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The biostructural theory versus the chemiosmotic theory

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Abstract

An alternative explanation for the observations that: (i) anoxia causes a large current through K_{ATP}-channels and (ii) it decreases the protein synthesis rate has been advanced. In the current research we considered the role of the biostructured matter in the living cell, which may be broken down under the hypoxic conditions and fast restored upon reoxygenation. Therefore Ca^{2+} and Na^{+} influx, K^{+} and amino acids efflux, as well as low ATP production can be directly related to the breakdown of biostructure. The lack of oxygen results in the breakdown of the cell biostructure, leading to a low protein synthesis. Besides, the chemiosmotic theory has failed to give a coherent explanation to these experimental data. Suitable experiments using Saccharomyces cerevisiae support the theoretical considerations as well.

Keywords*:* chemiosmotic theory; biostructural theory; yeast; K_{ATP}-channels.

Introduction

Since the discovery of ATP-sensitive K^{+} channels in the myocardium [1,2] there has been an extensive debate as to whether or not these channels contribute to the extra cellular accumulation of K^{+} during ischemia [3]. The opening of K_{ATP}-channels has been described to appear also in isolated heart cells by application of inhibitors of the oxidative metabolism [4] or by anoxia [5]. Generally, it is believed that a sufficient drop of the cytosolic ATP could explain the opening mechanism. Still, progressive opening of these channels cannot explain the triphasic time course of K^{+} accumulation in the ischaemic myocardium. In addition, half maximum inhibition of the channels activity is reached at tens to hundreds of μM ATP [6,7] whereas measured ATP levels in the ischaemic myocardium are in the range of several mM[3] which would allow only a few channels to open.

In order, to identify factors responsible for the down-regulation of mitochondrial biosynthetic processes during anoxia, the effects of oxygen limitation and pH on protein synthesis were also investigated in isolated mitochondria [8]. It appears that *in vitro* there is an overall suppression of the capacity for translation within the mitochondrion in response to either anoxia or changes in pH. Currently, the precise mechanism by which oxygen limitation influences mitochondrial gene expression is still unclear.

While scanning the literature for a plausible explanation for these findings we noticed that a so-called biostructural theory [9] could serve the best our purposes.

According to Macovschi's hypothesis [9], living organisms possess two forms of matter: a biostructured form (also named biostructure) and the coexistent molecular matter.

The biostructure features a specific organization, characteristic of living matter. It exists in living matter exclusively and breaks down concurrently with death, releasing, as simple molecules, the components it is consisted of [9]. Also, it features a remarkable characteristic of breaking down partially and reversibly, under the influence of metabolic inhibitors, as a heat effect, by electrical stimulation, ultra violet irradiation or may occur spontaneously, and under various physiological, pathological and experimental conditions. Besides, we have shown for the first time the breaking down of the biostructure both under hypoxia and the influence of some metabolic inhibitors such as dinitro-o-cresol [10,11]. A large amount of theoretical provided us and experimental facts have been brought in support of the biostructural theory [9-12].

Both ATP-sensitive K^+ channels and the effects of oxygen limitation and pH on protein synthesis occur when showing that the biostructure is damaged under anoxic conditions. The biostructural alterations result in the observed molecular processes.

Protein synthesis and K_{ATP}-channels

While searching the literature on the role of hypoxia on protein synthesis and the production of ATP [13-20], on the one hand, and the relationship between the state of the biostructure [9,10] and the concentration of metabolic inhibitors or oxygen level, on the other, we noticed that the biostructure breakdown coincides with the decrease in protein synthesis and ATP production, or especially with the decay of K_{ATP}-channel current. It therefore seems reasonable to hypothesize that the breakdown of cell or mitochondrial biostructure is the main cause for the observed phenomena. Figure 1 depicts our hypothesis on the breakdown of cell biostructure under hypoxic conditions as follows: oxygen acts directly upon the biostructure, maintaining it unimpaired. Biostructure can be considered as the rich energy complex in the living cell, characterized by continuity and a high level of organization. It does consist of a polimolecular system of proteins, nucleic acids, amino acids, minerals, water etc. The biostructure features a specific organization, characteristic of the living matter; it has a higher development and organization; it is the bearer of the biological features that are assigned to the living it belongs to. Electron microscopy studies [12] supported Macovschi's conception bringing evidence that the cytoplasmic ground substance is apparently constructed as a finely divided lattice of slender trabeculae.

