Contamination of Malic Dehydrogenase with Apotransaminase

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Transaminases are among the enzyme requiring pyridoxal-5 phosphate as a co-factor (1). In the course of studies on the presence of the apoenzyme of glutamic oxaloacetic transaminase (GOT) in the serum, it was found that the malic dehydrogenase preparations, which are used in the Karmen (2) transaminase assay system, had variable amounts of apotransaminase. The studies reported below present the evidence for this conclusion.

METHODS

Purified malic dehydrogenase extracted from pig heart bearing the lot numbers* 774, 11856, 39725, and 118660 was studied. GOT was assayed at 37° by the spectrophotometric method of Karmen (2). In this method the transamination reaction is coupled with oxaloacetate reduction. The reaction rate is measured by following the disappearance of DPNH+ at 340 mμ in the Beckman DU spectrophotometer. The results have been expressed as millimicromoles† of DPNH+ oxidized, per minute, per 2000 units of malic dehydrogenase.

The malic dehydrogenase extracts were suspended in a volume of

*Obtained from Sigma Chemical Company, St. Louis, Mo.
†One millimicromol of DPNH+ oxidised is equivalent to 2 Karmen units.
Table 1. REACTION MIXTURE

<table>
<thead>
<tr>
<th>Concentration or quantity</th>
<th>Volume (ml.)</th>
</tr>
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<tbody>
<tr>
<td>Malic dehydrogenase</td>
<td>400 U.</td>
</tr>
<tr>
<td>Pyridoxal 5-phosphate</td>
<td>*</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.2 M</td>
</tr>
<tr>
<td>Alpha-ketoglutarate</td>
<td>0.1 M</td>
</tr>
<tr>
<td>DPNH+</td>
<td>0.2 mg.</td>
</tr>
<tr>
<td><strong>TOTAL VOLUME</strong></td>
<td><strong>3.0</strong></td>
</tr>
</tbody>
</table>

*Buffer solution (pH 7.5) with 0.02, 0.04, 0.08, 0.16, 0.24, 0.32, and 0.40 μM of pyridoxal 5-phosphate.

Table 1. REACTION MIXTURE

Malic dehydrogenase was found to contain 1 to 2 mμM of GOT activity per 2000 units of malic dehydrogenase (Fig. 1, curve A). This figure was comparable to the manufacturer's assay of the transaminase activity of the malic dehydrogenase preparations. Preincubation of the malic dehydrogenase preparation with 0.02, 0.04, 0.08, 0.16, 0.24, 0.32, and 0.40 μM of pyridoxal 5-phosphate resulted in an increase of transaminase activity of the malic dehydrogenase preparation (Fig. 1, curves B through H). The increase was progressive with a level of 9.5 mμM reached when 0.08 μM of pyridoxal 5-phosphate was added (Fig. 2). Beyond this point the curve became asymptotic. Approximately 60 per cent of this increased activity was observed when 0.02 μM of pyridoxal 5-phosphate was used. This experiment was repeated 3 times; the results are summarized in Fig. 2.

Figure 3 shows the results of the experiment designed to test the possibility that the enhancement of GOT activity by pyridoxal 5-
phosphate might be the result of nonenzymatic transamination by pyridoxal 5-phosphate and metal contaminants of malic dehydrogenase. Boiling the malic dehydrogenase solution before incubation with pyridoxal 5-phosphate resulted in loss of all transaminase activity.

Fig. 1. Kinetic curves of GOT activity of malic dehydrogenase extract (lot No. 118656) without added pyridoxal 5-phosphate (curve A) and after incubation with 0.02, 0.04, 0.08, 0.16, 0.24, 0.32, and 0.40 μM of pyridoxal 5-phosphate (curves B through H, respectively).

Five studies were conducted to determine the effect of the length of the period of incubation of pyridoxal 5-phosphate with the malic dehydrogenase suspension (Fig. 4). The GOT activity of the malic
Fig. 2. Pyridoxal 5-phosphate saturation curve demonstrating amount of apo-transaminase present in malic dehydrogenase extract. The curve is a composite of 3 experiments; each point in the 3 experiments was determined in triplicate.

Fig. 3. GOT activity of malic dehydrogenase extract without (a) and with (b) pre-incubation with pyridoxal 5-phosphate (2.4 \mu M). The results with unboiled malic dehydrogenase are shown in section A and the results after boiling the malic dehydrogenase extract in section B. Loss of transaminase activity is obvious in section B.
Fig. 4. Summary of the experiment to study the influence on GOT activity of the length of time of preincubation of pyridoxal 5-phosphate (0.24 μM) with malic dehydrogenase extract. Maximal activation occurred in 10 minutes. This experiment was performed 5 times; each point in the 5 experiments was determined in triplicate.

dehydrogenase preparation was 1 mμM. Addition of pyridoxal 5-phosphate (0.24 μM/ml.) to the malic dehydrogenase and immediately thereafter of the substrates (permitting no preincubation of enzyme with co-enzyme) yielded a transaminase activity of 5 mμM. Preincubation of co-enzyme with apoenzyme for periods of 5 to 45 minutes yielded GOT activity of 8 to 9 mμM.

The effect of incubating either of the main substrates (aspartate or alpha-ketoglutarate) with the malic dehydrogenase prior to the addition of pyridoxal 5-phosphate was studied (Fig. 5). Incubation of the malic dehydrogenase with alpha-ketoglutarate or aspartate before incubation with pyridoxal 5-phosphate resulted in approximately 65 per cent of the maximal rise observed when pyridoxal 5-phosphate had been incubated first with the malic dehydrogenase.

DISCUSSION

The enhancement of transaminase activity of the malic dehydrogenase preparations in this study appears to conform to the proper-
ties of apotransaminase observed in tissue extracts (3-7). The progressive increase with increments of pyridoxal 5-phosphate resembles the observations of these workers (3-7) in their studies of porcine heart muscle apoenzyme. The observation that incubation with either substrate before incubation with pyridoxal 5-phosphate appeared to result in lesser degrees of increased GOT activity than those observed when pyridoxal 5-phosphate was added first likewise conforms to the observations of O’Kane and Gunsalus (5). These workers also have shown that incubation for a period as brief as 10 minutes results in approximately 85 per cent of the transaminase activity achieved by longer periods of pyridoxal 5-phosphate incubation. Even when no preincubation of pyridoxal 5-phosphate and apoenzyme was permitted, some activation of GOT activity was observed (7).

Snell and his associates (8) have shown that transamination may occur in the presence of pyridoxal 5-phosphate when certain metallic ions are present. The disappearance of the transaminase (apotransaminase activity) after boiling would appear to preclude nonen-
zymatic transamination as an explanation for the enhancement of transaminase activity by pyridoxal 5-phosphate.

Attempts to demonstrate serum apotransaminase have yielded conflicting results (9, 10). Studies in this laboratory revealed that some sera show an increased transaminase activity after incubation with pyridoxal 5-phosphate (11). In attempting to demonstrate this phenomenon the amount of apotransaminase in the malic dehydrogenase should be measured before the presence of circulating apoenzymes can be determined.

While this communication was being prepared for publication, a brief report appeared by Rosalki and Wilkinson (12), also showing enhancement of transaminase activity of malic dehydrogenase preparation by pyridoxal 5-phosphate.

SUMMARY

Studies of 4 commercially available malic dehydrogenase preparations have shown very small amounts of transaminase activity. Incubation of these preparations with pyridoxal 5-phosphate has revealed an enhanced transaminase activity. The data reported here suggest that the enhanced transaminase activity represents apotransaminase contaminating the malic dehydrogenase preparation.

REFERENCES