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THE NATURE AND FUNCTION OF THE PLANT OXIDASES*

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One of the most noteworthy characteristics of living organisms is their ability to carry out many deep-seated chemical changes without the ordinary means of producing such reactions. In other words, the living cell is a laboratory equipped to provide the most varied chemical transformations, yet with none of the relatively crude and violent agents such as high temperatures and strong chemicals which we are forced to use in the test-tube experiments of our man-made laboratories. In no case is this power of the cell more striking than in the oxidative phenomena of plants and animals; the latter especially are continually oxidizing and transforming large amounts of material for the maintenance of their life, and yet these oxidations are accompanied by few of the physical effects associated with oxidation and combustion in daily life or in the laboratory. It is not surprising, then, that the attention of biologists and chemists was early attracted to the investigation of biological oxidations. Beginning with Schoenbein in the fourth decade of the last century, and continuing to the present, numerous have been the theories advanced in regard to these phenomena. However, before proceeding with a discussion of the factors involved in the oxidations of the plant, it is desirable to indicate the means which the cell

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has at its disposal for carrying out its chemical reactions with such wonderful efficiency.

The fermenting action of certain bacteria and yeasts upon sugars and other substances has long been known and used in the industries. These yeasts were called *organized* ferments, while chemical preparations like pepsin, etc., which exhibit a fermenting or digesting action, were called *unorganized* ferments. This distinction was retained until 1897 when Buchner performed his classical experiment on yeast, showing that by the action of pressure applied in a hydraulic press he was able to obtain a liquid possessing all the fermenting power of living yeast plants even in the absence of the living organisms. This substance or property of the expressed liquid Buchner called an "enzyme." He said that substances of like nature were products of the life-activities of cells, but were *not* dependent on the *living* cell for the exhibition of their characteristic fermenting action. It is to ferments or enzymes like this that the cell owes its great chemical efficiency. Enzymes are members of the class of substances known as "catalyzers" which, by processes that are not fully understood, cause reactions to take place with a speed not shown under ordinary conditions. Generally, catalysts are capable of causing or assisting in reactions without being themselves destroyed by the processes they propagate.

In discussing the oxidases or oxidizing enzymes a somewhat critical attitude is necessary in the face of many conflicting and even contradictory results. To take an example, several of the so-called oxidizing enzymes have been shown to be not enzymes but heat-withstanding inorganic or organic catalyzers. At the present time our knowledge of these substances is being increased almost daily, with the result that we are now in a sort of transitional period, the literature of the whole subject being filled with assertions and denials on the part of equally able investigators. The tendency at present seems to be to consider as enzymes those apparently complex organic substances of non-diffusable nature and of high catalytic power, which are produced during the life processes of plants and animals; but when investigation reveals *definitely* their exact chemical nature, such

as the "laccase" of alfalfa, which Euler and Bolin[1] have recently proved to be calcium salts of simple organic acids, then they are referred to as organic catalysts. Bearing this in mind, the writer will use the terms oxidizing enzyme and oxidase interchangeably for convenience and with no implication that they are enzymes according to the strictest definition, or that future investigation may not prove the action of all the classes of oxidizing enzymes to be due to the same substance or property.

In regard to the rôle and the nature of many of the oxidases, we are still ignorant in spite of the study that has been devoted to them. In the case of enzymes like pepsin, trypsin, and lipase, investigation has produced considerable advances in our knowledge of them, but this cannot be said of the oxidases. In fact, there are doubts in some cases whether certain of the oxidases are enzymes at all, because a number of them have been proved to be comparatively simple organic or inorganic substances. However, such oxidases as peroxidase and tyrosinase still hold their places in the category of enzymes. In classifying the oxidases several arrangements have been suggested, many of which led only to confusion. After 1903, a more accurate classification was proposed, for it was then that Bach and Chodat[2] showed that the so-called oxidases of Bertrand are really composed of three separate parts as indicated below:

1. Oxygenase; a preformed organic peroxide resulting from auto-oxidation.

2. Peroxidase; a true enzyme which activates the oxygenase or added H_2O_2, etc.'

3. Catalase; a substance decomposing H_2O_2 into $H_2O + O_2$. Since 1903, a great deal of work has been done which shows that this conception of the so-called oxidases is founded on fact.

