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ACTION MECHANISM OF ACTIVATING OR ANTAGONISTIC METALLIC CATIONS IN THE OXIDATION OF SH IN THIOPROTEINS

/Following is a translation of an article by Mme Andree Goudot and Michel Faguet, presented at the 24 April 1961 meeting of the French Academy of Sciences and published in the French-language periodical Comptes rendus de l'Academie des Sciences (Reports of the Academy of Sciences), Vol 252, 1961, pages 2557-2559, under the subject heading of Theoretic Chemistry./

Activity of cations of the transition metals in the formation of the S—S bond by oxidation. It depends on the electronic structure of the cation that allows or does not allow the formation of a bond by resonance between the atoms concerned, through the intermediary of a 3d orbit. The antagonism was studied during the exponential growth of bacterial colonies (M. Faguet and A. Goudot, Bull. Soc. Chim. Biol. /Bulletin of the Biochemical Society/, in press).

S—S bonds occur, in the formation of certain metabolites and especially of enzyme precursors (zymogens), after oxidation of the SH groups of thioproteins like cysteine and glutathione. Two molecules of cysteine or of glutathione give: $2SH + O_2 = S—S + H_2O_2$. This oxidation occurs only very slowly in the air, but it becomes very rapid if the cysteine or glutathione molecules are coordinated to certain metallic cations, while others inhibit the reaction. We find in L/s/cherichia coli primarily glutathione whose enzymatic synthesis occurs as follows:

$$\text{L-glutamic acid} + \text{L-cysteine} + \text{glycine} + \text{ATP} \xrightarrow{Mg^{2+}} \text{L-glutathione} + \text{ADP} + P_i.$$

Therefore, the S—S bond takes place between the SH groups of the cysteine residues.

1. Catalysis by Free Metallic Cations. In the formation of a hybridization complex, the glutathione is joined to the metallic cation by means of atoms belonging to groups that make them strongly coordinating. These atoms are the N of the NH_2 group in glutamic acid, the N and the S in the cysteine residue. Two molecules of glutathione (or three of cysteine) form, with the metallic cation, a hexavalent complex by $3d^2\ 4s\ 4p^3$ internal orbits. The neutral O_2 molecule no longer has anything but the 4d external orbits to be coordinated to the central cation for a bond that is all the more unstable since the occupied orbits serve as a screen.

Nevertheless, due to their electronic structure each of the cations of transition metals of a same group has a different action mechanism.

These action mechanisms may be: 1. active by fixing O_2 and permitting a resonance bond between the atoms that are to react, through the intermediary of one of their orbits; 2. merely fix O_2 without interaction between O_2 and the substrates; 3. not fix O_2 in the complex.

The two systems may be assumed in the plane XY with the SH groups depending on the adjacent axes X, Y in the complex.

Mn^{2+} (five 3d electrons): In the formation of a $3d^2\ 4s\ 4p^3$ complex, after pairing 3d electrons, the fifth 3d electron occupies orbit $3d_{xy}$ alone, which is a π orbit of the metallic cation. The formation of a chelated complex by means of strongly donor coordinating atoms increases the redox potential, tending to stabilize the central cation in its highest state of valence. It may be assumed, therefore, that the metallic cation uses its fifth $3d_{xy}$ electron for a π bo ' with O_2 which is weakly coordinated to it. This fifth $_{xy}$ electron resonates, therefore, between the central cation and O_2. But the $3d_{xy}$ orbit has some positive lobes depending on +X and +Y where the SH groups are joined. A bond that we shall call bond by resonance may, therefore, be established between O_2 and the two SH groups with the aid of the $3d_{xy}$ orbit.

The calculation of the distribution of charges within the complex indicates a positive charge on S which produces a rupture between S and H. The oxidation reaction,

therefore, is made possible. In addition, the resonance on the two S located on the same plane depending on +X and +Y permits the S—S bond.

