COENZYME ACTIVITY OF PYRIDOXAL PHOSPHATE DERIVATIVES ON GLUTAMIC-OXALOACETIC TRANSAMINASE

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The general theory of the catalytic mechanism for pyridoxal enzymes that require free or potentially free carbonyl group, has been proposed by Snell (1) and Braunstein, assuming that the mechanism of non-enzymatic reaction parallels those of enzyme process. On the other hand, Gonnard et al. (2) and Bonavita et al. (3) showed that certain carbonyl derivatives of PAL-P could act as a coenzyme. Gonnard et al. interpreted the result as indicating that isonicotinoylhydrazone functions coenzymatically per se, since they were unable to show the liberation of isoniazide in the course of the experiment. This interpretation is contradictory to the theory of Snell and Braunstein which requires a free carbonyl group of PAL-P.

We reported previously that the coenzyme activity of various carbonyl derivatives of PAL-P was tested on glutamic decarboxylase of mouse brain and since the enzyme activated by isonicotinoylhydrazone of PAL-P was less susceptible to the inhibition by penicillamine than PAL-P itself, we thought that isonicotinoylhydrazone activated glutamic decarboxylase by functioning as a coenzyme per se rather than being converted to PAL-P (4).

In this report we examined the coenzyme activity of some carbonyl derivatives on purified glutamic-oxaloacetic transaminase of which characteristic spectral changes of bound coenzyme could be used as an indicator of the reaction process.

EXPERIMENTAL

Methods and Materials

1. Sodium Borohydride
   The preparation of 99% purity was obtained from Daiichi Kagaku Yakuhin Co., Ltd.

2. Carbonyl Derivatives of PAL-P.
   They were synthesized by Mr. Kuroda of Wakamoto Pharmaceutical Company.

1 Following abbreviations are used. PAL-P, pyridoxal phosphate; GOT, glutamic-oxaloacetic transaminase; KGA, α-ketoglutaric acid; ApA, aspartic acid; OAA, oxaloacetic acid; PAM-P, pyridoxamine phosphate.

2 村上 安子，牧野 堅。
3. Glutamic-Oxaloacetic Holotransaminase (holo-GOT) (EC 2.6.1.1)

It was prepared from pig heart muscle. The first step of purification was made by the method of Jenkins (5). The enzyme of 70–80% purity so obtained was dialyzed against 0.005 M potassium phosphate buffer, pH 7.0, for 15 hours. The precipitate of inactive protein was removed by the centrifugation. The supernatant solution containing 100 mg protein was poured over DEAE-cellulose column (2×20 cm), and unadsorbed substances were washed out with 0.005 M phosphate buffer, pH 7.0. The enzyme adsorbed on the column was eluted with 0.03 M potassium phosphate buffer, pH 7.0. Then, solid ammonium sulfate was added to the effluent up to 70% saturation in the presence of 0.0025 M KGA. The resultant precipitate was dissolved in a small volume of 0.3 M maleate buffer, pH 6.0, and finally dialyzed against deionized water for 48 hours. The final enzyme preparation had a single sedimentation pattern, when ultracentrifugal analysis was carried out with a Hitachi UCA 1 type ultra-centrifuge as is shown in Fig. 1.

![FIG. 1 Sedimentation Pattern of the Enzyme Preparation](image)

After centrifugation at 55,430 rpm for 40 minutes. Protein concentration: 3 mg/ml. 0.005 M phosphate buffer, pH 8.0.

Apo-GOT was prepared by the method of Wada from the holo-GOT obtained as described above (6). The enzyme activity was determined at 20°C by measuring the formation of oxaloacetic acid as the increase in optical density at 280 mµ in Shimazu DU type spectrophotometer according to the method of Jenkins (5).

The standard reaction mixture contained 20 µmoles of KGA and of ApA, and 300 µmoles of phosphate buffer, pH 8.3, in a total volume of 3.0 ml.

4. Determination of Protein Concentration

It was determined by the biuret method or from the ratio of optical density at 280 and 260 mµ according to the method of Kalcker.

5. Determination of PAL-P.

It was assayed by the hydrazone method of Wada et al. (7).

