DPN-specific α-glycerophosphate dehydrogenase in insect flight muscle

Mitochondria (sarcosomes) of insect flight muscle have an α-glycerophosphate oxidase system with exceptionally high activity[1-7]. A very prominent role of this system in the intermediate metabolism is indicated[3,7]. Pyridine nucleotide does not participate in the reactions[4,7]. The present paper describes a distinct enzyme, found in the soluble fraction of the same muscle (sarcoplasm), which catalyses the oxidation of α-glycerophosphate by diphosphopyridine nucleotide (DPN).

House flies, *Musca domestica*, of both sexes from 3 to 7 days old were reared and maintained by the procedure described previously[8]. The soluble fraction of their flight muscle was obtained by grinding isolated thoraces in cold 1.5% KCl with a Potter-Elvehjem homogenizer for 1 min. The brei was filtered through a surgical gauze pad to remove pieces of cuticle and then was centrifuged at 0° C for 10 min at 10,000 *g*. The supernatant was refiltered to rid the preparation of lipid material floating on the surface, and then centrifuged again at 10,000 *g* for 10 min. The second supernatant was used as the sarcoplasm fraction. In some experiments soluble fractions of the entire fly were utilized. Except for this difference in starting material, these were prepared by the same procedure used for the sarcoplasm.

As shown in Fig. 1, the α-glycerophosphate dehydrogenase from the sarcoplasm reduced DPN. Other experiments demonstrated the remarkable activity of this enzyme from insects. Approximately 0.50 μmole DPNH/ml was formed/min/mg protein, equivalent to a Q_{O_2} of 336. Activity was dependent upon pH for at pH 8.6 the rate of DPN reduction was 20 times greater than at 7.4. Additional observations revealed that this dehydrogenase was especially concentrated in the flight muscle. A comparison of the rate of DPN reduction by soluble fractions of the thorax with that by analogous fractions from the whole fly showed a 2.5-fold increase in the specific activity of the thoracic preparations. This distribution is even more striking when one considers that the thorax has approximately one-third the weight of the intact fly. Triphosphopyridine nucleotide (TPN) did not serve as a cofactor with either the thoracic or whole fly preparations.

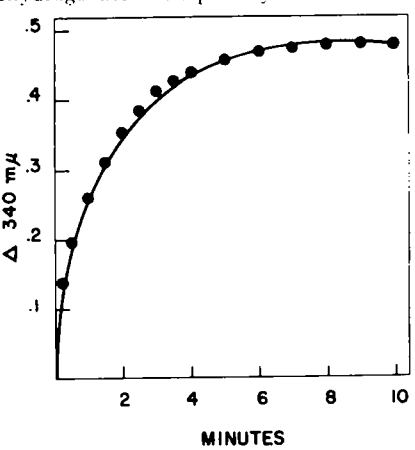

Fig. 1. DPN reduction coupled with the oxidation of α-glycerophosphate by the dehydrogenase found in the sarcoplasm of insect flight muscle. The reaction mixture contained: 0.2 ml DPN "65", 10 mg/ml; 2.6 ml tris(hydroxymethyl)aminomethane buffer, pH 8.6, 0.1 *M*; 0.1 ml enzyme preparation, 3.27 mg protein/ml; and 0.1 ml α-glycerophosphate, 0.5 *M*, added at zero time. Blank cuvette contained all constituents except that water replaced substrate. Room temperature.

The specificity of these preparations as observed now is in conflict with the report of CHEFURKA[9], who claimed that TPN, as well as DPN, was reduced by a similar preparation from whole flies. Perhaps this apparent discrepancy may be explained by the use in the present experiments of TPN at least 80% pure, whereas the coenzyme available to CHEFURKA was less than 8% pure, and by the fact that the optical density changes reported by CHEFURKA were exceedingly small despite the large quantity of enzyme preparation used.

The properties of the system described here; namely, its localization in the soluble fraction of the cell as well as its requirement for coenzyme, lends support for the probable identity of this enzyme with the α-glycerophosphate dehydrogenase so well known from studies with mammalian preparations.

BERTRAM SACKTOR
DONALD G. COCHRAN

Directorate of Medical Research, Army Chemical Center, Md. (U.S.A.)

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