AD NUMBER

AD855594

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; JUN 1969. Other requests shall be referred to US Army Biological Labs., Fort Detrick, Frederick, MD.

AUTHORITY

SMUFD/Fort Detrick ltr dtd 17 Feb 1972

Translation No. T-726-1

Authors: Otto Warburg and Walter Christian, Berlin-Dahlem, Germany

Title: Concerning a new oxidative enzyme (Über das neue Oxydationsferment).

Journal: Die Naturwissenschaften 20: 980-981 (1932).

June 1969

If one precipitates the cell juices of bottom yeast with lead acetate and then removes the excess lead from the filtrate with phosphate, then one obtains a solution of a new oxidative enzyme, which is still contaminated with a hemochromogen - the cytochrome C of Keilins.

However, it is possible to separate the hemochromogen from this enzyme. If one adds to the solution containing both of the pigments, a half volume of acetone and then allows the mixture to stand for 24 hours at 0°, the hemochromogen is precipitated out while the enzyme remains in solution. The enzyme can be further purified by additional acetone precipitations under the same conditions. If one saturates the solution with carbonic acid, then the enzyme will separate out as a yellow-red oil. When an aqueous solution of this oil is treated with methanol at 0°, the enzyme is precipitated out as a bright yellow powder which is contaminated with colorless proteins.

Since an aqueous solution of the yellow powder contains no pigments other than that of the enzyme, one can now determine the absorption spectrum of the enzyme directly. The spectrum obtained with the photoelectric cell and shown in Fig. 1 is in agreement with the "difference" spectrum that was determined from the difference in light absorption between solutions of oxidized and reduced enzyme.

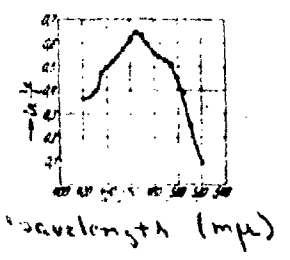

Fig. 1. Directly determined absorption spectrum of the enzyme.

The absorption spectrum of the enzyme has three bands. The long-wave bands at 465 and 495 mμ can be seen in a hand spectroscope using the light of a metallic filament filtered through copper ammonium oxide. Fig. 2 is a photograph of these bands.

Fig. 2. Bands of the enzyme in the blue-green (photograph can be seen in original paper).

In the enzyme, the pigment is bound to a protein. One can separate the pigment component from the protein component by shaking the enzyme at 38° with methanol-water (1 vol. water, 3 vol. methanol). In this manner, the protein is denatured and the pigments remains in solution. The protein-free solution of the pigment fluoresces green. It is catalytically inactive but has an absorption spectrum in the visible range that is very similar to that of the enzyme solution. The absorption spectrum of the protein-free pigment is shifted about 15 - 20 mμ from the spectrum of the enzyme.

If the protein-free pigment is dissolved in dilute sodium hydroxide, one can observe with a metallic filament lamp that it is extracted with chloroform when the solution is acidified but is not extracted from solution that are not acidified. Because of the noteworthy characteristic, one is able to separate the pigment from other materials and obtain it in pure form. It crystallizes from water as macrscopic needles which are shown in Fig. 3. It constitutes the chemically active component of an enzyme which has been isolated and purified.

Fig. 3. Crystals of the pigment component of the enzyme.