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Inhibition of Aldolase by Palladium

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Palladium is one of the metals collectively known as the platinum group of the noble metals class. Previously Pd was used in limited quantities, principally for the manufacture of electrical contracts, dental alloys, chemical catalyst and jewelry (BROWNING 1969). However, automotive manufacturers have recently used Pd in connection with platinum in automotive catalytic convertors. These devices are designed to reduce the concentrations of carbon monoxide and hydrocarbons in the exhaust stream by oxidizing them into carbon dioxide and water. It can be anticipated that noble metals associated with the use of catalytic convertors will not only be emitted into the air, but will also deposit on the ground. Accumulation of Pd and Pt in streams, rivers, lakes, and their sediments can result from discarded catalytic materials as well as from ground water contamination by exhaust attrition products. Further, microbial biotransformation in streams and lake sediment may serve to modify toxicity and promote food chain contamination. For example, methylmercury identified in Minamata disease is a typical case of this kind of contamination (NELSON 1971, WOOD 1974).

Currently, toxicological information concerning Pd is meager. However, because of the fact that this agent is expected to reach detectable levels in the ambient air when all the light-duty motor vehicles are expected to install the catalytic convertors after 1982, it is of importance therefore, to assess the potential health adversities associated with atomspheric contamination of various chemical forms of Pd.

It has been reported (WATERS et al. 1975) that human lung fibroblasts and rabbit alveolar macrophages were susceptible to the toxicity of soluble Pt. The toxicity was associated with a marked reduction of total ATP production. This report prompted us to investigate the possible effect of Pd on one of the metabolically important enzymes, aldolase (EC 4.1.2.13), which plays a critical role in the utilization of glucose as the principal energy source.

MATERIALS AND METHODS

Chemicals: Palladium chloride, fructose 1,6-diphosphate, 2,4-dinitrophenylhydrazine were obtained from Sigma Chemical Co. Chlorpromazine chloride was a product of Smith Kline and French Laboratory. Purified rabbit muscle aldolase (Sp.Act. 9.0 U/mg) was purchased from Boeringer-Mannheim Corp.

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Enzyme Assay: Aldolase activity was assayed by the colorimetric procedure based on the determination of dihydroxyacetone phosphate with 2,4-dinitrophenylhydrazine (PINTO et al. 1969).

Pd Binding Studies: Demonstration of the interaction between the accessible enzymatic sulfhydryl groups and Pd, using chlorpromazine-chromophore system was carried out as previously described (LIU et al. 1979).

RESULTS

When purified rabbit muscle aldolase was treated with various concentrations of Pd ions prior to the assay, a strong concentration-dependent inhibition of enzyme activity was observed, with a nearly maximal inhibition at a concentration of 20.0 μ moles of Pd (Fig. 1). As also seen from Fig. 1, the Dixon plot yields an inhibition constant, Ki, for aldolase of 4.0 μ mole, corresponding to a Pd concentration in the order of 0.5 ppm.

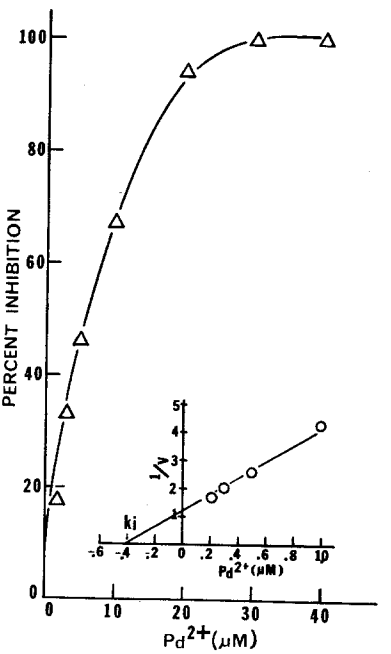

Figure 1. Concentration-dependent inhibition of rabbit muscle aldolase activity by Pd. Insert indicates a Dixon plot for estimation of Ki. The Ki for Pd-induced inhibition of aldolase activity was estimated to be 4.0 μ mole.