[1] Euler and Bolin. Zur Kenntniss biologische wichtiger Oxydationen:

(*a*) I. (Same title as the series, Zur Kenntniss, etc.), Zts. Physiol. Chem. **57**: 80. 1908.

(*b*) II. Ueber die Reindarstellung der *Medicago* laccase, Zts. Physiol. Chem. **1**: 1. 1909.

[2] Bach and Chodat. Zerlegung der sogenannte Oxydasen in Oxygenasen und Peroxydasen—V. Ber. Chem. Gesell. **36**: 606. 1903.

In the last edition of Oppenheimer's "Die Fermente"[3] he has adopted the following classification of the plant oxidases, which will be used in this paper:

1. Laccase; phenolase, etc.
2. Tyrosinase, melanin-forming enzymes.
3. "Oxidases."
   (*a*) Oxygenase.
   (*b*) Peroxidase.
4. Catalase.

Laccase

Schoenbein's interest in the problems of oxidation led him to investigate the cause of the coloration of certain mushrooms, and in 1856[4] he published his results. In *Boletus luridus* he found a substance soluble in alcohol that showed the same bluing from injury of the fungus or on treatment with oxidizing agents in the test-tube, that characterizes the bluing of the guaiac tincture; moreover, the same substances decolorize this blued extract as in the case of the blued guaiac tincture. Schoenbein saw the importance of the fact that spontaneous bluing *only* took place in the fungus *itself*, and concluded therefore that there was a substance present in the fungus with power to greatly increase the oxidizing power of the atmospheric oxygen. In *Agaricus sanguinareus* he was also able to find the same sort of spontaneously coloring substance that he noted in *Boletus luridus*. He concluded that, besides the chromogenic substance of these fungi, there is a substance present that can ozonize (activate) atmospheric oxygen; he called such an activating substance a "Sauerstofferreger," or literally an "oxygen-exciter."

The first really careful work on oxidizing ferments was done by Yoshida[5] who, in 1883, investigated the chemistry of lacquer.

[3] Oppenheimer. Die Fermente und ihre Wirkungen, "Die Oxydasen," chap. 7, pp. 337–391, Spezielle Teil, 3d ed. 1909. Also for an excellent treatment of oxidases in general see:

Kastle. The Oxidases. Bull. 59, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Serv. Washington, 1910.

[4] Schoenbein. Ueber die Selbstblauung einige Pilze, etc. Jour. Prakt. Chem. **67**: 496. 1856.

[5] Yoshida. Chemistry of Lacquer. Jour. Chem. Soc. **43**: 472. 1883.

The lacquer-work of the Japanese has long been a famous and beautiful product of that country. The milky latex of the tree *Rhus vernicifera*, rapidly oxidizes in a moist atmosphere to a black lustrous varnish which is not attacked by any chemical except concentrated nitric acid. In the latex Yoshida found a substance having the composition $C_{14}H_{18}O_2$ which he called urushic acid; besides this, he found a small amount of a nitrogenous constituent, "a peculiar diastatic matter," which rapidly caused the urushic acid to oxidize to the black oxyurushic acid ($C_{14}H_{18}O_3$). This peculiar diastatic matter of Yoshida lost its power to oxidize urushic acid after being heated to 63°; so Yoshida thought it a substance of enzymatic nature, which acted as an oxygen carrier in these oxidations.

Some years later, Bertrand[6] studied the lacquer formation more carefully. He called the substance an oxidizing ferment, which he believed brought about the oxidation of the mother-substance of the black lacquer. He found that the ferment was destroyed by boiling, and also that it was present in gum arabic and gum senegal, as well as in the latex of species of *Rhus*. He named this ferment "laccase" and tested numerous plants for it, finding it present in many cases. Bertrand used the tincture of guaiacum as a test for laccase.

In 1895, Bertrand with Bourquelot[7] tested a great many of the higher fungi for laccase, using guaiacum as a reagent. They found that laccase was widely distributed in these plants as well as in those containing chlorophyll. They also investigated those fungi which become colored when injured, and they believed the phenomenon was caused by a ferment identical with laccase. Bertrand[8] has shown that the oxidizing power of laccase is in some way connected with the manganese present; for, by repeated precipitation with alcohol, he divided his laccase preparation into three

[6] Bertrand. (*a*) Sur la latex de l'arbre à laque. Compt. Rend. Acad. Sci. **118**: 1215. 1894. (*b*) Recherches sur le suc laiteux de l'arbre à laque du Tonkin. Bull. Soc. Chim. [3], **11**: 717. 1894.

