〈Original Article〉

The relationship between the measurement error and the linearity of enzyme activity

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Summary The measurement error for the lag phase, which occurred due to the coupling enzyme used, was investigated. That error was determined by an assay of aspartate aminotransferase (AST) activity using malate dehydrogenase (MD) as a coupling enzyme. If the additional activity of the coupling enzyme declined, the measurement error became larger. However, the error was not compared with the additional coupling enzyme activity (of MD). The error became larger the higher the AST activity became. Moreover, when the coupling enzyme showed low activity, the linearity of the reaction became higher, showing that the lag phase would not be determined due to linearity of the reaction.

Key words: AST, Coupling enzyme, Linearity, Lag phase, Measurement error

1. Introduction

The lag phase develops[1-4)] when the enzyme activity is measured using the coupling enzyme. Without the measurement of the lag phase, the measurement of the reaction rate must be done when the response is progressing linearly. However, just because such a linear reaction took place does not mean the correct enzyme activity can be measured. Recently, an automated analyzer can check automatically whether a reaction is linear or non-linear. But even if a warning is given that the enzyme activity is low in a given specimen, the warning does not appear when high activity is measured. However, even when a lag phase should appear with a high activity assay, there is no reason why it should occur when measuring at low activity[3, 5)]. This makes it appear that the reaction linear check is not working because of the measurement error found owing to the occurrence of the lag phase. Thus, an experiment was undertaken to prove this.

There are many reports on the kinetics of coupling

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enzyme reactions. Bergmeyer, for example, reported that the coupling enzyme followed a zero-order reaction[6]. McClure et al. held that the coupling enzyme followed a first-order reaction[1]. Horio et al. succeeded in calculating the approximate value of a coupling enzyme in accordance with the Michaelis Menten formula[2, 3]. Ogawa et al. developed a computer program for the analysis of a zero-order Michaelis Menten reaction by computer[4, 5]. In the recommended method for aspartate transaminase (AST) assay by the Japan Society of Clinical Chemistry (JSCC), the waiting time, the additional malate dehydrogenase (MD) activity and the upper limit of AST activity were decided by this calculation method[7].

The relationship between the measurement error and the linearity of the coupling enzyme was clearly shown by this calculation approach.

2. Materials and Methods

1. Apparatus

For the photometric measurements, a Shimadzu UV 3100 PC with constant temperature curette holder was used. The temperature in the curettes (10 mm light path) was set to 37 ± 0.05℃. An automated analyzer, the Clinalyzer from JEOL, was used for the measurement of enzyme activities.

2. Reagents

All inorganic substances and glycerol were obtained from Katayama Chemical Industries. L-Alanine and 2-oxoglutaric acid were obtained from Peptide Institute. MD was from pig heart. All chemicals were of the highest purity, fulfilling at least the generally accepted requirements set forth by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)[8, 9].

3. Determination of AST activity

AST activities were measured using JSCC-recommended methods[10, 11]. Measurement error was calculated as

error (%) = (measured value - true value) / true value × 100

4. Determination of MD activity under AST assay conditions

MD activity was measured under AST assay conditions, because the activities have to be understood under those conditions. MD activity was

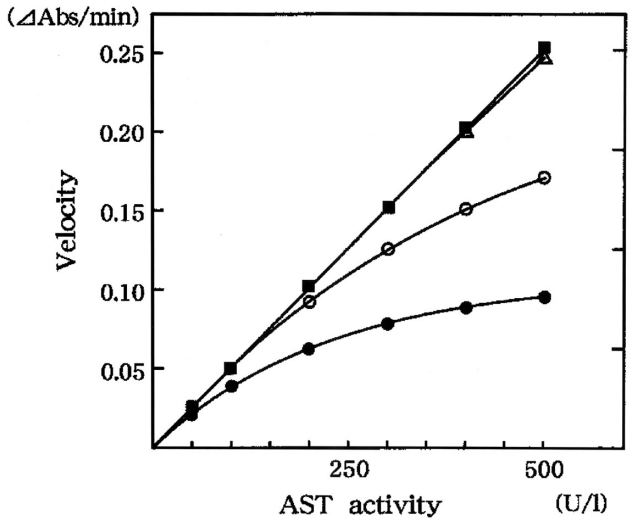

Fig. 1 Reaction rate of specimens in AST activity measurement with various MD activities
Reagents for measuring AST activity were prepared with the coupling enzyme MD at 50 (●-●), 100 (○-○), 250 (△-△), 420 (■-■)U/L. AST activity with 50, 100, 250 and 500 U/L was measured after 2.4 min reaction.