Cytochromes, the normal components of the mitochondrion biostructure, must be under a reduced state to react with oxygen. We suppose that the electrons are continuously transferred to oxygen molecules and the energy resulted could freely circulate within the cell or mitochondrion biostructure. Thus, a discontinuous molecular complex creates a continuous energetic system that becomes manifest on the biological structures. Furthermore, ADP molecules interact with this energetic biostructural complex forming ATP molecules, which are quantified. A hypoxic insult creates a breakdown of the biostructure, a lowering of the energetical level of the whole biostructure, and, therefore, reduces ATP levels to a minimum, as an indirect effect. As a result of the rapid fall in ATP levels and energy charge upon hypoxia, it was considered so far that Ca^{2+} homeostasis cannot be maintained, leading to Ca^{2+} overload [21,22]. We may consider that Ca^{2+} and Na^+ influx, K^+ and amino acids efflux, as well as low ATP production are directly related to the breakdown of biostructure. These are secondary effects of the breakdown of the cell biostructure. It is possible that hypoxia has an effect in diminishing intracellular pH, perhaps through elevated internal lactic acid production [13]. Generally, protease auto activation is triggered by acidic pH and its rate increases with increasing ionic strength [23]. Reoxygenation at this moment results in an altered state of biostructure. During the following minutes of maintained anoxia, total breakdown of the biostructure occurs, leading to the death of the cell.

There is a stepwise breakdown of the biostructure, under the action of metabolic inhibitors such as 2,4-dinitrophenol (2,4-DNP), dinitro-o-cresol, sodium azide etc. [9,10]. It is generally agreed that the uncouplers such as these above-mentioned ones act by causing the breakdown of some high-energy intermediates involved in the synthesis of ATP. Uncoupling agents also stimulate the activity of the enzyme ATPase, which is normally inactive as a hydrolytic enzyme in mitochondria. Actually, ATP is never formed in the presence of DNP, since high-energy intermediate, that is the biostructure, is attacked. Moreover, the addition of ATP can neither stop the breakdown of the cell biostructure, nor its restoration. The breakdown of the biostructure under the action of 2,4-DNP can easily be explained by energetically interaction with the triplet state molecular complex within the biostructure, leading to dissipate the energy from the biostructure as a heat effect. Adding ATP to the cultured cells under anoxia or under the action of the uncouplers has no effect because the biostructure of the higher organisms is maintained by the energy resulted from the interaction of oxygen molecules with the electron-transport complex containing the cytochromes under a reduced state.

While at the initial stages of hypoxia the biostructure breakdown is reversible, extended periods of oxygen deprivation result in irreversible biostructural damage. We may consider that this phenomenon occurs in brain cell or even in myocardial ones, but in embryo or other cells from metabolic organs such as liver, muscle, stomach, kidney, skin etc. These last-mentioned cells can survive longer under anoxia.

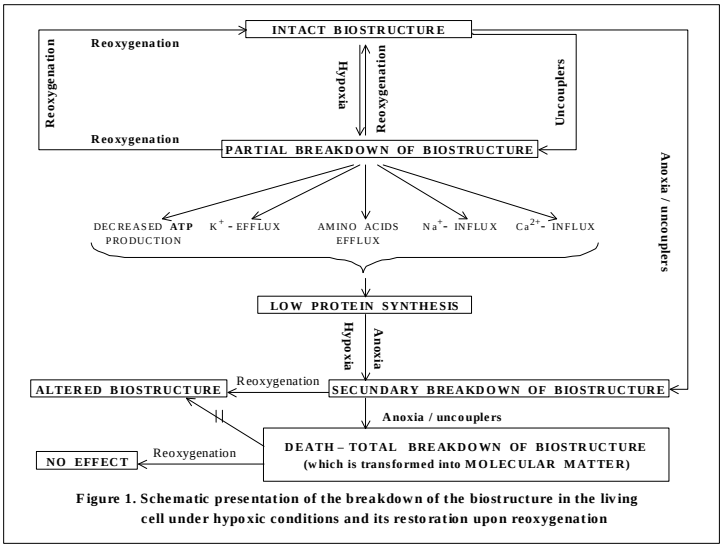

Figure 1. Schematic presentation of the breakdown of the biostructure in the living cell under hypoxic conditions and its restoration upon reoxygenation

ATP-sensitive K^+ channels. German scientists Thierfelder, Doepner, Gebhardt, Hirche and Benndorf [3] have shown that anoxia causes in ventricular myocardial cells of the guinea pig and the mouse, after a mean latency of 439 s and 129 s, respectively, a large current through K_{ATP}-channels. This current disappears within several seconds when reoxygenating the cells but decays also completely at maintained anoxia. The results suggest that in the ischaemic myocardium K_{ATP}-channels contribute only to the initial phase of extra cellular K^+ accumulation.

