

Review

The electric field induced by light can explain cellular responses to electromagnetic energy: A hypothesis of mechanism

Albert Amat^{a,b,c,*}, Josepa Rigau^a, Ronald W. Waynant^b, Ilko K. Ilev^b, Juanita J. Anders^c

^a *Histology and Neurobiology Unit, Faculty of Medicine and Health Sciences, Rovira i Virgili University, C. Sant Llorenç 21, 43201 Reus, Spain*

^b *Division of Physics, Centre for Devices and Radiological Health, Food and Drug Administration, 12725 Twinbrook Parkway, Rockville, MD 20857, United States*

^c *Department of Anatomy, Physiology and Genetics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, United States*

Received 25 July 2005; received in revised form 6 October 2005; accepted 9 October 2005

Available online 21 November 2005

Abstract

When cells are irradiated with visible and near-infrared wavelengths a variety of stimulatory effects are observed in their metabolism. To explain the observed light effects, researchers try to identify the chromophores that are involved in the processes. However, the mechanism of light absorption by a chromophore does not explain many of the experimental observations and therefore the primary mechanism for cellular light responses remains unproven.

In addition to the ability of photons to produce electronic excitation in chromophores, light induces a wave-like alternating electric field in a medium that is able to interact with polar structures and produce dipole transitions. These dipole transitions are analyzed in the present article at different cellular and biochemical levels, leading to the proposal that the primary mechanism for the observed light effects is related to the light-induced electric field.

Published by Elsevier B.V.

Keywords: ATP/ADP ratio; ATP synthesis; Ca²⁺ regulation; Light-induced electric field mitochondria; Na⁺/K⁺ ionic pump; Photochemistry; ROS

Contents

1. Introduction	153
2. Photoinduced biochemical and cellular effects	153
2.1. Light modification of enzymatic activity	153
2.2. Effect of light on adenosine phosphate molecules	154
2.3. Effect of light on the Na ⁺ /K ⁺ ATPase pump	155
2.4. Effect of light on intracellular free Ca ²⁺ concentration	155
2.5. Effect of light on mitochondrial ATP synthesis	156
2.6. Production of reactive oxygen species	157
3. General summary	157
3.1. Stimulatory effect of electromagnetic energy	157
3.2. Inhibitory effect of electromagnetic energy	158
3.3. Long-lasting effect of cellular irradiation	158

* Corresponding author. Tel.: +34 977 759 343; fax: +34 977 759 322/343.

E-mail address: albert.amat@urv.net (A. Amat).

4. Abbreviations	158
References	159

1. Introduction

The phenomenon of light absorption to produce electronic excitation of atoms and molecules has long been accepted by photochemists and photobiologists. When molecules are excited by light and immediately take part in chemical reactions, an improvement in the kinetics of the reactions is observed [1]. After the invention of the laser in the early 1960s, the field of laser chemistry developed and attention focused on the photochemical processes induced by laser light, such as multiphoton vibrational and electronic photochemistry [2]. Molecules that transform their electronic energy levels after light absorption are said to be *chromophores* [1]. For visible and near-infrared (NIR) wavelengths, chromophores are typically metals or molecules that contain metallic atoms in their structure [3]. Biological examples of these kinds of chromophores are proteins with metallic prosthetic groups, such as cytochrome *c* oxidase (COX), a fundamental enzyme for mitochondrial ATP synthesis [4]. COX contains a heme group (with iron), copper, zinc and magnesium [5]. The protein part of the molecule absorbs light in the UV range, and the metals absorb visible and NIR wavelengths [5].

To identify the mechanism involved when cells and tissues are light-irradiated, researchers look for the chromophore that is absorbing the photon. The chromophore must be involved in a biochemical pathway that connects to the observed experimental and clinic effects. However, identifying the chromophore and the biochemical pathway is difficult because in living cells thousands of different reactions are occurring simultaneously and the same final cellular state may be produced by different biochemical pathways. As a consequence, the mechanisms responsible for modulating cellular activity by light remain unidentified. The situation is even more unclear when light responses are obtained when irradiating systems *that do not have a chromophore*, i.e. enzymes with no metallic atoms [6,7] or adenine nucleotides molecules [8–10].

In addition to the ability of light to promote electronic excitation when absorbed, its electric characteristics also have to be considered. By definition, there is an intense oscillating electric field (E) associated with visible and NIR photons. This field produces electrical current if free electrons are present in the medium. The presence of the current leads to the absorption of the energy and conversion into heat (Joule heating) [11]. When there is a non-significant number of free charges in a medium, the medium is basically non-conducting, or an insulator. Polar molecules and bound electrons in chemical bonds, which also behave as exceedingly small dipoles, constitute a non-conducting medium [12]. These bound electrons are attached to the nucleus in a man-

ner that can be compared with an elastic spring and oscillate at their natural frequency – the resonant frequency [13]. When the electric field of a photon that oscillates at the same electronic resonant frequency approaches the electron, it is absorbed and electronic excitation occurs [13]. Any other frequencies are not absorbed, but they modify the frequency of oscillation of bound electrons. Because of this interaction, the light slows in the medium and is refracted [14], and some of its energy is stored in the medium in the form of electric potential energy [15]. This exchange of energy produces electric dipole transitions of the material, i.e. dipoles change their internal characteristics due to a displacement of bound charges [14,15]. Electromagnetic energy includes a magnetic field (B). Magnetic fields are produced by charges in movement when these charges are exposed to an electric field [13]. A cell is mostly formed by dipole structures, polar molecules and bound electrons that are able to experience dipole transitions when an electric field E is applied [12]. The light-induced magnetic component B is much less important because a cell is not a magnetic medium: free charges that can be displaced by an electric field will come from metals, and their concentration in cells is very low [16,17].

Cells respond to a variety of externally applied forces and physical phenomena, such as low intensity ultrasound and various types of electromagnetic energy. The electrical component of electromagnetic energy has an effect on dipolar molecules such as enzymes, ionic pumps, nuclear material and nucleotide molecules [18–22]. In the present review article, we will compare the effects of general electromagnetic energy to the effects of cellular light irradiation at different levels and discuss whether the effects of light fit better into the absorption and electronic excitation model or into the induced electric field model. The cellular processes we analyze are (1) enzymatic activity, (2) adenosine phosphate molecules kinetics, (3) the Na^+/K^+ ATPase ionic pump, (4) Ca^{2+} activation, (5) mitochondrial ATP synthesis and (6) reactive oxygen species (ROS) production.

