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SEMICONDUCTION OF PROTEINS AS AN ATTRIBUTE OF THE LIVING STATE: THE IDEAS OF ALBERT SZENT-GYÖRGYI REVISITED IN LIGHT OF THE RECENT KNOWLEDGE REGARDING OXYGEN FREE RADICALS

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Abstract—Oxyradicals have been considered as harmful byproducts causing molecular damage during aging. However, evidence is accumulating to show that the actual situation is more complex: the living state implicitly involves the production of oxyradicals. (1) Blast type cells produce much less oxyradicals than the differentiated ones, and an increased production of $OH^{\cdot}$ radicals induces differentiation of various lines of leukemia cells; meanwhile, their superoxide dismutase expression increases to a very high extent. (2) The supramolecular organization of the cells is developed by means of "useful" crosslinking effects $OH^{\cdot}$ radicals. (3) Repiratory inhibition of oxyradical production (KCN-intoxication, suffocation, etc.) would kill living organisms prior to the exhaustion of energy reserves. It is assumed that the continuous flux of $OH^{\cdot}$ radicals is a prerequisite for a constant electron delocalization on the proteins, which is a semiconduction of p-type, proposed already in 1941 by Albert Szent-Györgyi, and refuted on a "theoretical" basis. It has become clear by now that the carbon-based semiconduction is possible because diamond transistors are known to exist. The recently developed atomic force microscopy offers some real possibilities for experimental testing of this assumption. This concept may lead us to new horizons in interpretation of living functions, such as the basic memory mechanisms in brain cells and their impairment during aging.

Key Words: oxyradical flux, living state of proteins, semiconduction by carbon compounds, electronic interpretation of synapses, electron delocalizations on proteins

INTRODUCTION

ALTHOUGH HARMAN (1956, 1957) proposed long time ago that the oxygen free radicals may be the principal agents causing molecular alterations in the living systems, the true role of these radicals in cell physiology and biochemistry has not yet been sufficiently

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clarified. The central idea of the free radical theory of aging has always been that free radicals represent only harmful byproducts of the aerobic life and, as such, they are the basic cause of aging and of numerous diseases (Harman, 1968, 1988, 1992, 1994). The discovery of superoxide dismutase (SOD) (McCord and Fridovich, 1969a, 1969b) stimulated more meaningful consideration of the free radical effects in living systems, yet this assumption is still under debate. For example, on one hand, many biochemists neglect even today the biological role of oxygen free radicals because of their low concentrations in the cells. On the other hand, the harmful character of these radicals becomes more and more overemphasized by many scientists to such an extent that it became a central dogma of this field even for the nonprofessional public informed by the newspapers and other information media.

Oxygen free radicals unquestionably exist in biological systems and are involved in numerous biological phenomena such as cellular aging, mutagenesis, inflammation, and other pathologies (see, Zs.-Nagy, 1991, 1994). However, immediately one encounters a great contradiction, if considering the experimental facts. Namely, young individuals consume more oxygen per unit of mass and time than the old ones, i.e., also the radical formation must be more abundant in young systems, as compared to the old ones. In spite of this situation, the young cells and organisms are able to grow and differentiate, while older cells progressively deteriorate in their structure and functional performance. Although this paradox can be resolved as explained by the membrane hypothesis of aging (MHA) reviewed recently (Zs.-Nagy, 1994), it should be stressed that the implication of these radicals in the aging process leads to the necessity of analyzing the role of oxyradicals on a much wider basis. Such a consideration led us to the revision of some older suggestions regarding the semiconductive properties of proteins in living state (Szent-Györgyi, 1941a, 1941b).

FACTS AND PROBLEMS RELATED TO PROTEIN SEMICONDUCTION

The molecular components of living systems can be isolated in pure form and put in test tubes. However, life itself is lost during such a purification, because the living state involves supramolecular organization and interaction of the compounds within the cellular structure. The supramolecular organization is created through certain intermolecular reactions that are based on the interactions of the external electron orbits of the macromolecules. Starting from these and many other considerations, Szent-Györgyi (1941a, 1941b, 1977, 1978) concluded that the living state is bound to particular functions of the electrons. Consequently, it is plausible to assume that the processes of biological maturation and aging are also related to alterations of those electron orbits of the macromolecules. Because the free radical reactions usually take place on the same external electron orbits, it is obvious that these ideas and free radical biology have a number of common aspects.

More than 50 years ago, Szent-Györgyi (1941a, 1941b) explicitly suggested that the living state involves most probably a semiconductor function of the proteins in the cells. Although this was a very attractive hypothesis, it could not gain acceptance for two main reasons. (1) Most of the contemporary biochemists could not even understand the essence of this proposal, because the field of "semiconduction" was still largely unknown. (2) The hypothesis of Szent-Györgyi (1941a, 1941b) has been unanimously rejected by the contemporary physicists on an apparently solid, "theoretical" basis.

