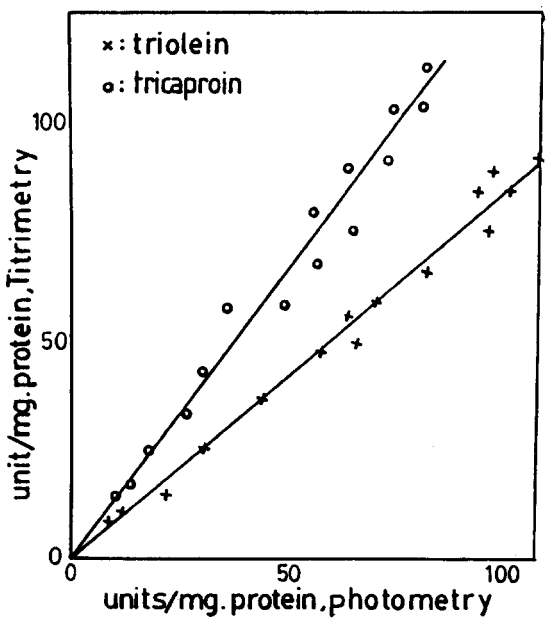

Fig 2. Comparison of lipase activity measured by photometry and titrimetry

ethanol (49:49:2, *v/v*) mixture was made as an extraction solvent and sodium diethyldithiocarbamate-ethanol solution (250*mg*/100*ml*) was prepared for color reagent.

Procedure for Enzyme Assay

Incubation mixture was prepared by mixing of 10*mM* substrate with 0.01% Tween 20, 1*mM* $CaCl_2$, and 5*mM* NaCl in 1*ml* of total volume. After incubation of 1*ml* of enzyme reaction mixture at pH 8.0 and 30°C, 2*ml* of copper reagent and 6*ml* extraction solvent were added, and the mixtures were shaken vigorously for 30*min*, and then centrifuged for 10*min* at 1,200xg, 3*ml* of clear upper layer were mixed with 0.25*ml* of collor reagent in test tubes rendered copper free by acid washing. Absorbance of reaction solution was measured at 440*nm* in a 1*cm* cell.

Results and Discussion

Fig. 1 shows standard assay curve constructed with oleic acid and caproic acid. It indicates that the intensity of color developed was linearly dependent on the amount of liberated fatty acid in the range from 0.05 to 1.5 and from 0.2 to 2.0 μ*moles* for oleic acid and caproic acid, respectively. This method was about 20 times more sensitive than the hydroxamate method for ester determination[6]. The regression equation for oleic acid and caproic acid is $Y = 1.27x - 0.031$ and $Y = 0.85x - 0.086$, where Y = absorbance at 440*nm* and $x = \mu eq$ FFA/assay tube. The results suggest that this method was effective for the estimation of lower fatty acid with improved reproducibility by increasing the ionic strength of aqueous phase. To confirm further this method, activities of 16 crude rice bran lipase preparations were measured by this method and titrimetric method. The results are illustrated in Fig. 2. The coefficiences of regression for using triolein and tricaproin as substrate were 1.33 and 0.84, respectively. And correlation coefficiences were 0.973 and 0.967 for triolein and tricaproin, respectively. The activity of lipase determined by photometric method was lower than titrimetric method in case of using tricaproin as substrate. On the other hand the reverse result was observed in the case of triolein. Apprarently, some part of the free caproic acid are remained in aqueous phase for some unknown reasons so that they are not extracted readily by the solvent. Although the free oleic acid in enzyme mixture existed in oil drop was not titratable, it was readily extracted into extration solvent.

From the above mentioned result, we assured that the present method is a simple and rapid procedure recommended for monitoring the low activity of lipase during the purification procedure and the inactivation study.

요 약

리파제 활성을 측정하는 간편한 방법을 개발하였다. 본 방법에 의하면 효소작용에 의해 생성된 유리지방산과 측정하는 색도와 서로 직선관계를 보였으며, 측정범위는 long chain 지방산인 경우 올레인산으로 환산하여 0.05-1.5 μ*moles* 이었으며 short chain지방산인 경우 카프로인산으로 환산하여 0.2-2.0 μ*moles*의 범위였다. 본 방법의 장점은 종래의 "Copper Soap" 추출방법에서 상부 용매층 제거에 많은 시간을 소비하는 과정을 클로로포름-핵산-에타놀 혼합용매와 NaCl로 포화시킨 copper reagent를 사용하므로서 손쉽게 해결하였다.

References

1. Wills, E.D. In *"The enzyme of lipid metabolism"* P. Desnuelle, ed., Pergamon, Oxford p.13 (1961)
2. Parry, R.M., Chandan, R.C., and Shabani, K.M.: *J. Dairy Sci.*, **49**, 356 (1966)
3. Duncombe, W.G.: *Biochem J.*, 88, 7 (1963)
4. Schmidt, F.H., Stork, H and Dahl, K.V.: in *"Methods of Enzymatic Analysis"*, Vol.2, Bergmeyer, ed. Academic, New York. p.819 (1971)
5. Hirayama, O. and Matsuda, H.: *Agr. Biol. Chem.*, **36**, 1831 (1972)
6. Noda, M and Kobayashi, K.: *Jap. J. Agr. Chem.*, **42**, 574 (1968)

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