2. Photoinduced biochemical and cellular effects

2.1. Light modification of enzymatic activity

Enzymes are proteins that catalyze chemical reactions in biological structures. Some of them contain metals (i.e. COX, glutamate dehydrogenase) [23,24], while others contain only amino acids (i.e. hexokinase, ATPases and ATP synthetases) [6,25]. Bolognani in 1992 found that myosin ATPases previously inactivated by CO_2 gas could be partially reactivated after irradiation with He–Ne (632.8 nm) and diode (904 nm) lasers [6,7]. Also, the irradiated enzyme

could act in the reverse mode (when appropriate conditions were met), synthesizing ATP from ADP and P_i . This ATP synthesis was enhanced by both visible and NIR laser irradiation and also by a pulsed electric field (110 Hz, 0.11 mV/cm). These effects were non-thermal, since the temperature of the sample did not increase and there was no absorption at the wavelengths studied. According to the authors, the effects of the low-frequency electric field may be produced by the electromagnetic interaction along the protein that favors dipole transitions. The effects of the light, as proposed by Lubart et al. and assumed by the authors, could be due to quantitatively low light absorption despite the lack of a chromophore [26]. In these experiments, laser light and the electrical device produce oscillating electric fields. Light oscillates at frequencies in the range of 10^{14} Hz [3] and in this case the electrical device oscillates at 110 Hz. However, it is possible for both electric fields to qualitatively produce the same kind of electrical interaction along the enzyme. Recently, it has been proposed that the effects of light on the ATPase enzyme are due to the fact that the active center of the enzyme experiences an internal resonant-type field that is trapped inside the structure; this field produces strong electrical interactions and conformational changes. The rise in energy in the active center produces a trigger action, with a step-like response similar to a phase transition [27]. Since this rise in energy is produced in the active center and this center is responsible for the chemical transformation of substrates into products, this electrical interaction hypothesis may explain the effects of light in non-absorbing enzymes.

COX experience sensitivity to visible and NIR wavelengths [5,28]. Light at 632.8 nm modifies the activity of the enzyme but does not change the electronic transfer rate of the isolated cytochrome chain, which is part of the structure of the COX complex and contains the metallic atoms [5]. The authors of [5] suggest that it is the micro-environment of the metal–protein (i.e. the apoprotein) that is affected rather than the metals or the heme group per se. This explanation is conceptually similar to what was proposed above for enzymes without an absorbing atom [27]. However, the presence of the metals explains the absorption of light by COX, and therefore absorption may play a role in this case, but why a change in structures that are not in the active center should improve the reaction kinetics is still not explained.

Summary. Enzymes without an absorbing atom and enzymes with absorbing atoms in their structure experience a modification in their activity when they are exposed to light. A low-frequency electric field produces the same kind of response as light in enzymes without an absorbing atom in their structure, so it may be possible that both electrical phenomena share the same mechanism.

2.2. Effect of light on adenosine phosphate molecules

ATP, ADP and AMP are molecules that are made up of adenosine and different numbers of phosphates (Fig. 1)

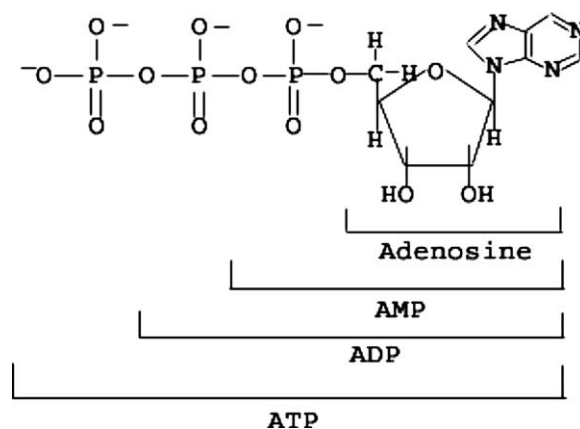


Fig. 1. Structure of the three adenosine phosphate molecules, ATP, ADP and AMP [29].

[29]. After a spectroscopic analysis of adenosine phosphate molecules, it is observed that the absorption of light occurs in the adenosine part of the molecules, typically at UV wavelengths. Visible and NIR frequencies are not absorbed [8]. However, when ATP, ADP and AMP are exposed to visible and NIR light, significant changes in the kinetics of the chemical reactions in which they are involved are observed [8–10]. The velocities of the luciferine–luciferase reaction increased when irradiated ATP was used as a substrate [9]. The kinetic parameters K_m and V_{max} (the Michaelis constant and the maximum velocity, which define the Michaelis–Menten-type enzymatic reactions) of ATPase, adenylate kinase and the ADP/ATP mitochondrial carrier reactions changed when irradiated adenosine-phosphate molecules were used as the substrates [8]. The kinetic parameters K_m and V_{max} of the hexokinase reaction also increased when the substrate ATP was exposed to light at specific wavelengths [10].

When a molecule is irradiated and the energy of light is absorbed, any excited state due to electronic excitation will decay in milliseconds if the energy is not used for a chemical reaction [2,30]. Decay will result in light emission or, usually, in an increase in temperature. When ATP, ADP and AMP in solution were irradiated, there was no increase in temperature. Also, the irradiated samples were added to the rest of the substrates and enzymes after 30 s, which is enough time for any electronic excited state to go back to the non-excited form. Therefore, the mechanism here must be completely different [8,9]. When the ATP molecule was analyzed, it was found that its last phosphate bond had different interconvertible resonance isoforms of similar but not equal energy (Fig. 2) [31]. As a result of these different isoforms, the double phosphor–oxygen bond is not in a definite position but travels around the phosphorus atom [31]. Other resonance forms of the orthophosphate are highly improbable and do not contribute significantly to the total resonance state of the molecule because two like charges are adjacent, which increases the instability of the structure (Fig. 3) [31]. Bonds with different resonance

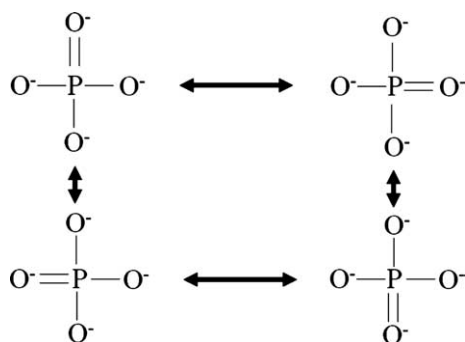


Fig. 2. Probable resonance isoforms of orthophosphate, where two negative charges are not in adjacent atoms [31].