The argument against this idea was the well-known fact that isolated proteins do not show any sign of conductivity, and even the possibility of semiconduction of carbon compounds was excluded in general on the following basis: it was claimed that the so-called valency and conduction bands in carbon compounds are energetically too far from each other. As a matter of fact, 5.5 eV energy would be required to push an electron from the valency band to the conduction band in carbon compounds (in Si, this energy gap is only 1.1 eV) (Angus and Hayman, 1988; Geis and Angus, 1992). Because the electron "excitation" in semiconductors is usually getting energy from heat agitation, the hypothesis of Szent-Györgyi was unanimously considered as a "dead duck" (see for details: Szent-Györgyi, 1978).

The objection of physicists, although it appeared to be very solid and convincing, did not prove to be really true for the carbon compounds in general. Recently, diamond transistors were developed, in which thin layers of artificial diamond crystals doped with the trivalent boron atoms resulted in excellent semiconductor properties (Angus and Hayman, 1988; Geis and Angus, 1992). One can conclude from these achievements that (a) in spite of the "theoretically" established barriers, semiconduction of carbon compounds is possible, if the structure and the circumstances are properly designed; and (b) this kind of "theoretical" predictions is obviously always of limited value, until a question is not explored sufficiently in its physical reality.

Szent-Györgyi (1977, 1978) had very seriously taken the objection of physicists against his hypothesis. Nevertheless, he believed that in spite of the mentioned energetic barrier, in living cells a mechanism for the formation of electron holes in the proteins may exist, and in case we can identify such a mechanism or mechanisms, the proteins can well function as semiconductors. He mentioned in general terms that suitable electron acceptors may take over electrons from the protein molecules, causing an "electron desaturation" of proteins, and he attributed the main role to a "one-by-one" electron transport where the terminal acceptor would be, e.g., some carbonyl groups. He also proposed the existence of a charge-transfer process maintained by compounds like methylglyoxal, etc. Although he and his coworkers presented considerable amount of experimental evidence supporting this concept (Együd and Szent-Györgyi, 1966; Szent-Györgyi, 1977; Pethig and Szent-Györgyi, 1977; Pohl *et al.*, 1977; Bone *et al.*, 1978; Fodor *et al.*, 1978), the basic hypothesis remains to be unequivocally proven, mainly because of quantitative problems. Indeed, semiconduction can be visualized only if the electron desaturation takes place continuously with a proper speed. Therefore, the idea of protein semiconduction in living state has practically been forgotten.

THE LINK BETWEEN SEMICONDUCTION OF PROTEINS AND OXYGEN FREE RADICAL BIOLOGY

Szent-Györgyi (1977, 1978) himself felt that a link between the semiconduction hypothesis and the existence of free radicals should exist. As a matter of fact, he reported that a living mouse placed in an ESR spectrometer gives a very complex ESR signal, and this signal immediately disappears if the animal is killed by KCN. This observation was considered by Szent-Györgyi as an argument in favor of the existence of life-dependent electron transitions.

There is, however, another possibility that has not been mentioned explicitly by

Szent-Györgyi (1977, 1978). Namely, the electron desaturation of proteins may well be caused and maintained by the continuous flux of $OH^{\cdot}$ free radicals. This would mean that the practically continuous suction of electrons by the $OH^{\cdot}$ radicals may represent the ubiquitous mechanism that helps to overcome the energetic barrier between the valency and the conductive bands of the protein molecules. In this assumption, the continuous radical flux is as important for the maintenance of the living state, as the voltage power supply is essential for the functioning of the computer. Considering Eq. (1)

$$OH^{\cdot} + RH \xrightarrow{k} R^{\cdot} + OH^{-} \qquad (1)$$

where RH may represent any organic compound, the estimates for k are in the range of 10^7–10^{10} $M^{-1}s^{-1}$ (Walling, 1975). This implies that the $OH^{\cdot}$ free radicals formed continuously via $O_2^{\cdot}$-SOD-H_2O_2-Fenton reaction pick up electrons from any of the surrounding molecules extremely quickly, in the upper range of k within about 2 molecular collisions. Although it is true that practically all organic molecules react with the $OH^{\cdot}$ radicals, the rate of such reactions may be different by about three orders of magnitude. The experimental evidence for the biological reality of Fenton reaction is strong: (a) sufficient amounts of Fe^{2+} are available in vivo (Floyd and Lewis, 1983); (b) the $OH^{\cdot}$ radicals formed by this reaction are able to attack the amino acids and proteins even under mild chemical conditions (Zs.-Nagy and Nagy, 1980; Floyd and Zs.-Nagy, 1984; Zs.-Nagy and Floyd, 1984).

