ZUSAMMENFASSUNG

Das Phosphagen der Protozoen ist weder mit Creatinphosphat, noch mit Argininphosphat, noch mit dem Phosphagen der Ringwürmer identisch.

Eine Untersuchung der Molybdat-Hemmung der Hydrolyse dieses neuen Phosphagens weist darauf hin, dass dieses wahrscheinlich dem Argininphosphat chemisch ähnlicher ist, als allen anderen bisher beschriebenen Phosphagenen.

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Short Communications and Preliminary Notes

CUPRIC ION INHIBITION OF ASCORBIC ACID OXIDASE

by

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In a study of the mechanism of activation of ascorbic acid oxidase (AAO) by thyroxine and related substances[1,2], it was observed that low concentrations of cyanide ion could also activate the AAO system. Higher concentrations of cyanide ion inhibited the enzyme as previously reported[3,4]. The relationship between the logarithm of the cyanide concentration and ascorbic acid oxidase activity is shown in Fig. 1. Maximum activation of the AAO** employed in these experiments occurs at $2\text{–}3 \cdot 10^{-5}$ *M* cyanide with virtually complete inhibition at $1 \cdot 10^{-3}$ *M* cyanide. The fact that cyanide concentrations below 10^{-4} *M* are required to show activation may explain why the activating effect of cyanide has not been noted earlier. The presence of extraneous protein which might activate AAO could also mask cyanide activation.

The activation by cyanide suggested the possibility of metal ion inactivation of AAO. That the enzyme is extremely sensitive to cupric ion is indicated in Fig. 2. Complete inactivation of approximately $2 \cdot 10^{-8}$ *M* AAO occurs with about $5 \cdot 10^{-7}$ *M* cupric ion. The residual activity is ap-

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** Ascorbic acid oxidase, Lot No. E521C, purchased from the Reheis Co., Berkeley Heights, New Jersey, had 0.24% Cu, 1000 Lovett-Janison units/mg; this preparation is estimated to be approximately 50% AAO based on the homogeneous preparation of DUNN AND DAWSON[6].

parently due to the well-known cupric ion catalysis of ascorbic acid oxidation also shown in Fig. 2. This cupric ion catalysis is even greater when no enzyme protein is present but not large enough to interfere with the interpretation of these data. The amount of copper required for inhibition is very sensitive to the amount of enzyme protein present. At constant cupric ion concentrations, a plot of the rate of ascorbic acid oxidation against the enzyme concentration gave the familiar non-competitive antagonist "titration" curves of ACKERMANN AND POTTER[5].

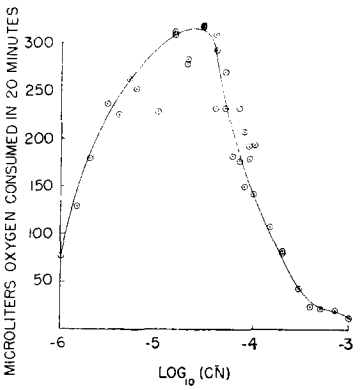

Fig. 1. The effect of cyanide on the activity of ascorbic acid oxidase. Conventional Warburg technic was used at 30.0° C with air as the gas phase. Initial concentrations in 3.00 ml reaction volume were AAO**, 2.0 γ/ml; sodium ascorbate, 0.067 M; pH 7.2; phosphate buffer, 0.100 M.

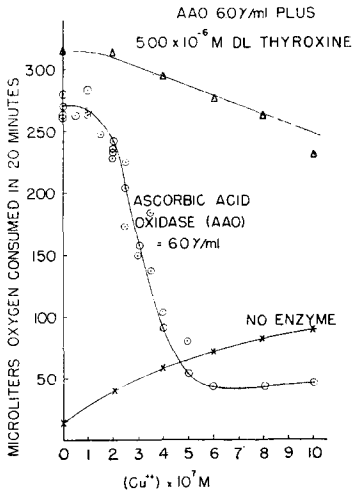

Fig. 2. The effect of cupric ion on the non-enzymatic, AAO catalyzed, and AAO with $5 \cdot 10^{-6}$ M thyroxine catalyzed oxidation of ascorbic acid. Conventional Warburg technic was used at 30.0° C with air as the gas phase. Initial concentrations in 3.00 ml reaction volume were AAO**, 6.0 γ/ml (except in no enzyme experiments); sodium ascorbate, 0.067 M; pH 7.2; phosphate buffer, 0.0100 M.

As indicated in Fig. 2, DL-thyroxine at $5.0 \cdot 10^{-6}$ M reverses the effect of as much as $8.0 \cdot 10^{-7}$ M cupric ion presumably by complexing the copper. Previously GEMMILL[1] has shown that thyroxine interacts with cupric ion and prevents the copper-catalyzed oxidation of ascorbic acid. It is also possible that the ability of thyroxine to counteract cupric ion inhibition of AAO is related to the ability of thyroxine and other substances to activate the enzyme.

Preliminary results indicate that Hg^{++}, Zn^{++}, and Ni^{++} also inhibit AAO activity though at considerably higher concentrations. Many other metal ions, *e.g.*, Mn^{++}, Co^{++}, are inert at 10^{-3}–10^{-4} M.

Another complete copper enzyme, the tyrosine-tyrosinase system, was not inhibited by cupric ion at 10^{-3} M using the test conditions of LERNER[7].

The significance of the fact that AAO, a copper containing enzyme[8], is particularily sensitive to cupric ion inhibition will be discussed in a more detailed report to appear later.

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