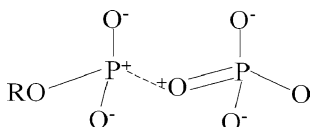


Fig. 3. Improbable resonance isoform of orthophosphate, unstable because of two adjacent like electrical charges, which contribute to the breakdown of ATP's terminal bond [31].

forms usually have electrons that are less tightly bound to the nucleus, and light produces larger responses when it interacts with them [32]. It is therefore possible that the displacement of the bound charges that the induced electric field produces in the molecule leads to the formation of a new, unstable, resonant state of ATP, which is easily cleaved by the enzyme. Since the effects of light do not vanish in a very short time [8], we can hypothesize that the new forms are metastable and that activation energy is needed to complete the process.

Summary. AMP, ADP and ATP do not absorb visible and NIR wavelengths. However, irradiation at non-absorbed wavelengths modifies the kinetics of a chemical reaction in which these light-exposed molecules are a substrate. A metastable new isoform of the phosphate can be produced when the light-induced electric field changes the charge distribution in the phosphate structure. This new isoform may be easily cleaved by the enzymes.

2.3. Effect of light on the Na^+/K^+ ATPase pump

Na^+/K^+ ATPase pump is a membrane enzyme that hydrolyzes ATP only if both Na^+ and K^+ are present [33]. The split of ATP provides the energy to transport the Na^+ and K^+ across the membrane and maintain the Na^+/K^+ gradient. In animal cells, the Na^+/K^+ gradient controls cell volume, drives the active transport of sugars and amino acids, and renders nervous and muscular cells electrically excitable. The fact that more than a third of the ATP consumed by an animal at rest is used to operate this pump underscores the importance of this mechanism [33].

Visible wavelengths (632.8 nm) are reported to increase the activity of Na^+/K^+ ATPase in erythrocytes [34,35].

No chromophore for this wavelength is present in the pump, and known cellular chromophores do not have an effect on the enzyme's function [35]. An interesting observation is that, due to a transmembrane potential induced by the electric field that provides the energy for operation, the Na^+/K^+ ATPase can be activated by electrical currents and function without ATP [36,37]. In other studies, alternating electric fields also modify the activity of the pump [21]. The increased activity of the Na^+/K^+ ATPase after light irradiation may be due to the Na^+/K^+ ATPase functioning without ATP, with the induced transmembrane electric potential produced by the light-induced electric field. Moreover, ATP that exists in the cytoplasm may change its chemical properties due to irradiation, thus increasing the effectiveness of the hexokinase reaction (as described in [10]) which is the ATP source in red blood cells [38]. In cells that do have mitochondria, the operation of the Na^+/K^+ ATPase without ATP due to irradiation in concrete cellular metabolic states will lead to an increased cellular ATP concentration and therefore ATP synthesis will stop. This hypothesis is supported by the experimental observation that ouabain, a substance that blocks the Na^+/K^+ ATPase, stops mitochondrial respiration by increasing cellular ATP concentration [39], which shows the importance of ATP synthesis regulation by the final product of the process, ATP.

Summary. Transmembrane potentials operate, without ATP splitting, the Na^+/K^+ ATPase pump when cells are exposed to electric fields. Light permits the operation of the Na^+/K^+ ATPase without using ATP. The light-induced electric field may generate transmembrane potentials to operate the Na^+/K^+ ATPase, sharing the mechanism with low-frequency electric fields.

2.4. Effect of light on intracellular free Ca^{2+} concentration

Ca^{2+} is a fundamental second messenger in cells that regulates many functions and initiates important biochemical pathways [40]. It is stored in the endoplasmic reticulum (ER) and released to cytoplasm after phosphatidyl inositol biphosphate (PIP_2), a structural phospholipid in the membrane, is hydrolyzed by phospholipase C into inositol diphosphate (IP_3) and diacylglycerol (DAG) [41]. IP_3 opens the calcium channels in ER, and this leads to phosphorylation of proteins and gene expression [42]. Ca^{2+} plays an important role in mitochondrial ATP synthesis. The difference in voltage between the outer and inner part of the mitochondrial membrane, permits the uptake of Ca^{2+} by the organelle via a simple mechanism of electrochemical gradients. Ca^{2+} uptake by the mitochondria progressively diminishes the membrane potential and activates ATP synthesis [43].

The effects of light on Ca^{2+} signaling both at cellular membrane and at mitochondrial level have been described. When plant cells and mammalian cells are irradiated with red and NIR light, oscillations in calcium concentration are induced [44,45] by releasing Ca^{2+} stored in the ER.

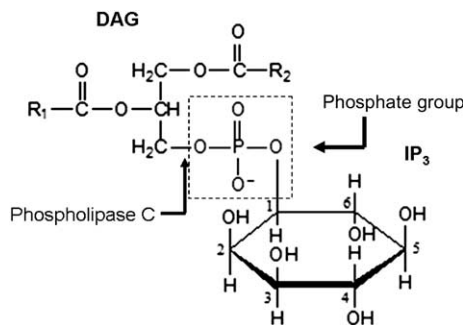


Fig. 4. This scheme shows where the enzyme phospholipase C breaks down PIP₂ to form IP₃ and DAG. A phosphate structure in IP₃, the same as in ATP, bonds to CH₂ terminal in DAG [44].

Since phosphorus is incorporated into the membrane after light irradiation, it is suggested that the phosphorus is used to synthesize new PIP₂ that was previously split due to the light and, therefore, that IP₃ is involved in the process. No chromophore is involved in this process.

IP₃ is attached to DAG to form PIP₂ by a phosphate structure like that in ATP (Fig. 4).

As happens with this phosphate structure when it is part of an ATP molecule, the bounding structure is expected to be unstable due to a displacement of charges induced by the electric field. The new configuration will lead to an easier cleavage by the enzyme phospholipase C.