[7] Bertrand and Bourquelot. Laccase dans les champignons. Compt. Rend. Soc. Biol. **47**: 579. 1895.

[8] Bertrand. Sur l'action oxydante des sels manganeux et sur la constitution chimique des oxydases. Compt. Rend. Acad. Sci. **124**: 1355. 1897.

fractions of different manganese contents, which with hydroquinone solutions showed activities proportional to their percentages of manganese. Bearing this in mind, other investigators have used mixtures of protein substances and manganese salts to prepare artificial oxidases giving many of the reactions of the natural preparations. It should be noted, however, that Bach and other investigators have prepared oxidases from various plants which, although active, did not contain manganese or iron.

During the last year, Euler and Bolin[9] have shown that the laccase prepared from alfalfa (*Medicago sativa*) is not an enzyme according to the commonly accepted usage of the word. They found that heating did not destroy the activity of the oxidase, and that the protein thus precipitated could be filtered off without lowering the activity in the least. This so-called laccase proved to be mostly calcium glycollate, with traces of the calcium salts of citric, malic, and mesoxalic acids.

If, as Bach and Chodat say, laccase consists of organic peroxides activated by the enzyme peroxidase, then it is the peroxidase part which confers upon laccase what specificity it has. However, laccase is not a specific enzyme in the narrow sense because, besides the laccol of *Rhus spp.*, it will oxidize guaiacol, hydroquinone, guaiac tincture, phenolphthalin, and many phenols and cyclic amino derivatives; still, it is not able to oxidize tyrosin or any of the tyrosin derivatives upon which tyrosinase exerts a truly specific action. So then, laccase is a specific enzyme, in that it acts only upon substances containing a certain grouping in their structure. The fact that laccase acts upon guaiac tincture and upon many other reagents usually employed to detect peroxidases, etc., makes one skeptical in regard to the nearly universal occurrence of laccase claimed for it by the earlier investigators.

Tyrosinase

After Bertrand and Bourquelot had shown that the bluing of *Boletus cyanescens* upon injury was due to the effect of laccase acting with the atmospheric oxygen upon the "boletol" in the

[9] *Loc. cit.*

fungus, they turned their attention to the case of *Russula spp.*, especially *R. nigricans*, the color change of which upon injury is from pink or reddish to black. In different researches they showed that laccase could not produce the same effect, and further, that it was an oxidation of a definite chemical substance in the fungus. Bertrand[10] next showed that the crystalline chromogen in *Russula spp.* was tyrosin and that it was also present in beets, potatoes, etc.; accordingly he named the enzyme which caused this change "tyrosinase," and said that laccase and tyrosinase were two representatives of the group of "oxidases." About this time it was found that rosettes of tyrosin crystals were present in the tissues of the fungus *Russula nigricans.*

At first it was thought that tyrosinase was as wide-spread an enzyme as laccase, but later results show this to be unlikely. Lehman and Sano[11] examined bacteria and higher plants for tyrosinase. A few species of bacteria showed the presence of tyrosinase, but in no case could it be separated from the living bacterial cells. Among the higher plants tyrosinase is present in wheat, barley, potatoes, *Papaver orientale*, *Rhus spp.*, etc. Thus we see, this enzyme is probably concerned in the formation of the black wound-covering over injured areas on potatoes.

The action of tyrosinase results in a yellowish pink coloration, then reddish, then brown, and finally black. This reddish black oxidation or condensation product is called melanin and is closely related to the natural animal pigments in dark hair, etc., and also in the so-called melanotic tumors. This action of tyrosinase and the resulting melanin have attracted a great deal of attention. The first investigators said that the action of the tyrosinase was simply the oxidation of tyrosin to melanin, and that the production of a black coloration in a plant was due to the action of its tyrosinase on tyrosin. However, it soon became clear that the matter was not so simple as at first thought. Certain experiments seem to show that the early change of tyrosin to a pink color

[10] Bertrand. Sur une nouvelle oxydase ou ferment soluble oxydant d'origine végétale. Compt. Rend. Acad. Sci. **122**: 1215. 1896. Also Bull. Soc. Chim. [3], **15**: 793. 1896.

