A Simple and Rapid Procedure for the Detection of Glutamate-Oxaloacetate Transaminase (GOT) Isozymes of Human Tissues by Agar Gel Electrophoresis

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(Received May 30, 1967)

Transaminase is the enzyme which carries out the amino group transformation between amino acid and α keto acid. This enzyme which is widely distributed in animal and plant tissues consists of many kinds of enzymes. However, those having clinical significance are glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT). GOT content is the highest in the heart, followed by that in the liver. It also is contained in skeletal muscles, the brain, the kidney, the stomach, the small intestine and the pancreas. It is the least in the lung[1].

In serum, Fleischer[2] has demonstrated on paper electrophoresis that there are two kinds of GOT. Subsequently, Boyd[3] separated GOT isozyme from the serum of rat by using agar gel electrophoresis. For the detection of GOT, Fleischer extracted the enzyme from the filter paper after electrophoresis while Boyd used expensive reagents such as malic dehydrogenase and DPNH and a special fluorescence apparatus. In either case, the procedure is complicated.

In a previous report, we obtained good results with agar gel electrophoresis for the separation of isozyme from among serum enzymes[8]. This method has now been applied to the separation of tissue GOT isozyme. Using the property that oxaloacetate produces azo-dye by reacting with diazonium salt, oxaloacetate in tissue extracts and serum can be measured[6][7]. Therefore, an attempt was made to detect the oxaloacetate produced by the action of GOT on aspartate and α-ketoglutamate substrate through the use of diazonium salt. We succeeded in developing a simple method for the separation and identification of tissue GOT isozyme. This method used in a study on the GOT isozyme in extracts from human organs and it has been found that this method is no less effective than previous complicated methods.

MATERIAL AND METHOD

Portions of heart, liver, stomach, intestine, pancreas, spleen, lung and skeletal muscles were obtained from 5 autopsy cases within 4–5 hours after death.

Extracts of these organs were prepared by the following method and examined. These organs were frozen and small sections (about 2 mm x 2 mm x 2 mm) taken. Each of these was weighed. An equal amount of cooled distilled water was added and homogenized for about 10 minutes.

This homogenate is taken into a hematocrit tube, centrifuged at 12,000 r.p.m. for 5 minutes, and the supernatant is taken. This supernatant is examined by agar gel electrophoresis.

Agar gel electrophoresis was conducted according to modified Shibata-Iuchi's method[9] (150 volt, 40 mA for 40 minutes). In this method, a mixture of Difco Bacto Agar and pH 8.4 Veronal buffer μ 0.05, solution in proportions of 0.75 % is heat dissolved, spread over a slide glass and permitted to solidify.

The staining of GOT may be done simply by allowing GOT to react with the following reagent for 40 minutes at 37°C and then washing it several times with running water. The reagent to be used is prepared as follows:

2 g of dl-aspartate and 29.2 mg of a-ketoglutarate is dissolved in 100 ml of pH 7.4 phosphate buffer solution. To 10 ml of this solution is added 40 mg of Fast Blue B salt (Sigma) dissolved in 15 ml of distilled water. This is used after filtration.

RESULTS AND DISCUSSION

The portion having GOT activity appears on agar as a purple band against a light yellowish brown background. The visceral GOT isozyme which was run together with serum as shown in Fig. 1 showed two bands of activity, one at an intermediary position between the α_2 globulin fraction and β fraction of the serum (this is tentatively called Isozyme $\alpha_2\beta$) and the other at a position corresponding to the γ fraction.

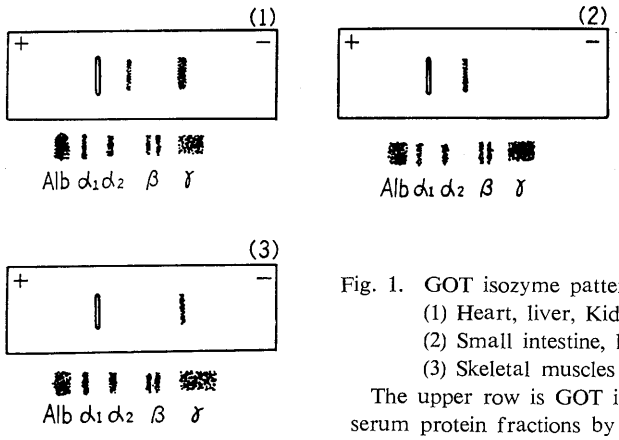

Fig. 1. GOT isozyme pattern of human tissues
(1) Heart, liver, Kidney, spleen, stomach and pancreas.
(2) Small intestine, lung
(3) Skeletal muscles
The upper row is GOT isozyme pattern and the lower is serum protein fractions by amidoblack 10 B stain.

The heart, kidney, spleen, stomach and pancreas showed the presence of both isozyme $\alpha_2\beta$ and isozyme γ, but the skeletal muscle showed only isozyme γ while the intestines and lung contained only isozyme $\alpha_2\beta$. In the organs having both $\alpha_2\beta$ and γ isozyme, the width of the isozyme γ band is wider and the purple coloration is also stronger as compared with those of isozyme $\alpha_2\beta$. In the lung, the band of activity ($\alpha_2\beta$) is barely noted and the activity is weak. In the skeletal muscles, intestined and lung, only one GOT isozyme was noted. However, it was impossible to determine whether there is only 1 GOT isozyme in these organs or whether the other one could not be demonstrated because of its low activity or because of the extraction process.