Mitochondrial Ca²⁺ is also affected by light irradiation. Visible light increases the mitochondrial membrane potential and mitochondrial Ca²⁺ concentration when hepatocytes are irradiated [46]. Interestingly, however, when the medium was Ca²⁺ depleted, light irradiation still changed the membrane potential [47]. The light-induced electric field is able to change the membrane potentials in the mitochondrial membrane by transferring charges from the outer side to the inner side or directly by a polarization of membrane dipoles. The light effect in the mitochondrial membrane may then be independent of Ca²⁺ concentration.

Summary. Ca²⁺ changes after light irradiation may be produced by the PIP₂–IP₃ pathway. The structure that will be affected is a phosphate group, like that in ATP, which is not a chromophore but can experience a charge displacement produced by the light-induced electric field. The light-induced electric field can directly change the electric potential in the mitochondrial membrane.

2.5. Effect of light on mitochondrial ATP synthesis

The effect of light on the synthesis of adenosine triphosphate, one of the most important cellular molecules, has been a major focus of study. In the 1980s, Passarella showed that in isolated mitochondria exposed to 632.8 nm laser light, ATP synthesis increased [47]. The study of this light response continued with the identification of mitochondrial chromophores for red light. The chromophores were found in the cytochromes that are part

of the electronic transport chain, and specifically in the enzyme COX [48]. COX is involved in the last step of ATP synthesis and has fundamental regulatory properties on respiration [49]. COX changes its spectrum when it is irradiated with red and NIR light [50]. If the absorption of light is responsible for ATP synthesis, COX seems to be involved. However, several reported findings should be considered. When HeLa cells are irradiated, ATP synthesis starts 25 min after the irradiation has stopped [51]. How can this delay be explained? A photochemical process produced by electronic excitation would be:

Irradiation → Absorption

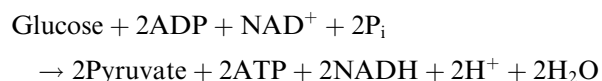
→ Conversion into chemical energy/heat/light

This would occur within milliseconds [2,30] and would happen independently of the cellular state if ADP and P_i were available. If they were not available, ATP synthesis is not possible. It has been proposed that the delay may be due to the delayed synthesis of adenine nucleotide molecules, i.e. ADP [51]. However, ADP is stored in the cell by binding other molecules and becomes free when needed but the most important source of ADP is the breakdown of ATP [49]. It may be possible that after the cells are exposed to light, irradiated ATP in the cytoplasm reacts with enzymes and substrates, free ADP concentration is increased and the ATP/ADP ratio is decreased. Increased free ADP levels and decreased ATP/ADP ratios are important stimuli for starting mitochondrial ATP synthesis [49,52–54]. This effect on ATP and, therefore, on ADP concentration and the ATP/ADP ratio, may explain the delay in ATP synthesis. The ATP synthesis caused by the breakdown of ATP may also explain why COX in irradiated cells has 28% more activity than non-irradiated cells 18 h after irradiation has stopped. This sustained activity cannot be explained by light absorption by the enzyme.

Light can also have a direct effect in the cellular organelles that are responsible for ATP synthesis. A fundamental mechanism that converts light energy into chemical energy (ATP) is photosynthesis. When chloroplasts are exposed to light pulses and electric pulses, the observed effects on ATP synthesis are exactly the same [55]. ATP synthesis has also been reported for in vitro mitochondria when the solution containing the organelles was exposed to electric pulses [56]. These effects were not due to an absorption of the current (Joule heating) but to an induced transmembrane potential. More recent studies have demonstrated how electric forces are essential for ATP synthesis. Specifically generated membrane potentials are able to act as a motor in mitochondrial ATP synthase, so ATP synthesis is enhanced [57]. From this evidence, the question that arises is why would a light-induced electric field not have the same effect? When cells are treated with oligomycin and are light-irradiated, ATP synthesis is blocked but the increase in membrane potential is not [58]. This increase in the mitochondrial membrane potential is also observed when isolated mitochondria are irradiated, which leads to an

increased exchange of ATP/ADP from inside the organelle to outside it [59], and when hepatocytes are irradiated in a Ca^{2+} -independent manner [46]. Is it reasonable to propose that light, through its induced electric field, and electric pulses share the same mechanism in ATP synthesis by altering the mitochondrial membrane potential?

The theory of energy absorption and ATP synthesis by COX does not explain why light also has an effect when the cells are in an anaerobic state [60]. The cell must then use glycolysis as source of ATP [61]. Glycolysis is started by the hexokinase reaction that converts glucose into glucose-6-phosphate. At the end of the process, from one molecule of glucose, two molecules of ATP are synthesized [62]. The initial and final states are:



Pyruvate is used in respiration and NADH in reduction reactions. In an anaerobic situation, the effect of the light may be linked to the effect on cytoplasmic ATP or hexokinase. Irradiated ATP reacts faster in the hexokinase reaction [10]. Also, the enzyme may be affected by the induced electric field, as happened with other ATPases [6,7].

Summary. (1) Light alters the mitochondrial membrane potential, which is a stimulus for ATP synthesis. Since electric pulses produce the same effect, the mechanism may be related to the light-induced electric field. (2) Extramitochondrial ATP synthesis may also be enhanced by light irradiation, which increases the kinetics of the hexokinase reaction when cellular metabolism is in anaerobic conditions. (3) The breakdown of light-irradiated ATP in cytoplasm will change free ADP concentration and ATP/ADP ratios, which is a stimulus for starting ATP synthesis.