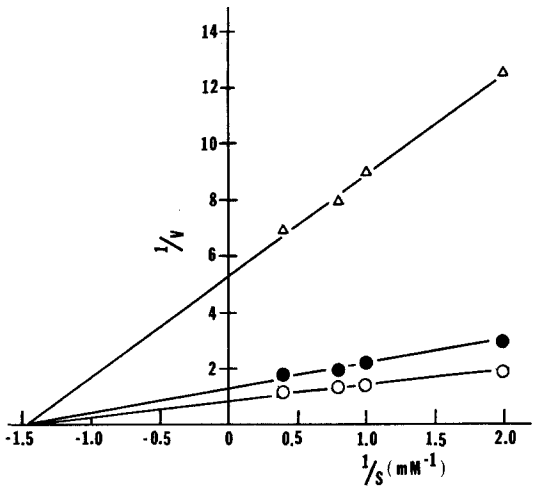

Figure 2. Non-competitive inhibition of rabbit muscle aldolase by Pd. O-O: no Pd; ●-●: + 4.0 μ mole Pd, and Δ-Δ: + 8.0 μ mole Pd.

To investigate the nature of Pd inhibition of rabbit muscle aldolase activity, a double reciprocal plot was constructed at varying concentrations of substrate with or without the treatment of Pd. As indicated in Fig. 2, the inhibition of rabbit muscle aldolase was clearly non-competitive with respect to substrate binding. This indicates that the interaction of Pd with the enzyme occurred at a site different from the one involved in substrate binding.

Pd form a complex with chlorpromazine (LEE et al. 1975) and this complex has a maximum absorption at 565 nm. This complex has also been shown to dissociate in the presence of reagents such as glutathione and cysteine. In the presence of these sulfhydryl-containing agents, Pd in the Pd-chlorpromazine complex can be extracted, resulting in a decrease in absorbance at 565 nm. The decrease is directly proportional to the concentration of added sulfhydryl groups (LAI et al. 1971). Therefore, it is of interest to investigate the possible interaction between aldolase and Pd by using the principle involving the displacement of Pd from Pd-chlorpromazine complex by accessible sulfhydryl groups. The actual binding of Pd to the enzyme can be monitored by the measurement of the decrease of absorbance at 565 nm. It is evident from Fig. 3 that there was indeed an interaction between enzymatic sulfhydryl groups and Pd. At fixed enzyme concentration, the degree of interaction appears to be dependent upon the concentration of Pd.

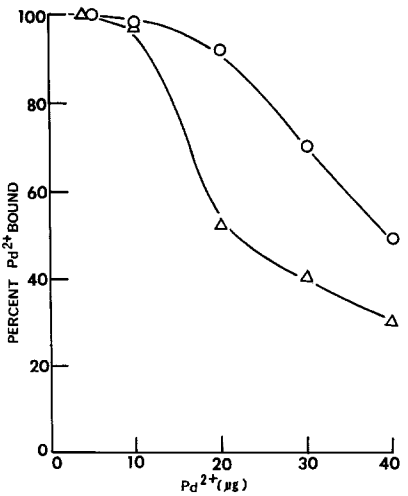

Figure 3. Binding of Pd to rabbit muscle aldolase. Δ-Δ: 200 μg enzyme per sample, and **O-O**: 500 μg enzyme per sample.

DISCUSSION

The binding study using chlorpromazine-chromophere system clearly demonstrated the interaction between the accessible enzymatic sulfhydryl groups and Pd. Although sulfhydryl groups of rabbit muscle aldolase are not directly involved in the substrate binding, two of the four "exposed" sulfhydryl groups in rabbit muscle aldolase subunits have been shown to be essential for maintaining the proper conformation for the enzyme activity (LAI et al. 1971). It can be speculated that Pd-induced inhibition of rabbit muscle aldolase activity may be a consequence of altering the enzymatic conformation resulted from the binding of Pd to these essential sulfhydryl groups. However, our data cannot preclude the possibility of interaction between Pd and other negatively-charged functional groups of the enzyme, such as -COOH and -OH groups.

Aldolase is a key enzyme in glycolysis, catalyzing the breakdown of fructose 1,6-diphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. The Ki for the inhibition of purified rabbit muscle aldolase was 4.0 μ mole, about 0.5 ppm Pd. The most obvious consequence of inhibiting tissue aldolase would be the disturbance in the utilization of glucose as the principal energy source.

With increasing usage of Pd compounds in catalytic convertor devices, the baseline level of Pd in the blood of residents living near a freeway and other polluted environments, by a cumulative process,

may reach critical levels. Additional investigations will be required to determine more precisely the nature of inhibitory effects of Pd ions on aldolase and other enzymes and to assess the possible consequence of acute toxic exposure as well as long-term low-dosage effects, on animals and man.

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