6. Detection of PAL-P and Its Oxime

It was carried out by paper chromatography as follows: To the reaction mixture containing 4–8 mg of protein per ml one-ninth volume of 50% trichloroacetic
acid was added and it was kept at 0°C for 20 minutes. After removal of the denatured protein by centrifugation, trichloroacetic acid was extracted from the supernatant solution with ether until the solution showed no acidity against Congo red. The solution was further subjected to the deproteinization procedure of Sevag. Alternative procedures were made in some cases for the deproteinization.

To the reaction mixture 1N KOH was added to give a final concentration of 0.1 N for the resolution of PAL-P from the holoenzyme and then the protein was precipitated with perchloric acid in a final concentration of 0.5 N. After the solution was neutralized with KOH, the resultant precipitate was removed. The supernatant solution was concentrated to approximately 1 ml, and 2 ml of ethanol were added to it. The precipitate was removed. These deproteinized solutions were concentrated under reduced pressure at 40°C and applied to Toyo Roshi No. 51 paper and developed by ascending paper chromatography in n-propanol: water: 1 M acetate buffer of pH 5.0 (70:20:10) at room temperature. The dried paper was exposed to ammonia and then PAL-P and its oxime were detected under ultraviolet lamp. By this method 1 μg each of PAL-P and its oxime was detectable.

RESULTS

1. Coenzyme Activity of PAL-P Derivatives

After various amounts of PAL-P or its derivatives were preincubated with the apoenzyme at 35°C for 60 minutes, the substrates were added to the incubation mixture and the enzyme activity was determined. A correction was made by appropriate blanks without the enzyme. The relative enzyme activities were given.

![Graph](image-url)

**Fig. 2 Coenzyme Activity of PAL-P and Its Derivatives**

1. PAL-P; 2. isonicotinoylhydrazone; 3. thiazolidine; 4. thiosemicarbazone; 5. oxime; 6. semicarbazone; 7. phenylhydrazone; 8. hydrazone.
in Fig. 2, where the maximum activity in presence of PAL-P was taken as 100. The GOT activity could not be detected when PAL-P or its derivatives were not added. PAL-P derivatives such as isonicotinoylhydrazone, oxime, semicarbazone, phenylhydrazone, thiosemicarbazone and penicillamine-thiazolidine showed the coenzyme activity. The following points differed from the case of glutamic decarboxylase: (a) Oxime and thiazolidine acted inhibitorily on glutamic decarboxylase. (b) Phenylhydrazone had no effect on the enzyme. (c) Other PAL-P derivatives, i.e. isonicotinoylhydrazone, thiosemicarbazone, semicarbazone and hydrazone could activate the apodecarboxylase in the same degree as PAL-P. However, the strength of their coenzyme activities for apo-GOT was found different. In high concentrations strong inhibition of the GOT activity was observed.

It is interesting that, although hydroxylamine itself is a strong inhibitor on GOT, PAL-P oxime which was prepared by combination of PAL-P with hydroxylamine could act as a coenzyme of the enzyme. It seems to show that the inhibitory action of hydroxylamine is caused by the combination with apoenzyme portion rather than with PAL-P. Therefore, the nature of the coenzymatic action was investigated using the oxime enzyme to know whether the oxime of PAL-P itself functions coenzymatically or PAL-P functions after decomposition of the oxime. It was also convinient for these studies that the oxime of PAL-P did not develop color with excess phenylhydrazine, differing from PAL-P.

2. Preparation of Oxime Enzyme

Apo-GOT, $7 \times 10^{-5}$ to $1.5 \times 10^{-4}$ M, and oxime of PAL-P, $3.3 \times 10^{-4}$ M in final concentration, were incubated in 0.1M phosphate buffer, pH 8.3, for 35 minutes at 35°C. Following procedures were carried out under 5º. Ammonium sulfate was added to the solution to 70% saturation and the resultant precipitate, after being washed with saturated ammonium sulfate solution, was dissolved in 2 volumes of the same buffer. The solution was again saturated to 70% with ammonium sulfate and the resultant precipitate was dissolved in an appropriate volume of the same buffer and used as the oxime enzyme.