During the following minutes of maintained anoxia, the large K_{ATP}-channel current decayed until disappearance. In addition, at the time when the K_{ATP}-channel current decreased a current with less specific conductance developed which becomes obvious by the downwardly deflecting current at –80 mV. This current was regularly accompanied by a progressive hypercontracture and the death of the cell.

In analogy to the experiments with anoxia, also the uncoupler 2,4 dinitrophenol induced after a delay a large K_{ATP}-channel current, which decayed in the time range of minutes after

reaching a peak. The transient opening of K_{ATP}-channels induced by DNP was indistinguishable from that during anoxia.

Reversible opening of elementary K_{ATP}-channels was observed in some investigated cells. Also, the closing of K_{ATP}-channels during the decay phase is not an irreversible alteration in the channels but a reversible mechanism.

German scientists have shown that K_{ATP}-channels close again during maintained anoxia and that the period of their opening is in the range of several minutes. They suggested that the closure of the channels during anoxia be not generated by an irreversible alteration of the channel molecules. The transient opening of K_{ATP}-channels at repeated anoxia and the rapid closure of the channels following reoxygenation favor the idea that the cytosolic ATP level controls the open probability of the channels and that this level may drop in individual cells to very low values. ATP in this concentration range is essential for sufficient phosphorylation of K_{ATP}-channels to open. The putative sequence of events in the cell after the onset of anoxia is: block of oxidative ATP-synthesis, inhibition of glycolysis leading to massive opening of the channels, further decrease of ATP thereby stopping phosphorylation by protein kinase A and closing of K_{ATP}-channels. These authors gave no explanation for the latency until the first opening of K_{ATP}-channels during anoxia is of such great variance [5].

Things are quite simple: there are many kinds of biostructures and each cellular or mitochondrial biostructure breaks down differently under anoxic conditions. Thus, brain biostructure is more sensitive to anoxia than the myocardial biostructure and, especially, the embryo one. The large current through K_{ATP}-channels disappears within several seconds when reoxygenating the cells because the biostructure becomes intact but decays also completely at maintained anoxia when an altered biostructure can be observed. Finally, at the time when the K_{ATP}-channel current decreases a current with less specific conductance develops. This current is regularly accompanied by a progressive hypercontracture and the death of the cell that means the total breakdown of biostructure. At this stage, the biostructure completely disappears, releasing its potassium content.

The transient opening of K_{ATP}-channels induced by 2,4-DNP is indistinguishable from that during anoxia. In fact, 2,4-DNP attacks the biostructure, which is broken down, releasing the amount of potassium it contains. Potassium is liberated into the intracellular molecular solution and, due its gradient of concentration passes outside through cell membrane. No special K_{ATP}-channels are necessary.

Oxygen and pH regulation of protein synthesis. American scientists Kwast and Hand [8] have found that, at the optimal pH of 7.5, exposure of mitochondria to anoxia decreases the protein synthesis rate by 79 %. Rates are suppressed by a further 10 % at pH 6.8. Intramitochondrial purine nucleotides vary little as a function of pH. The effect of anoxia is reversible so that the rate of protein synthesis upon reoxygenation after a 30-min bout of anoxia is comparable with the pre-anoxic rate.

Also, mitochondrial protein synthesis *in vivo* would be operating at a pH close to the pH optimum determined *in vitro*. After 1 h of anoxia, concentrations of both adenine and guanine nucleotides were not different among the pH treatments, except for higher ATP concentrations at pH 7.5 versus pH 6.8. Thus, no net loss of intramitochondrial purine nucleotides was observed during 1 h of anoxia.

Rates of mitochondrial protein synthesis were not enhanced by treatment with ATP compared with controls. In response to anoxia, there was a rapid (within 5 min) and severe suppression of protein synthesis rates at all pH values examined, which was fully reversible upon reoxygenation.

Because there was also a decrease in the intramitochondrial energy status during anoxia, they attempted to rescue protein synthesis with an ATP-regenerating system or with

the addition of 1-mM ATP at the onset of anoxia. However, identical rates of protein synthesis were observed.

