Inhibition of glutamic dehydrogenase of rat liver mitochondria by the carcinogen, N-2-fluorenyldiacetamide

In a previous communication[1] it was observed that in isolated rat liver mitochondria the fluorene carcinogens exerted an inhibitory effect on pyridine nucleotide-linked oxidations, especially on glutamate oxidation which was nearly completely inhibited by the carcinogen, N-2-fluorenyldiacetamide (2FdiAA). The purpose of the present note is to report that the mechanism of this inhibition appears to involve directly inhibition of the glutamic dehydrogenase of mitochondria by the carcinogen which acts as a powerful competitor of diphosphopyridine nucleotide (DPN^+) for the enzyme.

Extracts of mitochondria were prepared by suspending the particles[2,3] from 5 grams of liver in 20 ml of $0.14M$ KCl — $0.05M$ phosphate buffer, pH 7.6, freezing, thawing and homogenizing twice and finally centrifuging the suspension at $110{,}000 \times g$ for 30 minutes. The clear yellow supernatant extract contained glutamic dehydrogenase. Crystalline glutamic dehydrogenase (C. F. Boehringer, West Germany) centrifuged from 1 ml of suspension in ammonium sulfate solution was dissolved in 5 ml of $0.2M$ phosphate buffer, pH 7.6, and thoroughly dialyzed against the buffer. Glutamic dehydrogenase activity was determined from the rate of reduction of DPN^+. The test system contained 0.2 ml of $0.4M$ phosphate buffer, pH 7.6, an amount of $0.015M$ DPN^+ as indicated in the figures, 0.05 ml of enzyme diluted when necessary in KCl-phosphate buffer, 0.1 ml of $0.3M$ K glutamate, pH 7.6, 2.0 ml of $7.8 \cdot 10^{-5}M$ N-2-fluorenyldiacetamide when added and water to make a final volume of 3 ml. Glutamate was always added last to start the reaction. The change in optical density at 340 mμ for 2 minutes was taken as the activity reading. The initial reading for zero time was taken 30 seconds after the reaction was started.

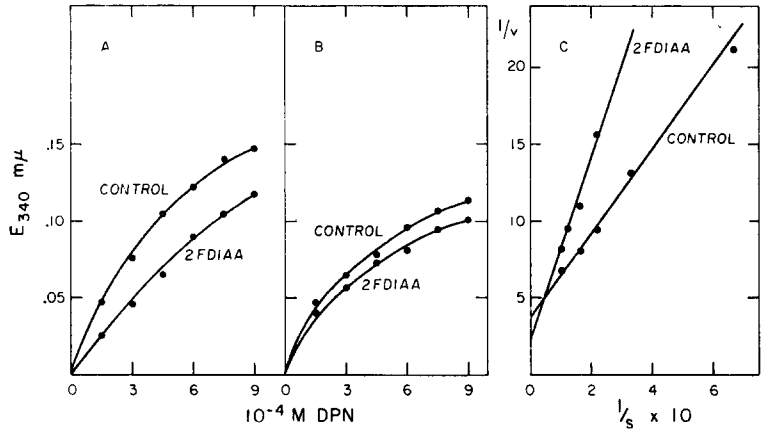

Fig. 1. Relation between glutamic dehydrogenase activity and DPN^+ concentration in the presence and absence of the carcinogen, 2FdiAA. (A) Mitochondrial extract, (B) crystalline glutamic dehydrogenase and (C) Lineweaver-Burk plot of data of Fig. 1A.

In Fig. 1A the relation between glutamic dehydrogenase activity of mitochondrial extract and DPN^+ concentration is shown; the behaviour of crystalline glutamic dehydrogenase under similar conditions is shown in Fig. 1B. It is evident that the carcinogen inhibited the mitochondrial enzyme to a significantly greater extent than the crystalline enzyme, particularly at low DPN^+ concentrations that are likely to be in the physiological range. An estimate of the carcinogen/DPN^+ ratio at 50% inhibition of the mitochondrial glutamic dehydrogenase activity is about 1/3, which indicates that the carcinogen has a considerably greater affinity for the enzyme than does the natural cofactor. A Lineweaver-Burk plot of the data of Fig. 1A for the mitochondrial enzyme is shown in Fig. 1C. The results indicate that a competitive type of inhibition is essentially involved. The slight inhibitory effect of the carcinogen on the crystalline enzyme does not appear to be competitive.

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