The oxime enzyme showed the same specific activity as the original holo-GOT. PAL-P enzyme was reconstructed by the combination of the apoenzyme with PAL-P instead of the oxime of PAL-P by the above method. This enzyme showed the same specific activity and the same PAL-P content as the original holoenzyme. The above facts seem to indicate that the oxime enzyme did not contain excess oxime.

3. Chemical State of Bound Oxime before the Addition of Substrates

The Changes of Absorption Spectra of the Enzymes with the Addition of Amino Acid — By using the oxime enzyme, firstly, spectrophotometrical experiments were carried out in the substrate level enzyme concentration in order to pursue the changes of the bound coenzyme. While the original holo-GOT showed the absorption maximum at 360 mµ in 0.1 M phosphate buffer, pH 8.3, the oxime enzyme showed the absorption maximum at 370 mµ.

Spectral changes of both enzyme solutions with different concentrations of ApA were given in Fig. 3. The absorption maxima of both enzymes shifted to 330 mµ, but the spectra in various concentrations of ApA were distinguishable one another. These results indicated that the PAL-P oxime itself combined with apo-GOT.
**FIG. 3** *The Changes of Absorption Spectra of the Enzymes with the Addition of Amino Acid*

ApA (20 μmoles/0.2 ml, pH 8.3) was added to the enzyme solution (in 0.1 M phosphate buffer, pH 8.3) at 20°C. 1, none; 2, 1.0 μmole; 3, 6.0 μmoles; 4, 11.0 μmoles; 5, 16 μmoles; 6, 20 μmoles.

**PAL-P Content of the Enzyme** — To the solution of the original holo-GOT and the oxime enzyme which contained the same amount of protein, one-ninth volume of 50% trichloroacetic acid was added, and each clear supernatant solution was used for the determination of PAL-P content. As is shown in Table 1, PAL-P content of the oxime enzyme was one-seventh of the original holo-GOT. This result also indicated that the oxime of PAL-P combined apo-GOT and the most part of the coenzyme in the oxime enzyme prepared by the described method was oxime itself. This however is not always the case; it depends on PAL-P derivatives. For example, when isonicotinoylhydrazone of PAL-P was incubated with apo-GOT, it was found from the spectral change that the coenzyme could combine with the apo-GOT after conversion to PAL-P, as was also reported by Torchinsky (8). Semicarbazone also was apt to liberate semicarbazide. It is not clear in other derivatives.

**TABLE 1**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>PAL-P content (μmole/8.6 mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original-GOT</td>
<td>0.099</td>
</tr>
<tr>
<td>Oxime enzyme</td>
<td>0.013</td>
</tr>
<tr>
<td>Apo-GOT</td>
<td>0.000</td>
</tr>
</tbody>
</table>

4. **Chemical State of Bound Coenzyme after Addition of Substrates**

*The Spectral Change of the ApA-treated Enzyme by KGA* — We examined whether the absorption spectra of the enzymes which had been treated with ApA returned to the original spectrum by adding KGA. After 20 μmoles of ApA was added to both holo-GOT and oxime enzyme in phosphate buffer at pH 8.3, the enzymes were precipitated by adding ammonium sulfate and again dissolved in the same buffer. When 10 μmoles of KGA was added to those solutions, the absorption spectrum of each enzyme solution returned to the original spectrum (Fig. 4).
When 20 µmoles of ApA was added to the enzyme "1" in 0.1 M phosphate buffer pH 8.3, at 20°C, the absorption spectrum of the enzyme changed to "2". Under cooling, "2" enzyme was precipitated by adding ammonium sulfate and dissolved in the same buffer. The enzyme showed the spectrum "3". When 10 µmoles of KGA was added to the "3" enzyme at 20°C, the spectrum changed to "4".

**Table 2**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>PAL-P content (µmole/8.6 mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original GOT</td>
<td>0.058</td>
</tr>
<tr>
<td>Oxime enzyme</td>
<td>0.019</td>
</tr>
<tr>
<td>Apo-GOT</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Determination of the Coenzymes by Paper Chromatography** — The coenzyme bound in the enzyme was determined before and after the reaction by paper chromatography. PAL-P was detected from the original holo-GOT and the PAL-P enzyme before and after the enzyme reaction. PAL-P oxime was detected in the oxime enzyme before the reaction. PAL-P oxime and a trace of PAL-P were detected in the oxime enzyme after the reaction. These results seem to show that the coenzyme of oxime enzyme, even after addition of substrates, was the oxime of PAL-P.

