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Kazuko Yamauchi, Tchan Gi Bak,*1 and Shigeaki Kuwano : Failure of Berberine to Inhibit Pig Heart Glutamic-Aspartic Transaminase.

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Previous studies from this laboratory[1~3] have established that the alkaloid berberine inhibits two typical vitamin B_6-enzymes of bacterial origins, i. e. tyrosine decarboxylase of *Streptococcus faecalis* and tryptophanase of *Escherichia coli*. Evidence has also been reported to indicate that berberine inhibits these enzymes by competing with the coenzyme pyridoxal phosphate. It has further been shown that the alkaloid, once combined with the apoenzymes, causes slow and irreversible inactivation of the enzymes.

The present investigation was undertaken to examine if berberine behaves similarly toward pig heart glutamic-aspartic transaminase, a typical B_6-enzyme of mammalian tissues. The results obtained were, however, rather contrary to expectation; no inhibition by berberine could be observed in spite of many attempts. It appears, therefore, that the susceptibility of the mammalian B_6 enzyme is significantly different from that of bacterial B_6 enzymes for reasons to be elucidated in future.

Materials and Methods

Glutamic-aspartic holotransaminase was purified about 35 fold from pig heart muscle according to the method of Lis[4] with slight modifications. Attempts to remove pyridoxal phosphate from this preparation were, however, not successful owing to intensive inactivation of the enzyme. The coenzyme-free apotransaminase was, therefore, prepared from the same source by the method described by O'Kane and Gunsalus[5] in which pyridoxal phosphate was detached from the protein during the initialsteps of purification. The apoenzyme thus obtained was about 16 fold purified and 97.5% resolved with respect to the coenzyme.

The transaminase activity was usually measured at 37° by spectrophotometrically determining the rate of oxaloacetate production as described by Cammarata and Cohen.[6] Spectrophotometric measurements were made with a Hitachi model EPU-2A spectrophotometer. A unit of the enzyme was defined as that amount of enzyme that would give a calculated initial velocity of one absorbancy unit per min. At appropriate concentrations of enzyme and substrates, the time-activity curve was linear for at least 2 min.

In inhibition experiments, however, oxaloacetate was estimated manometrically according to the method of O'Kane and Gunsalus,[5] since berberine strongly absorbs at 280 mμ and therefore interferes with the spectrophotometric determination of oxaloacetate. In the manometric assay corrections were made for the net amount of CO_2 evolved from oxaloacetate by decarboxylating it with aniline-citrate.

Protein was determined spectrophotometrically at 280 mμ[7] using crystalline horse serum albumin as the standard.

Berberine hydrochloride and pyridoxal phosphate used were the same preparations as described previously.[1]

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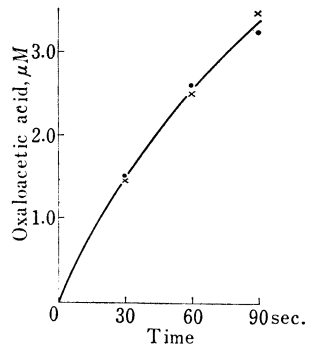
Results and Discussion

Fig. 1 shows that berberine had no inhibitory effect on the activity of holotransaminase even if the alkaloid had been preincubated with the enzyme before the addition of the substrates. Since this failure of berberine to inhibit the enzyme may be due to the firm binding of pyridoxal phosphate to the apoprotein, the effect of berberine on the coenzyme-free apoenzyme was then studied at pH 7.5. The apoenzyme was thus preincubated with berberine for 60 minutes and then for another 15 minutes with pyridoxal phosphate*[3] followed by the initiation of enzyme reaction by adding the substrates. No inhibition was again observed. This negative result might be accounted for by assuming that the inhibition of apotransaminase by berberine can be completely eliminated by the incubation with pyridoxal phosphate in the absence of substrates. This possibility was, however, excluded by further experiments in which the enzyme reaction was started by the simultaneous addition of coenzyme and substrates after the apoenzyme was preincubated with berberine for 60 minutes. As can be seen from Fig.2, be rberine completely failed to inhibit the reaction even under these conditions. It is therefore likely that the alkaloid possesses no inhibitory action at all toward the transaminase regardless of the presence and absence of the coenzyme. It has previously been shown that the inhibition of *S. faecalis* tyrosine decarboxylase by berberine is greatly augmented when the pH of reaction medium is raised to pH 8.0.[1] This is the reason why the experiments recorded in Fig. 2 were conducted both at pH 7.5 and 8.0.



The reaction mixture contained α-ketoglutaric acid, $1.33 \times 10^{-2} M$; L-aspartic acid, $1.33 \times 10^{-2} M$; K-Na-PO_4 buffer, $5 \times 10^{-2} M$; holoenzyme protein, 160 γ/cc. respectively. Berberine was added at $6 \times 10^{-3} M$ during preincubation and $5 \times 10^{-3} M$ during reaction. Total volume was 3.0 cc. Enzyme preparation was preincubated with berberine at 37°, pH 7.5, for 60 min.

Reaction was stopped by addition of 0.5 cc. of aniline citrate soln. and after shaking for 15 min. the evolution of CO_2 was estimated.

·——· Control
×——× +Berberine

Fig. 1. Action of Berberine on Holotransaminase Activity

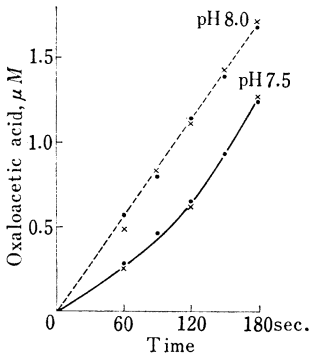


Fig. 2. Action of Berberine on Apotransaminase Activity

The reaction mixture contained pyridoxal phosphate, 1 γ/cc.; apoenzyme protein, 135 γ/cc. respectively. Enzyme preparation was preincubated with berberine at 37°, pH 7.5 or pH 8.0, for 60 min. and the reaction was started by the simultaneous addition of substrates and pyridoxal phosphate. Other conditions were the same as of Fig. 1.

·┈┈· Control
×┈┈× +Berberine

*[3] In order to attain maximum activity of the enzyme, it is necessary to preincubate the apoenzyme with more than 30 γ of pyridoxal phosphate for at least 15 min. before the reaction starts. If not, as shown in Fig. 2, the slight lag appears at the onset of the enzyme reaction at pH 7.5 and the enzyme activity is also finally lowered as compared with the optimum.

In the case of the mammalian transaminase, however, no inhibition by berberine could be observed even at pH 8.0.

From all these findings, it was concluded that pig heart glutamic-aspartic transaminase, unlike the bacterial B_6-enzymes, is not inhibited by berberine under any experimental conditions. In view of the facts that the pig heart enzyme is also a pyridoxal phosphate-requiring enzyme and berberine competes with pyridoxal phosphate in bacterial enzyme systems, this failure of berberine to inhibit the pig heart transaminase is interesting in certain respects. Firstly, this finding suggests that the action mechanism of the mammalian transaminase considerably differs from that of bacterial B_6-enzymes in certain fundamental points which are to be elucidated in future. Secondly, such different behavior of berberine toward mammalian and bacterial systems may provide a clue to the pharmacological effect of the alkaloid as a useful drug.

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