Is *Mycobacterium phlei* Glutamotransferase endowed with Glutamine Synthetase Activity?

In a previous paper[1] we reported the finding of glutamotransferase (an enzyme catalysing the transfer of the γ-glutamyl residue of glutamine to hydroxylamine)[2] in cell-free extracts of *M. phlei*. The enzyme required the presence of manganese ions, which could not be substituted by magnesium ions. No glutamine synthetase (an enzyme catalysing the synthesis of glutamine from glutamic acid and ammonia) activity was found in the *M. phlei* preparations, when tested in the presence of magnesium ions under conditions optimal for this enzyme in preparations of bacterial[2], plant[3] and animal[4] origin. The *M. phlei* extracts possessed only traces, if any, of glutaminase activity and did not hydrolyse glutamohydroxamic acid at all. On the other hand, these preparations showed very high asparaginase activity, lacking any aspartotransferase and asparagine synthetase activities. Aspartohydroxamic acid was very strongly hydrolysed by the *M. phlei* extracts; however, this activity was dissociated from asparaginase activity. We have, therefore, concluded that in *M. phlei* the reactions of transfer, synthesis and hydrolysis are due to the action of different enzymes.

It was reported recently[5] that glutamine synthetase and glutamotransferase of sheep brain were activated by either manganese or magnesium. The *p*H optimum of the manganese activated synthetase was in the acid range (*p*H 4·5), while that of the magnesium reaction was 7·2. Increase of the cation concentration caused a shift to the left of the *p*H optima of the manganese-activated synthetase and transferase. Thus the same enzyme seemed to catalyse both the synthesis and transfer reactions.

In light of these findings, and in view of the fact that the *M. phlei* transferase showed a specific requirement for manganese, it was of interest to see whether this organism also possesses a manganese-dependent synthetase.

Table 1. Testing for Glutamine Synthetase Activity of *M. phlei* Extracts with Emphasis on Concentrations of Manganese, Magnesium and Hydrogen Ions

Reaction mixture No.	$MnCl_2$	$MgSO_4$	*p*H
1	$2{\cdot}25 \times 10^{-2}$ *M*	—	4·5
2	$2{\cdot}25 \times 10^{-3}$ *M*	—	5·0
3	$4{\cdot}5 \times 10^{-4}$ *M*	—	5·5
4	$2{\cdot}25 \times 10^{-4}$ *M*	—	6·0
5		$2{\cdot}25 \times 10^{-2}$ *M*	6·0
6		$2{\cdot}25 \times 10^{-2}$ *M*	6·5
7		$2{\cdot}25 \times 10^{-2}$ *M*	7·0
8		$2{\cdot}25 \times 10^{-2}$ *M*	7·5
9		$2{\cdot}25 \times 10^{-2}$ *M*	8·0

The reaction mixture contained: L-glutamic acid, $2{\cdot}25 \times 10^{-2}$ *M*; hydroxylamine sulphate, $1{\cdot}10 \times 10^{-2}$ *M* (calculated as NH_2OH); ATP, $2{\cdot}5 \times 10^{-3}$ *M*; $MnCl_2$ and $MgSO_4$ as indicated; *p*H as indicated; *p*H values below 7·0 were obtained by the use of acetate buffer, 0·1 *M*, while *tris* (hydroxymethyl) aminomethane buffer, 0·05 *M*, was used for *p*H values of 7·0 and above; bacterial extract, 9·1 mgm. protein. Final vol., 2 ml. All solutions added were adjusted to the appropriate *p*H with sodium hydroxide. Experiments were performed in the absence and presence of L-cysteine, 0·04 *M*, which was added before sonication. The mixtures were incubated at 37° for 90 and 180 min.

The experiments were performed with cell-free extracts at various manganese, magnesium and hydrogen-ion concentrations as indicated in Table 1. All the experiments invariably gave negative results: no synthetase activity could be detected under any of these conditions employed. With manganese, but not with magnesium, these extracts showed high glutamotransferase activity as already reported[1]. The results confirm our previous assumption, that the glutamotransferase of *M. phlei* is not associated with any synthetase activity.

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PHYSIOLOGY

Flow of Portal and Hepatic Blood in Normal and Cirrhotic Liver

Hepatic blood-flow measured by the bromsulphthalein method, the colloidal radiogold method, or other methods is the sum of both portal blood-flow and hepatic arterial blood-flow. As to the relative portal and arterial blood-flow, one-third to one-fourth of the total hepatic blood-flow was estimated, in animal experiments, to be derived from the hepatic artery under basal conditions[1]. In some cases with cirrhosis of the liver, in which portocaval shunting operations were performed or portal venous collaterals were accessible, 25–75 per cent or 54 per cent of the total hepatic blood-flow was estimated to be from the hepatic artery[2,3]. It is desirable to develop a new method which enables the actual measurement of the portal blood-flow and the hepatic arterial blood-flow in normal subjects and cirrhotic patients, but there has been no report except that of Ueda[4].

Ueda was the first to describe a method of measuring portal and hepatic arterial flow of blood in clinical cases. His method was based on the fact that the hepatic arterial blood was diluted with the portal blood when the hepatic arterial blood appeared in the hepatic veins, and that a dye concentration curve of the peripheral arterial blood could be a substitute for that of the hepatic arterial blood. Relative portal and arterial flow of blood was calculated from T_{1824} concentration curves of the peripheral arterial blood, and of the hepatic venous blood after the injection of T_{1824} into the cubital vein. The ratio of the hepatic arterial blood-flow to the total hepatic blood-flow was calculated as the ratio of the area of the first (hepatic arterial) component of the hepatic venous concentration curve to that of the peripheral arterial concentration curve.

This method is theoretically attractive. However, it involves a difficulty in the actual measurement. According to our experience, the differentiation between the first (hepatic arterial) component and the second (portal) component in the hepatic venous curve, which is needed for the calculation, is impossible by the original method. Hence, we attempted to improve the method, and were successful in the actual measurement of the portal and hepatic arterial flow of blood by our improved method.

Instead of the original method of injecting a T_{1824} solution into the cubital vein, 50–100 μc. of radioactive iodinated human serum albumin is injected into the pulmonary artery by means of a venous catheter, and blood is taken at successive intervals of 2–3 sec. from the hepatic vein through a hepatic venous catheter and also from an arterial needle placed in the brachial

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