From these results, it is plausible that under our experimental condition, oxime itself is bound to the apoenzyme even after the addition of the substrates, functioning as a coenzyme, and is not converted to PAL-P during the enzyme reaction.
5. Inhibition of the Enzyme Activity by Sodium Borohydride

It is necessary to examine the coenzyme action of the bound oxime in such a lower enzyme concentration that the reaction rate increases linearly with the amount of the enzyme. Under this condition it was difficult to know the chemical states of the bound oxime from the ultraviolet absorption spectra and to estimate PAL-P content of the enzyme. Therefore, NaBH₄ was used to investigate the mechanism of the coenzyme action. This reagent was found to reduce PAL-P but not to affect its oxime. When treated with NaBH₄, the activity of the original holo-GOT (PAL-P form) was lost, but that of PAM-P enzyme, which had been prepared from PAM-P and apo-GOT by using the same method as the preparation of PAL-P enzyme as described above, was not affected (Table 3). This fact showed that NaBH₄ acted on the 4-carbonyl group of PAL-P in the holoenzyme.

### TABLE 3

**Enzyme Activity Before and After the NaBH₄-Treatment**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Enzyme activity (k min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td>Original GOT</td>
<td>0.490</td>
</tr>
<tr>
<td>PAM-P enzyme</td>
<td>0.490</td>
</tr>
</tbody>
</table>

6. The Chemical State of Bound Oxime before the Enzyme Reaction

Therefore, the effect of NaBH₄ on the original holo-GOT and oxime enzyme were examined. To the enzyme solution containing 60-80 µg of protein in 3.0 ml of 0.1 M phosphate buffer, pH 8.3, 0.2 ml of an aqueous solution containing 5 µg of NaBH₄ was added at 0°. After 5 minutes, the solution was saturated with ammonium sulfate to 70 % and kept standing for 30 minutes. The resultant precipitate was collected by centrifugation at 20,000 × g for 30 minutes and dissolved in 2.6 ml of the same buffer. These enzymes were used as NaBH₄-treated enzyme. NaBH₄-untreated enzymes were prepared by the same method without adding NaBH₄ in the solution. The activities of these enzymes were shown in Table 4 A and B. The NaBH₄-treated original holoenzyme lost its activity but the treated oxime enzyme showed 50-60 % activity of the untreated one. This result indicates that the oxime itself was combined with the apoenzyme in a diluted enzyme solution.

At high concentration of the enzyme, the same experiment was carried out. After precipitation of the enzyme protein with ammonium sulfate in order to

### TABLE 4

**Effect of NaBH₄ on the Activity (k min⁻¹) of “ApA-KGA-Untreated Enzyme” (Enzyme before Reaction) and “ApA-KGA-Treated Enzyme” (Enzyme after Reaction)**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Without NaBH₄ treatment (A)</th>
<th>Without ApA-KGA treatment (B)</th>
<th>With ApA-KGA treatment (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original GOT</td>
<td>0.483</td>
<td>0.036</td>
<td>0.036</td>
</tr>
<tr>
<td>Oxime enzyme</td>
<td>0.392</td>
<td>0.212</td>
<td>0.058</td>
</tr>
</tbody>
</table>
remove excess NaBH₄, the enzyme activity was determined. While the treated original holoenzyme lost its activity completely, oxime enzyme retained 50% of its activity. This was the same results as in the case of the low enzyme concentration. Spectral changes of both enzymes at high concentrations of phosphate buffer in the presence of different concentrations of NaBH₄ were shown in Fig. 5. Spectral changes of both enzymes in each concentration of NaBH₄ were distinguishable from each other similarly to the results in Fig. 3.

![Fig. 5 The Spectral Changes of the Enzymes with the Addition of NaBH₄](image)

Aqueous solution of NaBH₄ was added to the enzyme solution (in 0.1 M phosphate buffer, pH 8.3) at 15°. 1, none; 2, 0.025 µmole; 3, 0.05 µmole; 4, 0.075 µmole; 5, 0.1 µmole.