The authors of this investigation suggested that mechanisms might exist to suppress proton leak across the mitochondrial inner membrane during anoxia.

The biostructural theory may reach new explanations and conclusions superior to those established on the basis of other theories. Thus, the lack of oxygen has an effect in the breakdown of the cell biostructure, leading to a low protein synthesis. This effect is fully reversible upon reoxygenation due to oxygen interaction with the components of the biostructure.

The biostructural theory brings a logical explanation for the large current through K_{ATP}-channels and the decrease of the protein synthesis rate caused by anoxia. Considered here is the role of biostructure in the living cell, which is broken down under the anoxic conditions and fast restored upon reoxygenation. There are many published observations in the literature to support the above-mentioned hypothesis [24-31], even if they are not directly related to the biostructural theory. For example, it has been concluded that the NADH concentration is lower, while the NAD^+ concentration is higher in cancer tissue than in the normal one.[32] These data agree with the recent reports on the presence of NADH oxidase, predominantly in cell membrane and serum of cancer patients [33,34].

These tissues possess an altered biostructure and, of course, a low concentration of reduced species. Similarly, $FADH_2$ or coenzyme Q under the reduced state must be in a lower concentration in cancer cell than in the normal one.

The chemiosmotic theory

Mitchell [35] tried to explain the whole complexity of the living organisms by proton translocation through the biological membranes. He proposed that the function of the respiratory chain is to translocate protons thereby establishing a proton motive force, which consists of a ΔpH and a membrane potential. The chemiosmotic theory has been accepted as one of the great unifying principles of twentieth century biology [36]. The ATP synthesis in mitochondria and chloroplasts is based on this hypothesis in which transmembrane differences in proton concentration are central to energy transduction.

Nevertheless, we failed to explain the above-mentioned findings by Mitchell's hypothesis. Also, although this theory has stimulated much useful research in the last decades, it has so far lacked direct and convincing experimental support. At present, Mitchell's influence on the biochemical society is still so great that his formulation is almost blindly accepted in all textbooks.

Therefore, it should be clearly demonstrated that (1) a pH differential or a membrane potential can indeed lead to ATP synthesis and (2) that the operation of the respiratory chain can bring about a pH differential or a membrane potential.

Jagendorf [37] tried to demonstrate the feasibility of synthesizing ATP by building up a pH differential. Analogous experiments with rat liver mitochondria [38] have failed to show any synthesis of ATP, even if the pH was raised from 5.0 to 8.4.

In fact, Jagendorf considered Mitchell's chemiosmotic hypothesis for the mechanism of phosphorylation as a working guide for designing experiments [37]. Although he generated ATP under the experimental conditions, the source of ATP was not the pH differential as he claimed, but the consumption of the organic acids within the Krebs cycle. Thus, at the same pH gradient, ATP production was closely dependent on the type of organic acid (Table 1).

Table 1. ATP production by chloroplasts in the Jagendorf's experiment [37]

Acid	Acid in Pellet*		ATP* Yield
	Pellet I (pH 4.0)	Pellet II (pH 8.5)	
Succinic	1,016	156	82
Acetic	2,880	48	7

* µmoles per milligram of chlorophyll.

Moreover, Jagendorf did not measure the proton efflux or the anion influx during ATP production. In addition, the pH differential may create a stress to which the chloroplasts react by making ATP and also the shape of ATP production curves is different from case to case suggesting more complicated mechanisms than simple proton transduction. Thus, in another experiment [39], Jagendorf and Neumann showed that normally chloroplasts yield a higher amount of ATP at pH 8.0 than at pH 7.0 or, especially, at pH 6.6 in the absence of any pH gradient.

Mitchell and Moyle [40] showed that the operation of the respiratory chain causes a pH differential between the inside and outside of the membrane. This experiment was also carried out in exactly the same way by Tager, Veldsema-Currie, and Slater [38]. On adding 0.15 molar potassium chloride containing dissolved oxygen there was a rapid fall in the pH, followed by a slow return to the initial value. Nevertheless, they observed no oxidation of NADH to NAD^+ occurred on addition of oxygen as claimed the chemiosmotic hypothesis. On the other hand, the expected oxidation of NADH occurred only when ADP and inorganic phosphate were added together with the oxygen. Therefore, ATP production is not directly related with the fall in pH; there are two distinct phenomena but one. Piecing together the findings related to these phenomena, we are now able to postulate that during the chloroplasts illumination or by addition of oxygen to mitochondria a pH differential may occur due to the formation of an exited state, a true high-energy biological complex as stated by the biostructural theory. It is not a high-energy, non-phosphorylated intermediate as Slater claimed, but a biological structure, which cannot chemically be isolated. Most probably the excited state is a triplet state; a singlet state may result in a higher acidity. Thus, it was long ago discovered that the pK's of the photoexcited states of compounds such as phenols or amines differ enormously from the pK's of these molecules in the ground states [41-43]. Therefore, the first experimental data brought in support of the chemiosmotic hypothesis failed to demonstrate its validity, and the next ones proved to be questionable.