2.6. Production of reactive oxygen species

When photosensitizers absorb light, electronic excitation occurs. Photosensitizers in cells are endogenous chromophores such as porphyrins, flavins and mitochondrial cytochromes [63,64]. Because of their special characteristics, photosensitizers are able to transfer the energy to a nearby molecule that is in a triplet ground state [65]. One of the very few molecules with a triplet ground state is oxygen, O_2 . As a result of the transfer of energy from photosensitizers to oxygen, ROS, and particularly superoxide anion ($\text{O}_2^{\cdot-}$), are produced [66]. ROS are cytotoxic and, when their concentration is high, cells are killed [67]. When ROS are produced at low rates – about a few nM/min/mg protein [68] – they have a wide range of stimulatory effects [69]. Lubart et al. [70] have proposed that the mechanism for cellular light effects is via ROS production. H_2O_2 , a molecule produced after superoxide anion reacts with water, can increase Ca^{2+} concentration and therefore stimulate ATP synthesis. Nitric oxide (NO) is an important molecule because it is a cellular messenger in signal trans-

duction processes [71]. Lubart et al. [70] also proposed that light-produced ROS can stimulate NO production by NO synthase (NOS), promoting a cascade of cellular reactions. However, there is a delayed NOS activity in tissues such as arteries [72] and heart [73] following visible laser light irradiation. The hypothesis that ROS production is responsible does not fit in with these observations, since ROS molecules have a very short lifetime, varying from ns to ms [70]. It is also difficult to explain the observed light effects in tissues with a poor oxygen supply: O_2 will not be surrounding the photosensitizers, so ROS production through this mechanism will not be significant.

Associated with ATP synthesis is the production of superoxide anion by COX [74]. COX has been designed not to release $\text{O}_2^{\cdot-}$ but a small amount of the anion is unavoidably formed [74]. $\text{O}_2^{\cdot-}$ can be converted into a hydroperoxyl radical (HO_2^{\cdot}), which can react spontaneously with another superoxide anion to form H_2O_2 [75]. All these toxic derivatives of O_2 are scavenged by protective enzymes such as superoxide dismutase, catalase and peroxidase [76]. A light-induced electric field can produce a transmembrane potential, leading to ATP synthesis, as described above. Light-excited ATP can react faster with other substrates, which would increase ADP and decrease the ATP/ADP ratio, thus leading to increased ATP synthesis. Light-induced Ca^{2+} changes can also enhance mitochondrial ATP synthesis. All of these processes therefore increase ROS production. Under these circumstances, ROS would therefore not be the cause but the consequence of light-induced effects. However, the protective enzymes will absorb or diminish the ROS effects. This protection progressively decreases with age [77], which may explain the irregular effects observed in aged individuals due to irradiation [60].

Summary. ROS, which have stimulatory effects in cells, increase their cellular concentrations after light irradiation in the presence of O_2 molecules. ROS are also produced after enhanced cellular metabolism and ATP synthesis. Since ROS have a very short lifetime, varying from ns to ms, and after irradiation the proposed biochemical pathway requires several minutes to be active, the increase in ROS concentration after irradiation is likely to be produced by the enhanced ATP synthesis triggered by the light-induced electric field. They are therefore the consequence and not the cause of the effects of light.

3. General summary

3.1. Stimulatory effect of electromagnetic energy

The effects of a light-induced electric field will be non-specific because its intrinsic mechanism is to displace any bound charge in a wavelength dependent manner [13,14] or to create membrane potentials. This does not mean that any enzyme or chemical bond will be functionally affected by the induced electric field. The effect will depend on the cellular concentrations of substrates, products and

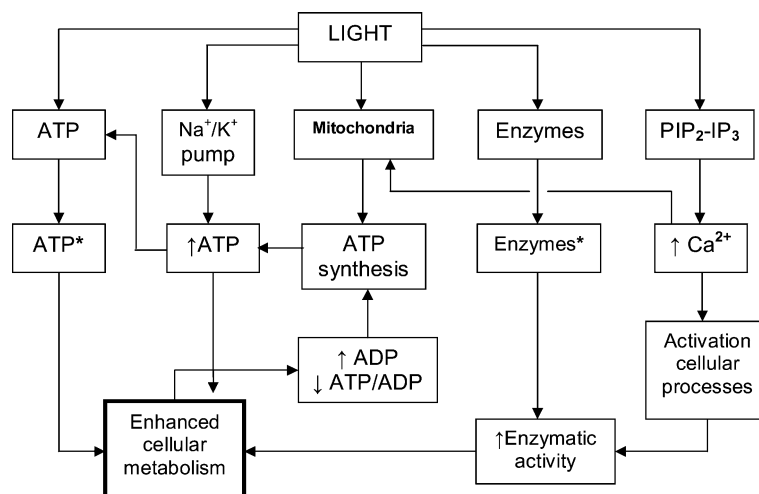


Fig. 5. Cellular pathways that can be activated when a light-induced electric field interacts with ATP, the Na^+/K^+ ATPase, mitochondria, enzymes and membrane phospholipids that regulate Ca^{2+} signal (legend: ATP^* , light-excited ATP; enzymes^* , light-excited enzymes).

enzymes. These concentrations will be determined by the biochemical pathways that are active in the cell when irradiation occurs. There, it will depend on the metabolic state of the cell.

The possible effects of a light-induced electric field on a cell are summarized in Fig. 5. An increase in Ca^{2+} concentration will initiate several metabolic processes. Enzymatic activity will be increased due to both the effects of Ca^{2+} and the activation of enzymatic molecules by the light. The metabolism will be powered by the direct action of light on mitochondria, the effects of Ca^{2+} on mitochondria, and the excitation of ATP in cytoplasm by the irradiation. If Na^+/K^+ ATPase functions without ATP due to the transmembrane potential, ATP concentration will be increased.

3.2. Inhibitory effect of electromagnetic energy

When ATP is hydrolyzed, ADP concentration will be increased and the ATP/ADP ratio will be decreased, thus stimulating ATP synthesis. The process of light-induced ATP breakdown/synthesis is circular and the energy to maintain it comes from the light. While cells are irradiated, the process continues until an excess of products will stop the biochemical pathways by a feedback mechanism. As a result, a saturation of the effect will be observed. In some cases, more incoming energy will force the cell to use substrates that are stored. When there are no more available substrates, the cell will stop some metabolic pathways and certain cellular functions will be disabled. A light-induced inhibition will then occur.

3.3. Long-lasting effect of cellular irradiation

When irradiation stops, the enhanced metabolism can continue for some time, depending on the concentration of substrates, products and enzymes in the cell at that

moment and on the active biochemical pathways. The energy required will be supplied by a previous light-induced ATP excitation and increased ATP synthesis, enhanced by any of the mechanisms described above.

