Résumé. Le sulfocyanate d'ammonium et l'aldéhyde formique, formés probablement au cours de l'évolution géochimique, fournissent les structures ressemblant à des cellules. La formation des ces structures, sous des conditions variables de concentration, est catalysée par les irradations UV. Ces formes protocellulaires expulsent leurs contenus vacuolaires et absorbent des matières colorantes. La transition de l'évolution géochimique aux protocellules semble être une étape admissible selon le résultat de nos expériences.

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Electron Spin Resonance Studies of Catalase and some of the Catalase Compounds

A magnetic study of the catalase and some of the catalase compounds has been made by THEORELL et al.[1–3]. According to these investigations, the iron of catalase, of catalase azide compound and of catalase fluoride is in ionic bond. Only the catalase-cyanide compound is a covalent complex; however, in the case of liver catalase only 3 hemin irons enter this covalent bond, while the fourth iron atom remains in an ionic bond. Compared with measurement of magnetic susceptibility, electron spin resonance (ESR) measurements permit a closer insight into the electron structure of hemoproteids[4–12].

This report presents the results of ESR studies of bovine liver catalase. The preparation of crystalline catalase was made according to the method described by SCHNUCHEL[13] with subsequent purification by gel filtration on Sephadex G 100 at pH 6.9. For ESR measurements $1–2 \times 10^{-4}$ valar catalase solutions were used. The measurements were carried out at a temperature of 77 °Kelvin.

The ESR spectrum of catalase (Figure 1) is an axial-symmetrical spectrum with the g-factors $g_{\perp} \approx 6.3$ and $g_{\parallel} \approx 1.92$ as found with the isolated prosthetic group of hemoglobin, the chlor hemin[14]. The additionally occurring absorption at $g = 4.2$ is due to an iron that is not bound to porphyrin – similar to what EHRENBERG[7] found in ESR measurements of myoglobin, MORITA and MASON[8] in peroxydase, YONETANI, SCHLEYER and EHRENBERG[12] in the cytochrome c peroxydase and BEINERT and SANDS[15] in the DPNH cytochrome c reductase. The iron with an absorption at $g = 4.2$ is to be conceived as a contamination of the catalase; it can be partially separated from the catalase protein by the gel filtration purification, that is the strength of absorption at $g = 4.2$ decreases after the gel filtration without altering the rest of the spectrum.

The absorption peak at $g_{\parallel} \approx 1.92$ is comparitively high for the axial symmetry of the ESR spectrum compared with the absorption of g-vertical. It can therefore be assumed that like other hemoproteids, myeloperoxydase[7] and plant peroxidases[8], there are still other paramagnetic cations, preferably copper, which contain catalase preparations and increase the absorption at $g = 2$.

In the repeated preparation of the catalase we always obtained, within the absorption band of $g \approx 6.3$, an additional peak which has been retained also in the ionic complexes of catalase. Such peaks at $g \approx 5.3$ have also been observed by EHRENBERG[7] with the alkaline form of myoglobin and with myeloperoxidase, and by MORITA and MASON[8] with peroxidases of horse-radish and of Japanese radish. The peak at $g \approx 5.3$ has not been interpreted by the authors; we are inclined to assume that this peak in the catalase suggests either 2 different possibilities of binding of the prosthetic group to the protein or that there is no longer an axial symmetry of the molecule as supposed by WATARI et al.[16,17] for the ferrihemoglobin M_{OSAKA}.

In ESR measurement catalase fluoride behaves very similarly to the catalase. So the 2 compounds – like methemoglobin fluoride[9] and the fluoride compound of

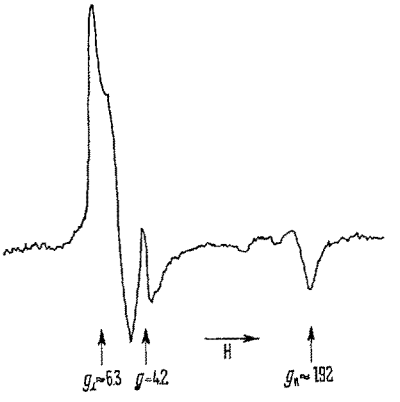

Fig. 1. First derivative of electron spin resonance absorption spectrum from catalase (pH = 6.9).