Some experimental confirmatory findings

We describe here a simple and suggestive experiment to show that when the biostructure is damaged by sodium azide a pH differential occurs. Thus, we used a wild strain of yeast (*Saccharomyces cerevisiae*), which was suspended in 0.35 molar sodium chloride. pH was varied by addition of 1 molar of hydrochloric acid or sodium hydroxide to vigorously stirred suspensions or sodium azide solutions. Suspensions containing from 0.5 g to 5 g yeast in 100 mL solution were continuously stirred and aerated and pH was monitored with a pH-Meter CG 838, Schott, Germany. Sodium azide from Merck, Germany was dissolved to obtain 0.1 or 0.2 molar solutions.

The pH value of both yeast suspension and sodium azide solution was brought to 5.32 with hydrochloric acid. On adding the sodium azide solution to the suspension, pH was suddenly increased up to 5.47 in less than 5 seconds. Then, it became 5.50 in a minute and slowly increased up to 5.59, when 0.18 mL of 1 molar hydrochloric solution was added to bring the pH to the initial value.

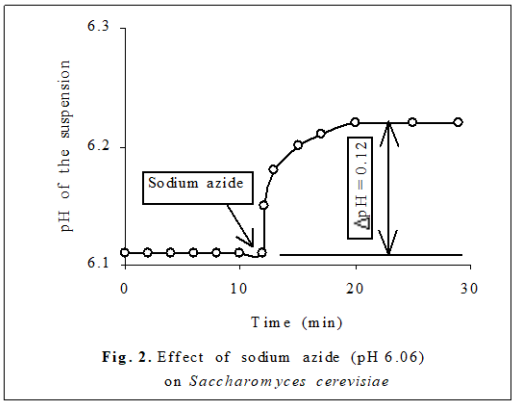

Fig. 2. Effect of sodium azide (pH 6.06) on *Saccharomyces cerevisiae*

We repeated the experiment at pH 6.11 at which ATP hydrolysis takes place without pH changes (Fig. 2). Similar results were obtained. Thus, on adding 5 mL of sodium azide solution to 10 mL yeast suspension, pH becomes immediately higher, increasing by 0.12 units. The experiment must also be repeated with DNP. According to Mitchell's hypothesis, the movement of the proton through membrane simply explains the facts. Nevertheless, we found an increase by three times in amino acid content of the suspension within the first minute after the treatment. The amino acid concentration increased by 6 times in the first hour to become almost normal the following day. Therefore, the mechanism of pH change is more complex than Mitchell's theory claims.

In brief, these experimental results that completely differ from those of Jagendorf's and Mitchell's teams are in good agreement with the biostructural theory, which takes into consideration the presence of a high-energy state characteristic of the living bodies. Therefore, sodium azide probably provoked the extinguish of the triplet states and, consequently, the proton consumption to generate the ground state. On forming these high energy states, a certain amount of protons was released. The simplest way to formulate this process is as follows:

$$(\text{GS-H}) \xrightarrow{O_2} (\text{TS}^{*-}\,\text{H}^+) \longrightarrow \text{TS}^{*-} + \text{H}^+ \xrightarrow{NaN_3} (\text{GS-H})$$

where, (GS-H) is the non-excited, ground state, TS is the triplet state, O_2 generates the triplet states under experimental conditions, and NaN_3 destroys these states to form the ground states. Obviously, such a biological, highly structured complex cannot be simply formulate as a chemical reaction, still this equation is very suggestive.

Conclusions

Searching for an explanation for the observations that anoxia causes a large current through K_{ATP}-channels and it decreases the protein synthesis, we remarked that the biostructural theory affords a valuable one. The chemiosmotic theory based on the supposition that a proton movement could explain the biological processes is not supported by most experimental findings; it cannot be a scientific fundament for the data investigated herein. Moreover, the experimental data that seemed to support Mitchell's ideas may be otherwise explained.

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