An interesting example of how a light-induced electric field is able to affect a living organism is the way that light guides neuronal growth. When a laser light (800 nm) approaches neurons, without being in direct contact with the cells, their dendrites grow towards the laser spot. The authors propose that dipoles in cellular cytoplasm feel a weak optical dipole force directed to the center of the laser spot. Due to this force, the dendrites are orientated and grow following the lines of force of the electric field E [78].

The absorption of light by chromophores such as hemoglobin or melanin plays a fundamental role when electromagnetic energy is used to heat target structures. Absorption may also play an important role in certain cellular processes, such as the direct effect of light on ATP production by the mitochondria. However, none of the proposed hypotheses to date accounts for all the observed light-induced effects. The effects of the light-induced electric field on molecules and cell structures provide a general explanation for the observed cellular light effects and imply that there is not just one but numerous primary mechanisms. These mechanisms depend on the various classes of molecules and structures that are affected and perhaps on the physical parameters that characterize the electric field, i.e., intensity, frequency and polarization of light.

4. Abbreviations

ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
B	magnetic field

COX	cytochrome <i>c</i> oxidase
DAG	diacylglycerol
<i>E</i>	electric field
ER	endoplasmic reticulum
K_m	Michaelis constant of enzymatic reactions
V_{max}	maximum velocity of enzymatic reactions
IP ₃	inositol triphosphate
NIR	near-infrared
NO	nitric oxide
NOS	nitric oxide synthase
O ₂ ^{•-}	superoxide anion
P _i	inorganic phosphate
PIP ₂	phosphatidyl inositol diphosphate
ROS	reactive oxygen species

References

- [1] I.N. Levine, Physical Chemistry, fifth ed., Mc Graw-Hill Inc., New York, 2001 (Chapter 18).
- [2] V.S. Letokhov, Laser-induced chemistry, *Nature* 305 (8) (1983) 103–108.
- [3] W.T. Silfvast, Laser Fundamentals, second ed., Cambridge University Press, Cambridge, 2004, pp. 23–37.
- [4] O.M. Richter, B. Ludwig, Cytochrome *c* oxidase-structure, function, and physiology of a redox-driven molecular machine, *Rev. Physiol. Biochem. Pharmacol.* 147 (2003) 47–74.
- [5] D. Pastore, M. Greco, S. Passarella, Specific helium-neon laser sensitivity of the purified cytochrome *c* oxidase, *Int. J. Radiat. Biol.* 76 (6) (2000) 863–870.
- [6] L. Bolognani, M. Cavalca, C. Magnani, N. Volpi, ATP synthesis catalysed by myosin ATPase: effect of laser and e.m. field, *Laser & Technol.* 2 (3) (1992) 115–120.
- [7] L. Bolognani, G. Majni, M. Costato, M. Milani, ATPase and ATP synthetase activity in myosin exposed to low power laser and pulsed electromagnetic fields, *Bioelectrochem. Bioenerg.* 32 (1993) 155–164.
- [8] S. Gagliardi, A. Atlante, S. Passarella, A novel property of adenine nucleotides: sensitivity to helium-neon laser in mitochondrial reactions, *Biochem. Mol. Biol. Int.* 41 (3) (1997) 449–460.
- [9] A. Amat, J. Rigau, R. Nicolau, M. Aalders, M.R. Fenoll, M.J.C. van Gemert, J. Toma's, Effect of red and near-infrared laser light on adenosine triphosphate (ATP) in the luciferine–luciferase reaction, *J. Photochem. Photobiol. A* 168 (2004) 59–65.
- [10] A. Amat, J. Rigau, R.W. Waynant, I.K. Ilev, J.M. Tomàs, J.J. Anders, Modification of the intrinsic fluorescence and biochemical behavior of adenosine triphosphate ATP after irradiation with visible and near-infrared laser light, *J. Photochem. Photobiol. B* 81 (2005) 26–32.
- [11] R.W. Ditchburn, Light, Dover Publications, New York, 1961, pp. 407–408.
- [12] E.H. Grant, R.J. Sheppard, G.P. South, Dielectric Behavior of Biological Molecules in Solution, Clarendon Press, Oxford, 1978 (Chapter I).
- [13] R.P. Feynman, R.B. Leighton, M. Sands, The Feynman Lectures on Physics, Addison-Wesley, Redwood City, 1963, vols. I & II and Chapters 31 & 32.
- [14] M. Born, E. Wolf, Principles of Optics, sixth ed., Pergamon, Oxford, 1984 (Chapter II).
- [15] C.Z. Tan, Electric potential energy of the incident light and the Hamiltonian of the induced oscillators in non-absorbing isotropic dielectrics, *Physica B* 269 (1999) 373–378.