[11] Lehman and Sano. Ueber das Vorkommen von Oxydations-fermenten bei Bakterien und höheren Pflanzen. Arch. f. Hyg. **67**: 99. 1908.

may be caused by an another enzyme and then it is upon this intermediate product that tyrosinase acts, finally giving the black melanin. The earlier workers considered that tyrosinase was a specific enzyme acting only on tyrosin, but in the course of time it has become evident that tyrosinase is specific in the same sense as laccase; namely, it acts upon a group of compounds closely related in structure.

Just as it is possible to obtain anti-toxins, research has shown that we may obtain anti-enzymes. In this place we are concerned only with the anti-oxidases, which have been produced in the usual manner, that is, by the repeated injection of small though increasing amounts of the enzyme preparation into a rabbit or other animal, and the withdrawal of some of the blood after immunity has been established to that particular enzyme. The blood serum from such immune animals prevents or retards the natural oxidizing action of the enzyme under investigation. Gessard[12] obtained anti-tyrosinase and anti-laccase that completely inhibited the oxidizing power of the corresponding plant enzyme preparations. We shall see later that anti-oxidases may play an important part in the physiology of the plant.

Generally speaking, tyrosinase seems to be the nearest to the true enzyme of any of the oxidases with which we are acquainted. It is most specific in its action, most sensitive to exterior conditions, and up to the present, has not been replaced by any artificial enzyme in the oxidation of tyrosin to a melanin. It is usually associated with laccase in plants, but the presence of laccase does not indicate the appearance of tyrosinase, while on the other hand, the latter is almost invariably accompanied by laccase.

As in the case of laccase, Bach[13] claims that the tyrosinase is really composed of two parts, oxygenase and the peroxidase. He found that by the use of alcohol precipitations he was able to reduce the activity of the tyrosinase of the potato, as previ-

[12] Gessard. (*a*) Anti-laccase. Compt. Rend. Soc. Biol. **139**: 644. 1904. (*b*) Sur la tyrosinase. Ann. Inst. Pasteur **15**: 593. 1901.

[13] Bach. Ueber die Wirkungsweise der Tyrosinase. Ber. Chem. Gesell. **41**: 221. 1908.

ously noted by Bertrand; but curiously enough, the addition of hydrogen peroxide to the enzyme solution restored it to its usual activity. This and many similar experiments led Bach to believe that tyrosinase contains the oxygenase and peroxidase complements.[14] Our final conclusion must be then, that tyrosinase may have the usual oxidase complements (oxygenase plus peroxidase) and that its peroxidase may be specific just as the peroxidase of laccase is specific in its action upon substances having a certain constitution.

(To be continued)

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REDISCOVERY OF TILLANDSIA SWARTZII BAKER

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In "Journal of Botany," **26**: 12, published in 1888, and in "Handbook of Bromeliaceae," 191, 1889, Mr. J. G. Baker described this species, based on a specimen collected many years ago by Swartz in the island of Jamaica and supposed by him to be *Tillandsia paniculata* L. Professor Carl Mez, in his Monograph of the family Bromeliaceae (DC. Mon. Phan. **9**: 884), published in 1896, states that he has seen this specimen, but regards it as doubtful, perhaps referable to the Liliaceae.

The type specimen is preserved in the herbarium of the British Museum of Natural History, and while there in the spring of 1910, I examined it and was inclined to agree with Professor Mez. But, on returning to New York immediately afterward, I found in a parcel of choice Jamaica plants collected early the same year by Mr. William Harris, fine specimens, which I recognized as of the same species, and on sending one of these to Mr. Edmund Baker at the British Museum, he confirmed my identification by a comparison with the type. Mr. Harris found the plant growing on rocks in the Rio Minho Valley, March 3, 1910 (*No. 10,885*), more than one hundred years after its collection in

[14] Recently he found that the salts of manganese, etc., could apparently replace the peroxidase part. In this connection see: Ber. Chem. Gesell. **43**: 366. 1910.