measured as follows: 2.66 ml of reagent-1, which contained 0.16 mmol/l NADH, 200 mmol/l aspartate, 500 U/l LD, 10 mmol/l 2-oxoglutarate and 80 mmol/l Tris-HCI buffer (pH 7.8), was incubated at 30℃. Then 0.24 ml of sample was added to reagent-1 and incubated at 37℃ for 8 min, after which 0.1 ml of 1.8 mmol/l oxaloacetate was added to start the MD reaction. The reaction occurred immediately after mixing. The absorbance at 340 nm was monitored to measure the decrease rate of NADH.

3. Results

1. Relationship among AST activity, additional MD activity and measurement error

AST solutions with the activity of 50, 100, 200, 300, and 500 U/L were determined with various reagents that added the coupling enzyme as MD of 50, 100, 200, and 500 U/L (Fig. 1). The measurement error became great so that AST activity was high, and most of the error did not show on the assay of low AST activity. The measurement error and standard deviation (SD) of various AST activities (24, 266, and 476 U/L) were determined using various reagents by adding various MD activity (26, 83, 420, and 500 U/L) (Table 1).

2. Relation among additional MD activity, waiting time and measurement error

At various times from the start of enzyme reactions (30 s, 1, 1.5, 2, 2.5, 3 min), AST activity was measured with various reagents that added MD (Fig. 2). The measurement error decreased as the measurement timing was delayed.

3. Relation of timing and linearity of enzyme reaction

Various AST samples (24, 266, 476 U/L) were measured by various reagents including various MD activity (26, 83, 420 U/L) at 2.4 min and 1.2 min from the start of the enzyme reaction (Table 2). The ratio of velocity (2.4 min/1.2 min after AST reaction) was small with high AST activity. In each condition, the reproducibility of each AST sample was determined 10 times. Then, the precision expressed SD. The SD never became large when the ratio was high, and there was thought to be no linearity (Table 3).

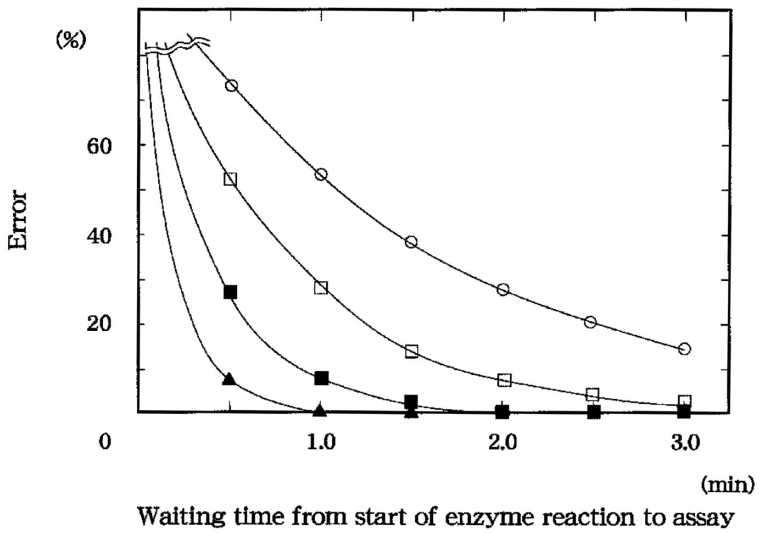

Fig. 2 Relation between timing of AST activity measurement and measurement error.
Reagents for measuring AST activity were prepared with the coupling enzyme MD at 50 (○-○), 100 (□-□), 250 (■-■), and 420 U/L (▲-▲). Measurement error was monitored after 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 min reaction.