- [16] C.C. Quintana, A.A. Olmedilla, N.N. Antoine, A.A. Ollacarizqueta, The occurrence of metals Al, Fe, Ni, Cu, Zn in the nuclei of animal cells: an ultrastructural, in situ, X-ray microanalytical study, *Biol. Cell* 61 (1987) 115–119.
- [17] F.D. Silva, R.J.P. Williams, The Biological Chemistry of the Elements: The Inorganic Chemistry of Life, Oxford University Press, Oxford, 2001 (Part II).
- [18] W.H. Chang, L.T. Chen, J.S. Sun, F.H. Lin, Effect of pulse-burst electromagnetic field stimulation on osteoblast cell activities, *Bioelectromagnetics* 25 (6) (2004) 457–465.
- [19] D. Quaglino, M. Capri, L. Zecca, C. Franceschi, I.P. Ronchetti, The effect on rat thymocytes of the simultaneous in vivo exposure to 50-Hz electric and magnetic field and to continuous light, *Sci. World J.* 4 (Suppl 2) (2004) 91–99.
- [20] Y. Liu, H. Yang, H. Takatsuki, A. Sakanishi, Effect of ultrasonic exposure on Ca(2+)-ATPase activity in plasma membrane from Aloe arborescens callus cells, *Ultrason Sonochem.*, Jun 1(2005) (Epub ahead of print).
- [21] M. Blank, Na,K-ATPase function in alternating electric fields, *FASEB J.* 6 (7) (1992) 2434–2438.
- [22] R. Cadossi, F. Bersani, A. Cossarizza, P. Zucchini, G. Emilia, G. Torelli, C. Franceschi, Lymphocytes and low-frequency electromagnetic fields, *FASEB J.* 6 (9) (1992) 2667–2674.
- [23] C.E. Cooper, The steady-state kinetics of cytochrome *c* oxidation by cytochrome *c* oxidase, *Biochim. Biophys. Acta* 1017 (1990) 187–203.
- [24] A. Ostuni, S. Passarella, E. Quagliarello, Photomodulation of glutamate dehydrogenase properties by red light, *J. Photochem. Photobiol. B* 20 (1993) 101–111.
- [25] O. Monasterio, M.L. Cardenas, Kinetic studies of rat liver hexokinase D ('glucokinase') in non-co-operative conditions show an ordered mechanism with MgADP as the last product to be released, *Biochem. J.* 371 (1) (2003) 29–38.
- [26] R. Lubart, Y. Wollman, H. Friedmann, S. Rochkind, I. Laulicht, Effects of visible and near-infrared lasers on cell cultures, *J. Photochem. Photobiol. B* 12 (1990) 305–310.
- [27] A.M. Bolognani-Fantin, M. Milani, M. Costato, Photon–enzyme interaction: The onset of a reactivation mechanism, *Il Nuovo Cimento, Note Brevi* 19 (1) (1997) 113–117.
- [28] T.I. Karu, Molecular mechanism of the therapeutic effect of low-intensity laser irradiation, *Lasers Life Sci.* 2 (1) (1988) 53–74.
- [29] M.A. Bianchet, P.L. Pederse, L.M. Amzel, Notes on the mechanism of ATP synthesis, *J. Bioenerg. Biomembr.* 32 (5) (2000) 517–521.
- [30] F.A. Popp, J.J. Chang, J. Fisch, Biophotons, Kluwer Academic Publishers, Dordrecht, 1998 (Chapter 1).
- [31] C.K. Mathews, K.E. van Holde, K.G. Ahern, Biochemistry, third ed., Benjamin/Cummings, San Francisco, 2000, pp.77–78.
- [32] R.W. Boyd, Non-linear Optics, second ed., Academic Press, Amsterdam, 2003 (Chapter 5).
- [33] P.L. Pedersen, E. Carafoli, Ion motive ATPases, *Trends Biochem. Sci.* 12 (1987) 146–150, and 186–189.
- [34] A.M. Moroz, Na⁺, K⁺-ATPase activity in erythrocytes after the effect of laser radiation, *Ukr. Biokhim. Zh.* 55 (6) (1983) 674–676.
- [35] E. Kilanczyk, D. Palecz, M. Bryszewska, Effect of red laser light on Na⁺,K⁽⁺⁾-ATPase activity in human erythrocyte membranes sensitized with Zn-phthalocyanine, *J. Clin. Laser Med. Surg.* 20 (2) (2002) 71–75.
- [36] J. Teissie, T. Yow Tsong, Voltage modulation of Na⁺/K⁺ transport in human erythrocytes, *J. Physiol.* 77 (9) (1981) 1043–1053.
- [37] E.H. Serpersu, T.Y. Tsong, Activation of electrogenic Rb⁺ transport of (Na,K)-ATPase by an electric field, *J. Biol. Chem.* 259 (11) (1984) 7155–7162.
- [38] M.C.L. Phillips, C.D. Moyes, B.L. Tufts, The effects of cell ageing on metabolism on rainbow trout red blood cells, *J. Exp. Biol.* 203 (2000) 1039–1045.
- [39] T.I. Karu, L.V. Pyatibrat, N.I. Afanasyeva, A novel mitochondrial signaling pathway activated by visible-to-near infrared radiation, *Photochem. Photobiol.* 80 (2) (2004) 366–372.
- [40] E. Carafoli, Calcium – a universal carrier of biological signals, *FEBS J.* 272 (5) (2005) 1073–1089.
- [41] H.J. Xia, G. Yang, Inositol 1,4,5-trisphosphate 3-kinases: functions and regulations, *Cell Res.* 15 (2) (2005) 83–91.

