ENZYME INHIBITION BY PALLADIUM CHLORIDE*

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In the course of examining palladium porphyrins (1) as possible sensitizers for the photodynamic inactivation of enzymes (2), we observed that palladium (+2) inactivated trypsin directly by a non-photochemical process. Although palladium (+2) has been shown to bind to proteins such as carboxypeptidase (3), casein (4), papain (5) and silk fibroin (6), and to interfere with plant growth (7), we did not find any reports in the literature on its action as an enzyme inhibitor. For this reason we carried out a preliminary examination of the effects of palladium (+2) on several enzymes.

One ml quantities of 80 u$\underline{M}$ enzyme were incubated in a 15^{o}C. water bath with 0.5 ml of sodium phosphate buffer of pH 8.9 or 4.2. The enzymic activity was assayed at zero time; 0.5 ml of 2 x 10^{-3} $\underline{M}$ palladous chloride was then added and samples of the mixture were removed for assay at 5 min intervals for 20 min. Enzyme assays were carried out spectrophotometrically as described in the references listed under (8). The substrates used were: hydrogen peroxide for catalase; acetyl-L-tyrosine ethyl ester (ATEE) for alpha-chymotrypsin; lyophilized $\underline{Micrococcus\ lysodeikticus}$ for lysozyme; hydrogen peroxide coupled with $\underline{o}$-dianisidine for peroxidase; cytidine-2', 3'-cyclic phosphate for ribonuclease; and benzoyl-L-arginine ethyl ester (BAEE) for trypsin.

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Of the enzymes listed above, only chymotrypsin and trypsin were inactivated by palladium (+2). The inactivation was time- and pH-dependent as shown in Fig. 1. As may be seen, trypsin was very rapidly inactivated at pH 4.2, but was not inactivated at pH 8.9. Alpha-chymotrypsin was also inactivated very rapidly at pH 4.2, but, in contrast to trypsin, was inactivated fairly rapidly at pH 8.9.

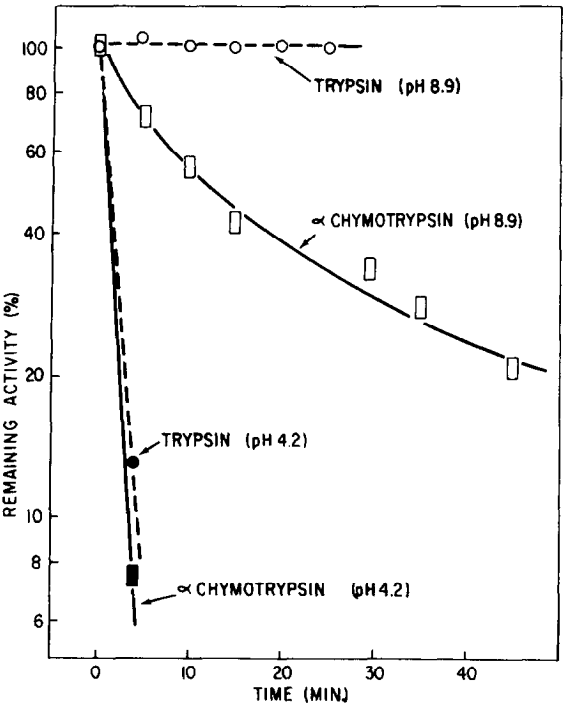

Figure 1. Inactivation of trypsin and alpha-chymotrypsin by palladous chloride at pH 4.2 and 8.9. The reaction system was 40 uM in enzyme, 5 x 10^{-4} M in palladous chloride and 0.125 M in sodium phosphate buffer. There was no loss of enzyme activity under the conditions described in the absence of the palladous chloride.

The mechanism of the inactivation of chymotrypsin and trypsin by palladium (+2) is not known. Other metals (copper, zinc, mercury) in the divalent form also inhibit these enzymes (9); mercury presumably inhibits trypsin by reacting with sulfhydryl groups. It has been suggested that mercury may inactivate chymotrypsin (which has no free sulfhydryl groups) by forming a stable chelate with the active-site histidine (10). Palladium

(+2) binds to papain with the same stoichiometry as does mercury (+2), which suggests that, for this enzyme, its binding is due to interaction with sulfhydryl groups (5). Palladium (+2) forms complexes with L-cysteine, L-cystine and L-methionine in solution, but not with L-histidine (11). Thus one could envision that palladium (+2) inactivates trypsin by combining with free sulfhydryl groups and/or with cystine groupings, while the inactivation of chymotrypsin might result from reactions with cystine groupings.

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