Fe^{2+} (six 3d electrons): As in the preceding case, the complex has a tendency to become stabilized in its ferric state. Therefore, it may be assumed that Fe^{2+} utilizes its sixth 3d electron for a bond with O_2. Since this electron occupies the π 3d orbit, a bond by resonance is produced between O_2 and the SH groups, as in the case of Mn^{2+}.

Experimentally, the amount of O_2 consumed depends on the thioprotein concentration when it is in excess (Michaelis and Barron, J. Biol. Chem., 83, 1929, p 191). Therefore the H_2O_2 product of the reaction is used as follows: $SH + SH + H_2O_2 = S—S + 2H_2O$.

Co^{2+} (seven 3d electrons): The seventh 3d electron has to be raised to an antibonding orbit of the metallic cation, in order that the $3d^2\ 4s\ 4p^3$ complex may be formed. O_2 coordinated to the central cation by a 4d external orbit may capture this electron. We then have a cobaltic complex to which the O_2^- ion is bound. Therefore, the complex fixes the oxygen in the air, but there is no interaction between O_2 and the SH groups. In fact, here the $3d_{xy}$ electron is completely displaced on O_2. There is no longer any resonance, therefore no oxidation reaction. Experimentally, the amount of O_2 consumed depends on the Co^{2+} concentration. Since the cobaltic complex that is formed is very stable, there is blockage of the thioproteins and of O_2, whence an inhibition of the synthesis reaction.

Ni^{2+} (eight 3d electrons): One single orbit may be freed for a bond through an external orbit. Therefore, there is no longer any possibility for a bond with the neutral O_2 molecule. Moreover, since the substrate molecules give a stable complex, there is inhibition of the reaction by blockage of the substrate.

Dissociation energy. The resonance energy is calculated in the 2d SH-metal and GS-SG-metal complexes. The complex toward which the reaction tends is the one in which the electrons occupy the lower energy levels. Therefore, this is the complex that has the lower resonance energy, because they both have the same number of electrons. The difference between these resonance energies gives, as an algebraic value, the dissociation energy of S—S.

1. With Fe^{2+}: The resonance energy is lower in the GS-SG-metal complex. Therefore, the reaction has a tendency to take place toward synthesis. The difference between the resonance energies corresponding to the synthesis is 62.2 kg. cal. (heat of formation of S—S is 63.8 kg. cal.).

2. With Co^{2+} and Ni^{2+}: The resonance energies are lower in the 2GSH-metal complex. The energy in favor of the SH + SH dissociation is 17.6 kg. cal. for Co^{2+} and 36 kg. cal. for Ni^{2+}.

2. Catalysis by means of the Cytochrome Oxidase-Cytochrome C Couple. The oxidation reaction of the sulfhdryl groups of the thioproteins is accomplished in tissues in vivo without production of H_2O_2 by the action of the cytochrome oxidase in presence of cytochrome C (Sumner, The Enzyme, pp 365 and 614). According to the hypothesis drawn up on the active role of cytochromes in the respiratory chain (A. Goudot, Comptes Rendus, Vol 251, 1960, pp 722 and 1194), that is to say that the cytochrome oxidase is not only an electron transporter but also performs the $O_2 = O + O$ dissociation, the oxygen is the utilizable for the oxidations.

We have, as in the respiratory chain: cyt. oxidase $Fe^{3+} + O_2$ = cyt. oxidase $Fe^{2+} - O^+ + O$ and in the presence of cyt. C, cyt. oxidase $Fe^{2+} - O^+ + O$ + 2 cyt. C Fe^{2+} = cyt. oxidase $Fe^{3+} + O^- + O^-$ + 2 cyt. C Fe^{3+}. The oxidation reaction, then, is accomplished without the formation of H_2O_2.

The oxidation of phenyldiamine in the cardiac muscle is accomplished in the same way.

Comment. The activity in vivo of these cations of the transition metals and of these enzymes on the exponential growth of bacterial colonies has been the object of an experimental study (M. Paguet and A. Goudot, loc. cit.): a. on aerobic bacteria; b. on anaerobic bacteria.

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