- [42] L. Stryer, Biochemistry, fourth ed., W.H. Freeman and Company, New York, 1995 (Chapter 13).
- [43] I.E. Scheffler, Mitochondria, Wiley-Liss, New York, 1999, pp. 232–235.
- [44] I.D. Volotovskiy, S.G. Sokolovskiy, E.L. Nikiforov, V.P. Zinchenko, Calcium oscillations in plant cell cytoplasm induced by red and far-red light irradiation, *J. Photochem. Photobiol. B* 20 (1993) 95–100.
- [45] E.E. Alexandratou, D. Yova, P. Handris, D. Kletsas, S. Loukas, Human fibroblast alterations induced by low power laser irradiation at the single cell level using confocal microscopy, *Photochem. Photobiol. Sci.* 1 (8) (2002) 547–552.
- [46] M. Greco, R.A. Vacca, L. Moro, E. Perlino, V.A. Petragallo, E. Marra, S. Passarella, Helium–Neon laser irradiation of hepatocytes can trigger increase of the mitochondrial membrane potential and can stimulate c-fos expression in a Ca²⁺-dependent manner, *Lasers Surg. Med.* 29 (5) (2001) 433–441.
- [47] S. Passarella, E. Casamassima, S. Molinari, D. Pastore, E. Quagliariello, I.M. Catalano, A. Cingolani, Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium–neon laser, *FEBS Lett.* 175 (1) (1984) 95–99.
- [48] S. Hallen, M. Oliveberg, P. Brzezinski, Light-induced structural changes in cytochrome c oxidase. Measurements of electrogenic events and absorbance changes, *FEBS Lett.* 318 (2) (1993) 134–138.
- [49] G.C. Brown, Control of respiration and ATP synthesis in mammalian mitochondria and cells, *Biochem. J.* 284 (1992) 1–13.
- [50] T.I. Karu, Photobiology of low-power laser effects, *Health Phys.* 56 (5) (1989) 691–704.
- [51] T.I. Karu, L. Pyatibrat, G. Kalendo, Irradiation with He–Ne laser increases ATP level in cells cultivated in vitro, *J. Photochem. Photobiol. B* 27 (3) (1995) 219–223.
- [52] S.K.B. Arnold, Cell respiration is controlled by ATP, an allosteric inhibitor of cytochrome-c oxidase, *Eur. J. Biochem.* 249 (1997) 350–354.
- [53] S.K.B. Arnold, The intramitochondrial ATP/ADP ratio controls cytochrome c oxidase allosterically, *FEBS Lett.* 443 (1999) 105–108.
- [54] J.K.B. Napiwotzk, Extramitochondrial ATP/ADP-ratios regulate cytochrome c oxidase activity via binding to the cytosolic domain of subunit IV, *Biol. Chem. Hoppe-Seyler* 379 (1998) 335–339.
- [55] H.T. Witt, Energy conversion in the functional membrane of photosynthesis. Analysis by light and electric pulse methods. The central role of the electric field, *Biochim. Biophys. Acta* 505 (1979) 955–1026.
- [56] J. Teissie, B.E. Knox, T.Y. Tsong, J. Wehrle, Synthesis of adenosine triphosphate in respiration-inhibited submitochondrial particles induced by microsecond electric pulses, *Proc. Natl. Acad. Sci. USA* 78 (12) (1981) 7473–7477.
- [57] G. Kaim, P. Dimroth, Voltage-generated torque drives the motor of the ATP synthase, *EMBO J.* 17 (20) (1998) 5887–5895.
- [58] C.S. Enwemeka, Laser biostimulation of healing wounds: specific effects and mechanisms of action, *J. Orthopaed. Sports Phys. Ther.* 9 (10) (1988) 333–338.
- [59] S. Passarella, A. Ostuni, A. Atlante, E. Quagliariello, Increase in the ADP/ATP exchange in rat liver mitochondria irradiated in vitro by helium–neon laser, *Biochem. Biophys. Res. Commun.* 156 (2) (1988) 978–986.
- [60] J. Rigau i Mas, Effect of low level intensity laser light on the modulation of cellular function, Doctoral Thesis, Rovira i Virgili University, Reus, Spain, 1996.
- [61] H. Westerblad, D.G. Allen, Cellular mechanisms of skeletal muscle fatigue, *Adv. Exp. Med. Biol.* 538 (2003) 563–571.
- [62] J.G. Pastorino, J.B. Hoek, Hexokinase II: the integration of energy metabolism and control of apoptosis, *Curr. Med. Chem.* 10 (16) (2003) 1535–1551.
- [63] H. Kale, P. Harikumar, S.B. Kulkarni, Assessment of the genotoxic potential of riboflavin and lumiflavin B. Effect of light, *Mutat. Res.* 998 (1) (1992) 17–23.
- [64] A.M. Edwards, E. Silva, Effects of visible light on selected enzymes, vitamins and aminoacids, *J. Photochem. Photobiol. B* 63 (1–3) (2001) 126–131.
- [65] D.R. Kearns, Physical and chemical properties of singlet molecular oxygen, *Chem. Rev.* 71 (4) (1971) 395–427.
- [66] Y.V. Savin, L.V. Goryachev, Y.A. Adamenkov, T.V. Rakhimova, Y.A. Mankelevich, N.A. Popov, A.A. Adamenkov, V.V. Egorov, S.P. Ilyin, Y.V. Kolobyani, E.A. Kudryashov, G.S. Rogozhnikov, B.A. Vyskubenko, Singlet oxygen production and quenching mechanisms in travelling microwave discharges, *J. Phys. D: Appl. Phys.* 37 (2004) 3121–3128.
- [67] G.Y. Fraikin, M.G. Strakhovskaya, A.B. Rubin, The role of membrane-bound porphyrin-type compound as endogenous sensitizer in photodynamic damage to yeast plasma membranes, *J. Photochem. Photobiol. B* 34 (2–3) (1996) 129–135.
- [68] N. Li, K. Ragheb, G. Lawler, J. Sturgis, B. Rajwa, J.A. Melendez, J.P. Robinson, Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production, *J. Biol. Chem.* 278 (10) (2003) 8516–8525.
- [69] I.A. Vladimirov, G.I. Klebanov, G.G. Borisenko, A.N. Osipov, Molecular and cellular mechanisms of the low intensity laser radiation effect, *Biofizika* 49 (2) (2004) 339–350.
- [70] R. Lubart, M. Eichler, R. Lavi, H. Friedman, A. Shainberg, Low-energy laser irradiation promotes cellular redox activity, *Photomed. Laser Surg.* 23 (1) (2005) 3–9 (Review).
- [71] C. Nathan, Nitric oxide as a secretory product of mammalian cells, *FASEB J.* 6 (1992) 3051–3064.
- [72] V. Borutaite, A. Budriunaite, G.C. Brown, Reversal of nitric oxide-, peroxynitrite- and S-nitrosothiol-induced inhibition of mitochondrial respiration or complex I activity by light and thiols, *Biochim. Biophys. Acta* 1459 (2–3) (2000) 405–412.
- [73] Q. Zhu, W. Yu, X. Yang, G.L. Hicks, R.J. Lanzafame, T. Wang, Photo-irradiation improved functional preservation of the isolated rat heart, *Lasers Surg. Med.* 20 (3) (1997) 332–339.
- [74] E.D. Kerver, I.M. Vogels, K.S. Bosch, H. Vreeling-Sindelarova, R.J. Van den Munckhof, W.M. Frederiks, In situ detection of spontaneous superoxide anion and singlet oxygen production by mitochondria in rat liver and small intestine, *Histochem. J.* 29 (3) (1997) 229–237.
- [75] M. Wikstrom, J.E. Morgan, The dioxygen cycle. Spectral, kinetic, and thermodynamic characteristics of ferryl and peroxy intermediates observed by reversal of the cytochrome oxidase reaction, *J. Biol. Chem.* 267 (15) (1992) 10266–10273.
- [76] J.A. Tainer, E.D. Getzoff, J.S. Richardson, D.C. Richardson, Structure and mechanism of copper, zinc superoxide dismutase, *Nature* 306 (5940) (1983) 284–287.
- [77] R.S. Balaban, S. Nemoto, T. Finkel, Mitochondria, oxidants, and aging, *Cell* 120 (4) (2005) 483–495.
- [78] A. Ehrlicher, T. Betz, B. Stuhmann, D. Koch, V. Milner, M.G. Raizen, J. Kas, Guiding neuronal growth with light, *Proc. Natl. Acad. Sci. USA* 99 (25) (2002) 16024–16028.