7. Chemical State of the Bound Oxime after Enzyme Reaction

The Effect of NaBH₄ on the Enzyme after the Reaction——The enzyme of low concentrations (80 µg protein) and the substrates were brought into reaction for five minutes under the condition mentioned above and ammonium sulfate was added to the mixture to 70% saturation at 0°. After thirty minutes the resultant precipitate was collected by centrifugation at 20,000 x g for thirty minutes and was dissolved in 3.0 ml of the same buffer (“ApA-KGA-treated enzyme”). On the other hand, “ApA-KGA-untreated enzyme” was prepared by the same treatment without adding the substrate. Both “ApA-KGA-treated and ApA-KGA-untreated” enzymes were reduced with NaBH₄ by the method mentioned above.

The results showed that “ApA-KGA-treated oxime enzyme” lost its activity by NaBH₄ treatment (Table 4 A-C), indicating that the bound oxime had been converted to some compound during the reaction. Then, we intended to determine which substrate, ApA or KGA, decomposed the bound oxime.

The Effect of NaBH₄ on the Oxime Enzyme after Addition of ApA——The enzyme was incubated with ApA for five minutes under almost the same condition as mentioned above, except that the reaction mixture did not contain KGA. The enzyme was precipitated from the incubation mixture by addition of ammonium sulfate. It was dissolved in phosphate buffer, treated with NaBH₄ and reprecipitated with ammonium sulfate. Using the final precipitate, the GOT activity was measured. As shown in Table 5 A-B, NaBH₄ did not inhibit the activity of the oxime enzyme treated with ApA. With the original holo-GOT, the same
Table 5

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Without NaBH₄ treatment (A)</th>
<th>With NaBH₄ treatment (B)</th>
<th>With ApA treatment (C)</th>
<th>With KGA treatment (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original GOT</td>
<td>0.483</td>
<td>0.415</td>
<td>--</td>
<td>0.030</td>
</tr>
<tr>
<td>Oxime enzyme</td>
<td>0.392</td>
<td>0.392</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

result was obtained. Absorption spectrum was also determined after the following treatment. After adding ApA under the same condition, the oxime enzyme was precipitated with ammonium sulfate and was dissolved in a small volume of the buffer. The spectrum of this enzyme resembled that of PAM-P enzyme. When these results are compared with those of Table 4, it is clear that the oxime is changed by ApA to some form resistant to NaBH₄.

The Effect of NaBH₄ on the Enzyme after Addition of KGA —— After addition of KGA instead of ApA, the enzyme was treated with NaBH₄. The enzyme lost its activity in this case (Table 5 A–C). It means that the bound oxime changed to some form which was much more susceptible than the oxime form to the inhibition by NaBH₄. From the above two experiments it is clear that the bound oxime was decomposed by addition of ApA or KGA.

The Effect of Carboxylic Acids and Carbonyl Compounds on the Oxime Enzyme —— It seems that the enzyme becomes active by combination with the substrates, whereby the oxime was decomposed. If this is true, other substances which can combine with the enzyme protein might decompose the bound oxime. Alternatively, the decomposition of oxime in the presence of KGA might indicate that transoximation occurred. Therefore, the effect of carboxylic acids and carbonyl compounds on the oxime enzyme was observed.

Oxime enzyme (80 μg) was incubated with carbonyl compounds, such as oxaloacetic acid, acetylacetone, acetone and pyruvic acid or carboxylic acids, such as malonic, propionic, fumaric, succinic and maleic acid, 20 μmoles each in 3.0 ml of 0.1 M phosphate buffer, pH 8.3, at 35° for five minutes. Then, ammonium sulfate was added at 0° to 70% saturation. The precipitated enzyme was dissolved in 3.0 ml of the same buffer. After treating with NaBH₄ by the method described above, the enzyme activity was determined. The direct inhibition by these carbonyl compounds and carboxylic acids is not necessary to consider, because after the addition of these compounds the isolated enzymes retained their whole activities. Each activity of NaBH₄-treated enzyme is shown in Table 6, where the activity of untreated enzyme is taken as 100. In the case of carbonyl compounds, i. e., KGA, acetone, acetylacetone and oxaloacetic acid, the bound oxime was decomposed to some form which was reducible by NaBH₄. Free oxime of PAL-P was also incubated with these carbonyl compounds in a final concentrations of 3 × 10⁻⁸ and 6 × 10⁻⁸ M respectively in phosphate buffer, pH 8.3, at 35° for one hour and the color development of the mixtures, due to the liberation of PAL-P, was examined by phenylhydrazine. The color intensities were the same with those without the addition of the carbonyl compound and the intensities
TABLE 6

