Purification and activation properties of pyruvate carboxylase from the fungus Aspergillus nidulans

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Previous studies on a partially purified pyruvate carboxylase from the related fungus, Aspergillus niger, have shown that this enzyme is fully active in the absence of acetyl-CoA but is inhibited by L-aspartate (Bloom & Johnson, 1961; Feir & Suzuki, 1969). We have recently reported that activation of a partially purified preparation of A. nidulans pyruvate carboxylase by acetyl-CoA can be observed in the presence of L-aspartate or of o-oxoglutarate (Osmani et al., 1981).

Homogeneous preparations of A. nidulans pyruvate carboxylase have been obtained by subjecting the partially purified preparation described previously (Osmani et al., 1981) to chromatography on hydroxylapatite, followed by gel filtration on Sepharose 6B and then chromatography on DEAE-Sephadex A-50 (SO₄²⁻ form). The purified enzyme migrates as a single band when subjected to polyacrylamide-gel electrophoresis in the absence and presence of sodium dodecyl sulphate. Under the former conditions a single band of enzymic activity, determined as described by Scrutton & Fatabene (1975), is coincident with the protein band. The subunit molecular weight of A. nidulans pyruvate carboxylase obtained as described by Weber & Osborn (1969) is 125000, in agreement with that found for other pyruvate carboxylase that are subject to activation by acyl derivatives of coenzyme A and inhibition by dicarboxylic acids (Utter et al., 1975). An antibody raised to the purified enzyme in rabbits gives a single precipitin line in Ouchterlony diffusion analysis and causes complete inhibition of enzymic activity.

By using this homogeneous enzyme preparation we have confirmed that acetyl-CoA activation is not observed in the absence of the regulatory inhibitors and in the presence of saturating substrate concentrations. However 2–3-fold activation by acetyl-CoA is observed at pH 7.4 in the presence of non-saturating concentrations of MgATP₂⁻ and pyruvate. This activation results primarily from a decrease in the apparent Kᵦ for pyruvate and a decrease in the Hill coefficient (h) describing the relationship between initial rate and pyruvate concentration. With MgATP₂⁻ as varied substrate, addition of acetyl-CoA causes an increase in apparent Vₘₐₓ, without affecting the apparent Kᵦ or h for this substrate. The properties of interaction of A. nidulans pyruvate carboxylase with HCO₃⁻ are unaffected by addition of acetyl-CoA. Variation of MgATP₂⁻ or HCO₃⁻ concentration has no significant effect on the apparent Kᵦ or h for acetyl-CoA.

These studies demonstrate that pyruvate carboxylase from A. nidulans has both molecular and activation properties resembling those of other regulated pyruvate carboxylase. However, the activating effect of acetyl-CoA is weaker than that described for any other pyruvate carboxylase that shows this response.


Effect of diethyl pyrocarbonate on normal human gastric-mucosa and stomach-carcinoma acid proteinases

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Acid-proteinase activity from human stomach carcinoma has been found to be more susceptible to inactivation by diazoyacetil-DL-norleucine methyl ester, when compared with acid proteinase activity from normal gastric mucosa (Dionyssiou-Asteriou & Rakitzis, 1978). In the present communication the effect of diethyl pyrocarbonate (pyrurocarbonic acid diethyl ester) on acid-proteinase activity of the fractions obtained by DEAE-cellulose column chromatography of homogenates from human normal gastric mucosa and stomach carcinoma is reported.

Samples of gastric mucosa (10–20g) were obtained from resected stomachs of patients undergoing gastrectomy for treatment of duodenal ulcer. Tissue samples were homogenized within a few hours after gastrectomy or were kept at –20°C for periods not exceeding 2 months, before homogenization. Proteolytic activity at pH 3.8, with haemoglobin as the substrate, was determined by a slight modification of the assay for cathepsin D activity used previously (Rakitzis, 1974); instead of treating the trichloroacetic acid filtrates with Folin–Ciocalteu reagent, the A₁₆₀ of these filtrates was read with a Beckman DK 2A spectrophotometer. Tissue samples were homogenized in a Sorvall (Omni-Mixer 17220) homogenizer, for a period of 5 min, and in a ratio of 2.5 ml of water/g of tissue. The preparation was centrifuged at 16000g for 10 min. The supernatant was used for column chromatography. The preparation was made 10 mM with respect to Tris/HCl pH 8.2, and was placed on a DEAE-cellulose column (2.5 cm x 40 cm) that had been pre-equilibrated with the same (10 mM, Tris/HCl, pH 8.2) buffer. Elution was with a linear NaCl gradient, with 0.5 litre of 1 M NaCl, in the same buffer, in the adjoining supply chamber.

The effect of diethyl pyrocarbonate on acid-proteinase activity of the elution fractions was studied by adding 0.3 ml of a 1:17 (v/v) dilution of diethyl pyrocarbonate in ethanol to 8.0 ml of enzyme preparation made 0.1 M with respect to sodium phosphate buffer, pH 6.0. The concentration of diethyl pyrocarbonate in the enzyme acylating-agent mixture was 6.5 mM in each instance. The enzyme/acylating-agent mixture was incubated at 25°C for various periods of time, and portions were used for the determination of proteolytic activity. The log (percentage of residual proteolytic activity) was plotted against reaction time with diethyl pyrocarbonate. Biphasic log (fractional enzyme activity)-versus-(reaction time) curves were obtained in each instance. The assumption was made that the enzyme 1982