As a consequence of the very quick electron delocalization caused by the $OH^{\cdot}$ radicals, a number of molecular alterations of $RH^{\cdot}$ take place in the living system, as detailed by Zs.-Nagy (1994). For example, formation of covalent crosslinks; the formation of protein radicals with various life spans (involved in catalysis: Stubbe, 1989; or functioning as second messengers: Schreck and Bauerle, 1991). Nevertheless, from the point of view of the semiconduction hypothesis, the most important possibility is that the electron hole of $R^{\cdot}$ can be refilled from the next adjacent available electron shell, resulting in a new hole, which is, again, refilled from another shell, etc. This kind of repetitions may create a situation in which the hole is moving from one place to another. This is nothing else than a semiconductive phenomenon on the protein structure, as suggested by Szent-Györgyi (1941a, 1941b).

Although this idea is fully speculative for the time being, there are some known facts that should be considered in this respect. For example, KCN is widely known as one of the most toxic substances causing an immediate death of the organism, because it blocks the oxygen supply of the tissues in virtually zero time. Everybody believes that the immediate lethal effect of KCN is due to the block of ATP synthesis, i.e., the cause of death is an energy shortage. However, this is probably not true, because, as a matter of fact, the ATP-reserves at the time of death would be still sufficient for considerably longer time (5–10 min) even in the brain. Obviously, the block of oxygen supply also stops the formation of the oxygen free radicals, the consequences of which are much more immediate than the exhaustion of the ATP reserves, because there are virtually no reserves of these radicals. To the best of my knowledge, nobody has ever explained the role of KCN as the quickest and most perfect oxygen radical scavenger, although such an interpretation may be justified. The death from suffocation may be described in the

same way, i.e., due to the block of oxygen radical formation, with the only difference from the KCN intoxication that it is somewhat less immediate, because the oxygen reserves in the lung and blood can still be utilized for the maintenance of the radical flux. In any case, even the suffocation-caused death takes place much before the exhaustion of the ATP reserves of the brain.

The above assumption implies that the oxygen supply would be necessary not only to produce ATP, but also to maintain a sort of "supply voltage" for the living cells through a continuous electron delocalization on the proteins. This is a completely novel suggestion. This idea implicitly contradicts the central dogma of the free radical biology, according to which oxygen free radicals are considered only as harmful byproducts. It must be emphasized, however, that this concept does not deny the possibility of damaging side effects of these radicals, but offers a much wider basis for interpretation of the radical functions.

CONSEQUENCES OF AND DEDUCTIONS FROM THE ABOVE HYPOTHESIS

If the electron movements on the protein structures take place in a regulated, dynamic way, in which the protein structure plays the decisive role, since the $OH^{\cdot}$ radicals "rape" electrons nonspecifically, one can imagine that a "p" type semiconduction may be a reality in the living material. Of course, we do not know yet, how we should describe a single neuron in such terms. One possible assumption is that every synaptic contact may represent the base of a single "transistor", whereas the other two electrodes (called collector and emitter in technical terms) of the "transistor" are located intracellularly. If we assume "p" type semiconduction for the collector and emitter, the base can be regulated by a very small negative input voltage. It is tempting to speculate on the implications of the fact that the resting membrane potential (-40–100 mV) falls exactly in the range of the opening voltage of a real p-n-p transistor. If this is not only a chance coincidence, one can imagine that in the resting state the elementary units called "transistors" are open, and, therefore, maintain a certain dislocation of electrons (current) on the protein structure. This flux of electrons derives from the substrates of food (like that which maintain the energy production), and ends on the $OH^{\cdot}$ free radicals, i.e., disappears in the water. This would mean a perfect "battery" for the living system, because there is practically no ballast to be eliminated by special mechanisms.

Let us speculate further: during an action potential, the negative voltage on the base goes to near zero, i.e., a short closure of the "transistor" comes into being, during which the electron flux between the collector and emitter may be stopped and/or deviated to some other places, as compared to the resting state. Such a deviation of the electrons may create more or less persistent changes of the protein molecular conformation, and such changes may represent the elementary physical basis of one type of immediate (short-term) memory records.

One has to emphasize the fact that the brain is very rich in SOD, but it is almost completely devoid of catalase, and its GPO activity is also relatively low. This fact may be explained by the necessity of the brain cells to produce more $OH^{\cdot}$ free radicals via SOD-H_2O_2-Fenton reaction, than, for example, of liver cells, in agreement with the assumption that $OH^{\cdot}$ free radicals are of particular importance in quantitative terms for the living state of the brain cells and for the maintenance of their memory function.

