〈Original Article〉

Relationship between measurement error and activity of lactate dehydrogenase added to aspartate transaminase enzyme assay reagent

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Summary Lactate dehydrogenase (LD) is generally added to aspartate transaminase (AST) assay reagent in many recommended methods, even with commercial reagents. We conducted a detailed investigation of the reason for adding LD and the measurement error that results from insufficient addition. No need was seen to add LD in order to eliminate negative error from decarbonation of the oxaloacetate produced by the AST reaction. In addition, to avoid positive error by pyruvate and LD present in the sample and NADH in the AST assay reagent, it was necessary to pre-incubate the sample and NADH and let them react for 5 min, and then start the AST activity measurement reaction.

Key words: AST, LD, Measurement error, Decarbonation, Pyruvate

1. Introduction

Lactase dehyrogenase (LD) (EC 1.1.1.27) is added to aspartate transaminase (AST) (EC 2.6.1.1) activity assay reagent for two reasons[1)]. One is that negative error can be avoided by adding LD, even if the oxaloacetate produced by the AST reaction is decarbonated and changed to pyruvate. The second reason is that the positive error from decreased NADH response due to the pyruvate and LD contained in the sample and the NADH in the AST activity assay reagent is eliminated[2)]. In the recommended method of GSCC, 1200 U/L of LD is added[3, 4)], in the recommended method of IFCC, 600 U/L is added[5)], and in the recommended method of JSCC, 500 U/L is added[6)]. Malate dehydrogenase (MD) (EC 1.1.1.37) is added to the AST assay reagent as a coupling enzyme. Generally, the Km value of MD against oxaloacetate is 8×10^{-5} mol/L, whereas the Km value with respect to LD pyruvate is 4×10^{-4} mol/L. The Km value of MD is lower. If decarbonation is done at the same rate as the rate of the reaction of oxaloacetate with MD and becomes pyruvate, some thought should be given as to whether LD, which has a larger Km value than MD,

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should be added and whether addition with the same activity is suitable for the purpose.

Second is that the positive error that occurs from pyruvate and LD present in the sample becomes larger as the pyruvate concentration in samples increases. The size of the positive error also changes depending on the measurement procedure. Positive error disappears if the pyruvate in the sample can be completely eliminated. Accordingly, we would like to investigate, using kinetic evidence and experiments, the kinds of error in order to avoid such error.

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, 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 from pig heart. All chemicals were of the highest purity, fulfilling at least the generally accepted requirements set forth by the IFCC[1, 2].

3. Determination of AST activity

AST activities was measured using JSCC recommended methods[3, 4]. Measurement error was calculated as

error (%) = (measured value − real value)/real value × 100

4. Determination of pyruvate concentration

10 mmol/L oxaloacetate was incubate for 2, 4 and 6 min at 37℃ under AST assay condition without MD and LD. Then each reaction-mixture of 2.5 mL were determinate the pyruvate concentration by the adding 0.5 mL LD.

Table 1 Pyruvate concentration with decarbonation from oxaloacetate under AST assay condition

Incubation time (min)	Pyruvate concentration (mol/L)
2	9.5×10^{-7}
4	1.1×10^{-6}
6	1.5×10^{-6}

3. Results

1. Concentration of decarbonated pyruvate from oxaloacetate preserved at 37℃

Oxaloacetate of 10 mmol/L was incubated for 2, 4, and 6 min at 37℃ under AST activity measurement conditions. Only a very small amount of pyruvate was detected within these reaction times (Table 1).

2. Intermediate oxaloacetate concentration generated in the AST activity measurement process from kinetic evidence

The concentration of oxaloacetate, a reaction intermediate, generated in the reaction between a sample of 500 U/L AST, which is the upper limit of activity measurements with the recommended method of JSCC, and AST assay reagent containing 500 U/L MD was calculated with kinetic program. The concentration was found to reach only about 2.0×10^{-5} mol/L.

3. Error that occurs in AST activity measurements of AST solution containing pyruvate

A sample of 5.0 U/L AST containing 1.0×10^{-3} mol/L pyruvate was reacted with AST assay reagent using the sample start method. The measurement error that occurred with each measurement time and the results of kinetic analysis are shown in Fig. 1.

