Note

Usefulness of Glucokinase from
Bacillus stearothermophilus
for the Enzymatic Measurement
of Magnesium in Human SerumMasayoshi TABATA, Takahiro KIDO,*
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For enzymes used in clinical analysis, stability of the solution is a prerequisite. We have developed a novel enzymatic method for measuring magnesium in serum, based on the following sequence of reactions¹⁾:

where G6PDH (EC1.1.1.49) stands for glucose-6-phosphate dehydrogenase. The concentration of magnesium can be measured using the increase in absorbance at 340 nm due to NADPH produced. The method is simple and rapid, giving results in excellent agreement with those obtained by atomic absorption measurements.^{2,3)} However, one drawback of the method was that the hexokinase (HK; EC 2.7.1.1) preparation which was commercially available (140 U/mg, from yeast as supplied by Boehringer-Mannheim-Yamanouchi, Tokyo, Japan) was not stable enough. Even when kept in a refrigerator, the solution of HK in 50 mM Tris-HCl buffer at pH 8.5 lost approximately 35% of its enzymatic activity in one week.

A glucokinase (GK; EC 2.7.1.2) preparation from *Bacillus stearothermophilus* (444 U/mg, Unitika Ltd., Osaka, Japan) has been shown to have much higher specific activity and heat stability than HK from yeast,⁴⁾ and it is now available on the market. This prompted us to examine whether *B. stearothermophilus* GK could not only replace yeast HK but also give better results in our enzymatic method for magnesium measurement.

The standard procedures we finally employed using GK and G6PDH (140 U/mg; Boehringer-Mannheim-Yamanouchi, Tokyo, Japan) are presented in Table I. The

NADP⁺ and ATP were products of the Kohjin Co., Tokyo.

This method with GK was essentially the same as that with HK reported previously. However, the advantages associated with the use of GK in place of HK are: (i) pronounced heat stability of the reagent enzyme, and (ii) much less of the enzyme used per assay tube. Figure 1 shows the difference in thermal stability between the GK and HK used for the magnesium assays. With GK, no loss of the activity occurred even after treatment of the enzyme solution at 55°C for 60 min. In contrast, HK completely lost its activity by treatment at 55°C for 45 min. We also found that the GK solution shown in Table I retained its

TABLE I. SERUM MAGNESIUM ASSAY USING
GK AND G6PDH

	Blank (ml)	Standard (ml)	Test (ml)
Substrate solution ^a	3.0	3.0	3.0
H ₂ O	0.02		
Standard Mg solution		0.02	
Serum			0.02
Incubate at 30°C for 5 min			
Enzyme solution ^b	0.05	0.05	0.05
Incubate at 30°C for 15 min			
Stopper solution ^c	0.1	0.1	0.1
Read the absorbance at 340 nm.			

^a 50 mM Tris-HCl buffer, pH 8.5, containing 1.0 mM ATP, 0.5 mM NADP, and 25 mM glucose.

^b 50 mM Tris-HCl buffer, at pH 8.5, containing 4.0 U/ml GK and 28.0 U/ml G6PDH.

^c 200 mM EDTA-4Na.

FIG. 1. Thermal Stability of GK and HK.

Standard magnesium solution (2.0 mM) was used for the assays under the conditions shown in Table I, except that GK(A) and HK(B) solution had previously been treated at (●) 65°C, (○) 55°C, (▲) 45°C, or (△) 35°C, for the period of time indicated in the figure.

full activity for at least three months when stored in the cold.

As shown in Table I, the standard procedure employed only 0.2 U or 0.45 μ g of GK per tube with 0.02 ml of serum, the blank value due to GK being nearly zero. Almost equally good results could be obtained using 0.4 U of GK with 0.01 ml of serum. Such an increase in the amount of enzyme to enhance the response necessary for the case of lessening the sample volume was not possible with HK, as much as 0.7 U or 5 μ g per tube of which had to be added even under the standard conditions.

The K_m value for ATP of GK at pH 8.5 was 5×10^{-5} M,⁴⁾ which was approximately one-sixth of that of HK.⁵⁾ However, twice as much ATP (1.0 mM) was used for the GK-G6PDH method (Table I) than for the HK-G6PDH method.¹⁾ This was a result of empirical optimization for making the standard curve completely linear up to 3.0 mM magnesium, which had been attained with HK.¹⁾

We compared these enzymes using serum samples from a hospital. Figure 2 illustrates the excellent agreement of the GK-G6PDH method with the atomic absorption method over a wide range of magnesium values. The calculated linear regression and correlation coefficient were: $Y = 0.990x + 0.006$ and $r = 0.993$. Furthermore, the method with GK gave satisfactory precision and reproducibility (within one day, $CV = 1.9\%$ for $\bar{x} = 1.00$ mM Mg, and $CV = 1.4\%$ for $\bar{x} = 2.29$ mM Mg; day to day, $CV = 2.3\%$ for $\bar{x} = 1.00$ mM Mg, and $CV = 1.7\%$ for $\bar{x} = 2.29$ mM Mg). It also gave as accurate reaction recoveries (98~101%) as those reported previously for the method with HK,¹⁾ and likewise, can be used in automated analyzers.

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FIG. 2. Correlation between Results of Measurement of Magnesium in Serum by the GK-G6PDH Method and by the Atomic Absorption Method. Superimposed points are not illustrated.

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