The GOT content of human organs is variable being predominant in the liver and low in other organs[1], but nevertheless there are almost no differences in their isozyme patterns. Particular attention has been given to the heart and liver GOT, but the patterns are similar.

Fleischer et al.[2], who have examined the extract of human heart and liver by paper electrophoresis, separated GOT into two components. However, no mention had been made about the location to which they migrated. Boyd[5] demonstrated 2 GOT isozymes (GOT II) in the liver of rat. On agar gel electrophoresis, GOT_I appears in the region corresponding the midway between the $\alpha_2$globulin fraction and β-gloaulin fraction while GOT_{II} appears in the region of γ-globulin fraction. This is consistent with the results of our experiments.

Kalnitsky and Tapley[7], utilizing the property that oxaloacetate almost completely forms a stable band with diazonium salt, measured oxaloacetate in the extracts of organs and in the serum. We have applied this method to the detection of GOT isozyme on agar. That is oxaloacetate produced by the action of GOT on aspartate and α-ketoglutarate is captured by Fast Blue B and precipitated as a purplish insoluble material by which GOT was detected. The reason why Fast Blue B had been selected as the diazonium salt is that the result of a comparative study with Fast Blue RR and Fast Garnet GBC revealed the deepest purple color was attained with Fast GBC but the background was also deeply stained yellowish brown, while coloration by Fast Blue RR was poor. The purple coloration by Fast Blue B is slightly weaker than that by Fast Garnet GBC, but at the same time the coloring of the background is light so that it is easy to detect GOT isozyme.

In a control series in which the GOT staining process had been carried out with either dl-aspartate, α-ketoglutarate or Fast Blue B excluded. Staining was not accomplished when any one of them was absent. Moreover, Kalnitsky & Tapley[7] state that these reagents do not show any coloration by reaction with diazonium salt. Therefore, the method in which oxaloacetate produced by the action of GOT on the substrate is identified by Fast Blue B seems to be highly specific for GOT.

This method had been conducted on serum GOT, but we failed to demonstrate GOT isozyme in human serum. Since GOT_{II} (isozyme γ) is said to rapidly lose its activity in serum within a short time[5)10)11)], it is quite natural that it is difficult to demonstrate GOT_{II}. However, that GOT also is not found seems to be due to the fact that activity in serum is lower than the sensitivity of this method. Nevertheless, in the case of the extract from organs, GOT isozyme can be demonstrated similarly at any time.

CONCLUSION AND SUMMARY

By combining the agar gel electrophoresis method with the method of specifically demonstrating oxaloacetate using diazonium salt, a simple method was developed for the detection on glutamate-oxaloacetate transaminase (GOT) isozyme of human organs.

This method showed two types of GOT isozyme to be present in human organs. In the heart, liver, kidney, spleen, stomach and pancreas there were the band between α_2 globulin fraction and β-globulin fraction and γ-globulin fraction. In skeletal muscle only one band in the position corresponding to γ-globulin fraction was noted. In the small intestine and lung there was also one band in the position intermediate between the position corresponding α_2 globulin and β-globulin fraction of the serum.

Acknowledgement : The author wishes to thank professor S. Shibata and associate professor H. Takahashi for constant interest and guidance in this study.

REFERENCES

1) Abderhalden, R: *Klinische Enzymologie, die Fermente in der Pathogenase Diagnostik und Therapie.* Georg Thieme, Stuttgart, 1958.
2) Fleischer, G. A., Potter, C. S. and Wakim, K. G.: Separation of 2 glutamic-oxaloacetic transaminases by paper electrophoresis. *Proc. Soc. Expt. Biol. & Med.*, **103**: 229-231, 1960.
3) Boyd, J. W.: The intracellular distribution, latency and electrophoretie mobility of L-glutamate oxaloacetate transaminase from rat liver. *Biochem. J.*, **81**: 434-441, 1961.
4) Fleischer, G. A. and Wakim, K. G.: Presence of two glutamic-oxaloacetic transaminase in serum of dogs following acute injury of the liver. *Proc. Soc. Expt. Biol. & Med.*, **106**: 283-286, 1961.
5) Boyd J. W.: Glutamate oxaloacetate transaminase isozymes in rat serum. *Clin. Chim. Acta.*, **7**: 429-431, 1962.
6) Walker, P. G.: A colorimetric method for the estimation of acetoacetate. *Biochem. J.*, **58**: 699-704, 1954.
7) Kalnitsky, G. and Fapley, D. F.: A sensitive method for estimation of oxaloacetate. *Biochem. J.*, **70**: 28-34, 1958.
8) Takahashi, H., Wajima, T. and Mizushima, A.: Alkaline phosphatase isozymes of normal human blood serum as examined by a combined procedure of agar gel electrophoresis and

diazocoupling staining. *Medicine and Biology.*, **66** (5): 224-229, 1963.
9) Shibata, S. and Iuchi, I.: Abnormal hemoglobin. *Saishin Igaku.*, **15**: 2567-2581, 1960.
10) Wakim, K. G. and Fleischer, G. A.: The fate of enzymes in body fluids. An experimental study II. Disappearance rates of glutamic-oxaloacetic transaminase I under various conditions. *J. Lab. & Clin. Med.*, **61**: 86-97, 1963.
11) Fleischer, G. A. and Wakim, K. G.: The fate of enzymes in body fluids. An experimental study III. Disappearance rate of glutamic-oxaloacetic transaminase II under various conditions. *J. Lab. & Clin. Med.*, **61**: 98-106, 1963.