Table 1 Measurement error with inadequacy of coupling enzyme, MD, in measurements of AST activity

AST activity	Additional MD activity			
	500 U/l	420 U/l	83 U/l	26 U/l
476 U/l	0%	0%	-25%	-70%
266 U/l	0%	0%	-5%	-52%
24 U/l	0%	0%	-2%	-30%

Reagent for measuring AST activity were prepared with MD of 26, 83, 420, and 500 U/L, and AST samples of 24, 266, and 476 U/L were measured 2.4 min after the start of the reaction.

Table 2 Ratio of velocity measured 2.4 and 1.2 min after start of AST reaction with various MD activities added to reagents for AST assay

AST activity	Additional MD activity			
	500 U/l	420 U/l	83 U/l	26 U/l
476 U/l	1.00	1.00	1.01	1.04
266 U/l	1.00	1.00	1.04	1.23
24 U/l	1.00	1.00	1.02	1.31

Table 3 Standard deviation with various MD activities added to reagents for AST activity

AST activity	Additional MD activity			
	500 U/l	420 U/l	83 U/l	26 U/l
476 U/l	3.21	3.09	3.18	1.62
266 U/l	1.86	1.93	2.32	0.84
24 U/l	1.02	1.07	0.82	0.07

4. Discussion

Unless the coupling enzyme activity is excessive, the product from the enzyme measured in reagents cannot be converted immediately into a measured substance. The measured enzyme reacts under zero-order reaction conditions, but a coupling enzyme reportedly reacts according to a zero-order mechanism[6] or first-order reaction[1]. Horio et al. thought that the coupling enzyme reacts according to the Michaelis Menten formula[2, 3]. They calculated the waiting time and additional coupling enzyme activity by approximating the value by computer. Ogawa succeeded in performing a complete simulation of a continuous reaction using the Michaelis Menten formula. Using the JSCC recommended method, the concentration of the reagent and the assay procedure were obtained from this calculation[10, 11].

The operator must await the completion of the non-linear phase of the enzyme reaction by the lag phase, because the reaction rate must be measured. However, one cannot prove a correct reaction rate with a linear reaction. Although a mechanism was equipped to check for a linear reaction by auto-analyzer, problems with linearity were pointed out only when the reaction rate was delayed; it was not checked in a sample with high activity. Ordinarily, the lag phase should occur when a high-activity specimen is measured. In this case, a clear contradiction developed even though the measured value was in error when a low value should have been obtained. Therefore, we conducted our experiment to clarify the relationship between the lag phase and the measurement error.

Using various reagents of AST assay that included 50, 100, 200, and 500 U/L MD, various AST samples (50, 100, 200, 300, 400 and 500 U/l) were measured. The higher the AST activity, the greater the error occurred (Fig. 1). Even when MD activity dropped to

50 U/l, one-tenth of the original level, a measurement of 50 U/l of AST activity could be made with only limited measurement error. The error of -35% occurred at the measurement of 500 U/L AST with the same reagent. This means that even with quality control for a low-activity sample, reagent abnormality cannot be detected. The higher the AST activity, the greater the measurement error became.

The relationship with the additional MD activity, waiting time and the measurement error was investigated at the determination of 200 U/l AST activity (Fig. 2), and the shorter the waiting time the higher the error in AST measurement. If additional MD activity was added in excess of 250 U/L, and the wait was more than two min, no measurement error occurred. When various AST solutions were measured, low AST activity did not show any error (Table 1). These results were in agreement with the theoretical result. But with the non-linear check system using the auto-analyzer, only the low AST specimen was found. It was a clear contradiction that a high AST specimen was not checked. Then, the reaction rates were detected at 1.2 min and 2.4 min after the ratio of 2.4 min/1.2 min (Table 2). At the assay of 476 U/L AST, using the reagent of only 26 U/L MD, this ratio indicated only 1.04, but this ratio became higher when low AST activity was measured. This ratio was not proportional to the measurement error. These facts clearly demonstrated that the linearity check was not for finding measurement error through the lag phase. Moreover, even when seen in terms of reproducibility (SD), there was not necessarily correspondence with the size of the lag phase. This means that, when there a few coupling enzymes, the reaction curve becomes linear because the reaction results when the coupling enzymes are at full capacity. Thus, the measurement error with this coupling enzyme decrease could be discovered faster and more certainly by assay of the high-activity specimen.

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