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activity per cent</th>
<th>Substance</th>
<th>Activity per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.0</td>
<td>Oxaloacetic acid</td>
<td>47.7</td>
</tr>
<tr>
<td>Malonic acid</td>
<td>104.0</td>
<td>Acetylacetone</td>
<td>38.3</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>101.0</td>
<td>Acetone</td>
<td>24.6</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>70.0</td>
<td>Pyruvic acid</td>
<td>19.4</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>60.0</td>
<td>α-Ketoglutaric acid</td>
<td>13.7</td>
</tr>
<tr>
<td>Maleic acid</td>
<td>55.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

were 1% of that of PAL-P in the same concentration. From these results, it is clear that the interaction between oxime and carbonyl compound occurs only in the presence of the enzyme protein. The reaction products were not investigated and the mechanism of the decomposition of the oxime is obscure. On the other hand, the effects of carboxylic acids on the activity of the oxime enzyme were weaker than those of carbonyl compounds. Maleic acid had the same effect as oxaloacetic acid, the weakest carbonyl compound. Succinic and fumaric acids had some effects, but propionic and malonic acids had no effect. Jenkins has observed that the addition of some dicarboxylic acids to the holo-GOT in an alkaline solution increased the absorption at 435 mµ of the enzyme, and the same dicarboxylic acids inhibited the enzyme activity (5). The carboxylic acids which decomposed the bound oxime in our experiment seem to correspond to the dicarboxylic acids reported by Jenkins. From these facts, it may be considered that the structure of bound oxime becomes more labile in the presence of not only the substrates but also the carboxylic acids capable of combining with the enzyme.

DISCUSSION

The above results suggest that at the substrate level enzyme concentration, the oxime combined with the apoenzyme remained nearly intact even after the enzyme reaction. In lower concentrations, where the reaction rate increased linearly with the amount of the enzyme, however, the oxime combined with the apoenzyme was decomposed after the enzyme reaction. PAL-P oxime may be changed to PAM-P or PAL-P by the addition of ApA or KGA, respectively. The fact that the bound oxime was decomposed by some carbonyl compounds and carboxylic acids which could combine with the holoenzyme, suggest that the chemical state of the bound oxime is labile. It can also be assumed from the fact that the addition of excess ApA brought the spectral pattern of the oxime enzyme (Fig. 3 right) to that of the original holo-GOT (Fig. 3 left). The concentration of the enzyme occurring in nature may be low. Whether the decomposition of the bound oxime is essential for the enzyme reaction or not remained undecided. If it is essential, the above results obtained with the enzyme of high concentration seems to show that only a small amount of the enzyme took part in the reaction, whereas the greater part remained unparticipated in the reaction, owing to the insufficiency of the absolute amounts of the substrates. If the decomposition of the bound oxime is secondary,
the use of the PAL-P oxime would not be suitable for investigating the mechanism of the present enzyme reaction.

SUMMARY

1. Isonicotinoylhydrazone, oxime, thiosemicarbazone, hydrazone, phenylhydrazone, semicarbazone and thiazolidine combined with penicillamine of pyridoxal phosphate activated glutamic-oxaloacetic apotransaminase.

2. From the absorption spectra and pyridoxal phosphate content of the enzyme it was found that oxime itself combined with apo-glutamic-oxaloacetic transaminase.

3. At higher concentrations of the enzyme, it was found from its ultraviolet absorption spectrum, its pyridoxal phosphate content and the detection of the coenzyme by paper chromatography that the bound oxime remained nearly intact even after the enzyme reaction. At lower concentrations of the enzyme, where the reaction rate increased linearly with the amount of the enzyme, however, it was found by using NaBH₄ as the means to see the state of bound oxime that the oxime was decomposed after the enzyme reaction.

ACKNOWLEDGEMENTS

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REFERENCES