This new concept also requires the revision of some generally used claims in the free radical biology. Namely, ever since its discovery, SOD was considered as an "antioxidant", i.e., a protective enzyme. This interpretation should be changed, because (a) SOD is an "antireductive" enzyme; and (b) a number of facts indicate that SOD cannot be considered as a protective enzyme (see for details, Zs.-Nagy, 1992, 1994). Its role in the differentiation of the blast-type cells (see for details, Nagy *et al.*, 1993, 1994) agrees with the assumption that this enzyme is an important factor in the mechanism of cell differentiation where it contributes most likely to the "useful" crosslinking of the cell components through an increased formation of $OH^{\cdot}$ free radicals.

POSSIBILITIES FOR EXPERIMENTAL TESTING

1. The above speculations can now be tested experimentally. Recent developments of biophysics would permit us to perform the critical experiments. The invention of the so-called scanning tunneling microscopy (Binnig and Rohrer, 1985) and the further developments in this field resulting in a completely new family of other "scanning probe" microscopes, like the atomic force microscope (AFM) (Binnig *et al.*, 1986; Rugar and Hansma, 1990; Ohnesorge and Binnig, 1993), has opened a completely new way for such experiments. AFM offers atomic resolution of the molecular structures in dimensions that have never been explored before by any kind of optical or electron microscopy. It reveals visual information regarding the electron clouds surrounding atoms and molecules. It is important that the AFM method allows the use of samples either in air or in water, and it is possible to study also proteins immobilized in a buffer (Weisenhorn *et al.*, 1989, 1990). AFM methods have already been successfully applied to the study of a variety of samples, including bacteriorhodopsin (Butt *et al.*, 1990), phosphorylase-phosphorylase kinase complexes (Edstrom *et al.*, 1990); electrostatic, Van der Waals, and hydration forces in electrolyte solutions (Butt, 1991); planar membrane bilayers (Singh and Keller, 1991); hydrated phosphatidyl-ethanolamine (Zasadzinski *et al.*, 1991); three-dimensional membrane protein crystals (Lacapére *et al.*, 1992); living cells immersed in a fluid (Kasas *et al.*, 1993); and in situ hybridization of human chromosomes (Putman *et al.*, 1993).

The semiconductive properties of proteins can be studied by AFM as follows: certain filamentous protein models should be immersed in a proper liquid in a specially designed specimen holder. AFM imaging will give the information about the static situation of the electron clouds around these molecules. In case we shall apply electron donors to one end of the protein filaments and generate $OH^{\cdot}$ free radicals on the other one, it is reasonable to expect that the AFM image will change, if these latter additions stimulate electron delocalizations on the protein chain. It cannot be predicted how difficult the application of this technique will be for this purpose, but it seems to be possible to reveal important information on the electron movements.

2. If the above speculations contain some elements of truth, and the AFM method will deliver convincing evidence for the basic phenomenon, one has to also find the mechanism of the long-term memory. If the short-term memory content is written into proteins by means of a kind of semiconductive mechanism, due to the much shorter lifetime of proteins compared to the individual itself, there must be another way assuring the maintenance of the memory content independently from the protein decomposition. Theoretically speaking, one has to suppose the existence of a kind of reverse

translation and transcription which is writing backward the altered protein structure in some way into the genome. This part of information then should be transcribed again to RNA and translated into new proteins, the incorporation of which to the proper place in the given nerve cells may assure the long-term persistence of the memory contents. The imperfections of the human memory, i.e., a considerable loss of the content with time may well be explained by a statistically predictable incompleteness of such a replacement of the damaged proteins. An even more attractive fact is that we need to switch off our brain from time to time, i.e., have to go to sleep. This may be necessary to assure the time to elaborate the information having filled in our "primary memory table" during the day, and transfer its content into the long-term memory compartments.

This concept assumes a kind of reverse flow of genetic information from the proteins to DNA, which is not entirely without a precedent at the present level of knowledge. Namely, molecular genetics has already discovered a process called "retroposition" (Rogers, 1983, 1985), which is nothing more than a reverse flow of genetic information from RNA to DNA. The result of this process is the formation of retroposons, which are classified into viral and nonviral superfamilies (Weiner *et al.*, 1986). An extraordinary variety of nonviral retroposons has been described, all of them corresponding to a partial or complete DNA copy of a cellular RNA species (Weiner *et al.*, 1986). It seems worth mentioning that although, according to some opinions, the retroposons represent a sort of molecular parasitism (Orgel and Crick, 1980; Doolittle and Sapienza, 1980; Wichman *et al.*, 1985), they can also confer a selective advantage on their host (Hartl *et al.*, 1983), i.e., cannot be regarded only as a ballast. Therefore, we have to accept that retroposition can shape and reshape the eukaryotic genome in various ways, which may represent a component of the imaginary process of reverse flow of genetic information in relation to the long-term memory content, as outlined above. The missing link is to determine how the primary information can be stored by proteins.

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