4. Discussion

To investigate in detail the reason for adding LD to AST assay reagent, the extent to which the oxaloacetate produced in the AST reaction was decarbonated and became pyruvate was examined experimentally. Oxaloacetate of 10 mmol/L was incubated at 37℃ for 2, 4, and 6 min under AST activity measuring

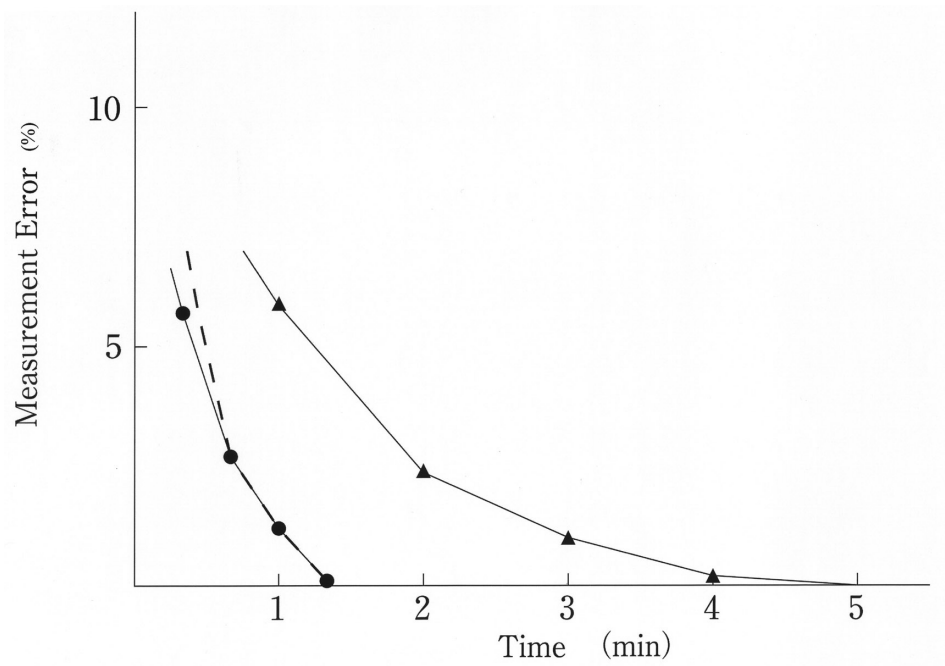

Fig. 1 Relationship between reaction time for elimination of pyruvate with LD and measurement error in AST activity measurement.
Two samples, which contained 5 U/L AST and 1 mmol/L pyruvate in albumin solution (●—●) and a serum (▲—▲), were prepared. AST activities in two samples were determined with AST assay reagent containing 500 U/L LD. The error in AST activity measurement were shown in figure. And the error was calculated by program in those conditions (----).

conditions. Pyruvate was barely detected under these conditions (Table 1). To understand the actual concentration of oxaloacetate produced under the AST activity measurement conditions, we conducted an analysis using the program developed by Ogawa et al.[7-9]. The concentration of reaction intermediate increase with increasing AST activity and decreasing levels of coupling enzyme MD. Then, calculations were done assuming MD activity of 500 U/L in AST assay reagent and AST activity of 500 U/L in the sample, which is the upper measurement limit in the recommended method of JSCC. The results showed that the oxaloacetate concentration reached only 2.0×10^{-5} mol/L during the reaction time. From these two results, oxaloacetate produced under normal AST activity measurement conditions was not thought to be decarbonated and negatively affect measured values. To further demonstrate this we prepared AST assay reagent with and without the addition of LD, and compared measurements of samples that exceeded the measurement upper limit activity of 500 U/L in the recommended method, but no difference was seen in the measured values obtained (data not shown). Thus, it was unlikely that the produced oxaloacetate was decarbonated and caused negative error, and addition of LD was unnecessary.

Pyruvate and LD exist in human serum. Here, an NADH decreasing reaction occurs when NADH is added, and positive error occurs in AST activity measurements. Because of this, a method has been proposed in which the measuring reagent is divided into two. NADH is added in the first reagent to eliminate the pyruvate completely before starting the AST reaction. The influence was calculated and the time and the additional activity of LD required for the elimination of pyruvate was obtained (Fig. 1).

Specimens with low AST activity or high pyruvate concentration are greatly affected. Therefore, calculations were made assuming the existence of 1.0 mmol/L pyruvate with AST activity of 5 U/L, the lower limit for the JSCC recommendation method. An experiment was conducted under the same conditions, and the experimental and theoretical results were compared. There was good agreement between the two, and this reaction was finished with reaction for 2 min. On the other hand, Figure 1 showed that 5 min was needed until NADH change was completely finished when human serum reacted with AST assay reagent containing 500 U/L LD. This is conjectured to be because a substance other than pyruvate in serum reacts with the LD. This substance has not been identified, but there is a strong likelihood that it is 2-oxobutyric acid. Even considering this result, the effect could be avoided by adding 500 U/L LD and reacting for 5 min.

In conclusion, the negative error resulting from decarbonation of oxaloacetate produced by AST reaction is below the level that needs to be considered. It is thought that a minimum of 5 min should be used for elimination of LD reaction substances in serum with 500 U/L LD adding AST assay reagent.

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