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peroxidase[8] – represent pure high-spin complexes[18]. In contrast to this, catalase cyanide shows a typical low-spin spectrum with the g-factors $g_1 = 2.78$, $g_2 = 2.15$, $g_3 = 1.60$ (Figure 2). The magnetic titration of the catalase with potassium cyanide made by THEORELL and AGNER[1] showed that only $^3/_4$ of the catalase iron was cyan-sensitive, while the last $^1/_4$, the non-hem iron, was not affected by cyanide. Accordingly, the ESR spectrum of the catalase cyanide shows that the total porphyrin-bound iron enters a binding with cyanide. The iron which is not porphyrin-bound, having a g-value of 4.2, does not react with cyanide, that is the absorption at $g = 4.2$ does not change upon reaction of catalase with potassium cyanide. The ESR spectrum of catalase azide indicates that this compound must be a mixed complex of the high-spin and low-spin form similarly as methemoglobin and some methemoglobin compounds[9], the peroxidase cyanid[8] and the cytochrome c peroxydase[12].

The g-values of catalase azide are $g_\perp \approx 6.5$ and for $g_1 = 2.66$, $g_2 = 2.6$, $g_3 = 1.7$.

Since the measurement of the magnetic susceptibility for the catalase azide complex gave a high value at 20 °C which corresponds to 5 unpaired electrons[1], it must be assumed that at low temperature the low-spin proportion increases as was also observed for methemoglobin[19].

If catalase azide is allowed to react with hydrogen peroxide or barium peroxide without oxygen and is then frozen immediately after the reaction, then the ESR measurement of this frozen solution gives a spectrum in which the absorption at $g \approx 6$ is strongly reduced compared with the catalase azide spectrum. At the same time an intensive narrow signal occurs at $g = 2$. This signal can be attributed either to a free radical or to ferro-catalase-nitric oxide which is found by FOULKES and LEMBERG[20] and by NICHOLLS[21] as a product of the reaction from catalase azide with hydrogen peroxide. Ferro-catalase-nitric oxide will give a narrow ESR signal of the same widths as a free radical like hemoglobin-nitric oxide[22].

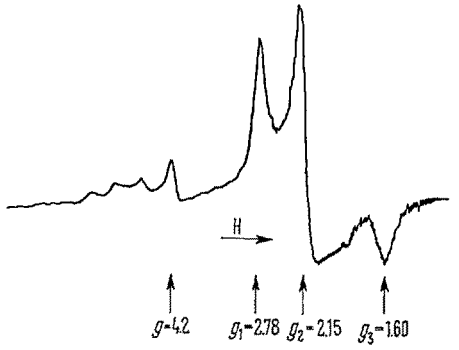

Fig. 2. First derivative of electron spin resonance absorption spectrum from catalase cyanide.

Free radicals have also been observed in the reaction of hydrogen peroxide with other hemoproteids[8,12,23]. This finding is of interest because THEORELL and EHRENBERG[3] have found that the azide catalase-H_2O_2-complex in nitrogen or CO atmosphere is a diamagnetic complex from which they conclude that a ferro complex has formed. However, the presence of a free radical or ferro-catalase-nitric oxide by the reaction of hydrogen peroxide with catalase azide indicates that a complete diamagnetism cannot be possible. Is it possible that both, a free radical on the protein and ferrocatalase-nitric oxide, are present in the compound formed on the addition of catalase azide with hydrogen peroxide in nitrogen.

ESR studies of the reaction of cytochrome c peroxidase with ethyl hydroperoxide, in which the ESR absorption of the porphyrin-bound iron disappears with simultaneous formation of a free radical[12], suggest that the present ideas about the process of azide catalase hydrogen peroxide reaction require further investigations.

Zusammenfassung. Rinderleberkatalase und einige Katalasekomplexe wurden bei einer Temperatur von 77 °Kelvin mit der Methode der Elektronenspinresonanz untersucht. Aus den Elektronenspinresonanzspektren ist zu entnehmen, ob es sich bei der Katalaseverbindung um einen Gross-spin-Komplex, um einen Klein-spin-Komplex oder um eine Mischform beider Komplexarten handelt.

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Striatal Bradykinesia Alleviated by Intracaudate Injection of L-Dopa

In former experiments it was shown[1] that prolonged chemical stimulation of the caudate nucleus in cats by injection of alumina cream into this ganglion induces first a bradykinesia and later a nearly complete akinesia and a catatonia-like condition. The chemical stimulation of the caudate nucleus seemed useful for a study of the effect of L-dopa, which is converted into dopamine in the tissues, upon this ganglion. Such a study seemed of interest in view of the findings that the dopamine content of the caudate nucleus is low in Parkinson's disease[2], and that therapeutic administration of L-dopa is able to relieve some symptoms of this disease, particularly the akinesia, at least temporarily.

Experimental. Since it takes several days until the effect of the alumina cream injections becomes obvious,

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