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RESEARCH ARTICLE

Colors as Catalysts in Enzymatic Reactions

Samina T. Yousuf Azeemi[1]*, Syed Mohsin Raza[1], Masoom Yasinzai[2]

[1]*Department of Physics, University of Balochistan, Quetta, Pakistan*
[2]*Institute of Biochemistry, University of Balochistan, Quetta, Pakistan*

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Abstract
We studied the effects of visible range irradiation (*in vitro*) on the enzyme solutions (glucose oxidase, cholesterol oxidase+cholesterol esterase and lipase) in order to infer the changes produced in the human body after chromotherapy. The glucose oxidase showed enhanced activity to the color purple (464 nm), while the activity of the other enzymes, cholesterol esterase+cholesterol oxidase and lipase, increased when exposed to dark violet (400 nm). Purple is being used in conventional chromotherapy for diabetes, as supported by the experimental observation in which purple enhanced the activity of enzymes responsible for the oxidation of glucose. Specific wavelengths regulate living processes by acting as catalysts in enzyme activity, while some wavelengths may reduce enzyme activity. The irradiation of specific wavelengths effect enzymatic processes, which as a consequence, accelerated biochemical reactions. This particular frequency when provided to the enzymes (*in vitro*) lead to changes which may well be occurring *in vivo*.

1. Introduction

Every living cell needs energy. The energy necessary for the complex functions of the cell comes from nutrients absorbed by the organism. In its primary form, however, the chemical energy of the nutritive compounds is not directly usable for the cell, but has first to be converted biochemically into a cellular usable form. The cellular energy transfer takes place in the mitochondria, and therefore these organelles have a key function for the cells. Examination of the energy range, which is relevant for the energy transfer from the nutrient into the high energy adenosine triphosphate (ATP) in the cell, shows also the influence of electromagnetic radiation in the form of visible light. This range corresponds to metabolic energy as far as the energy intake and energy release systems of the whole process are concerned. Because radiation phenomena, in consequence of the wave particle dualism, are part of the fundamental nature of electrons, the electron flow linked with the mitochondrial energy transfer can also be described as a radiation process; therefore the classical particle concept of the mitochondrial electron flow can be regarded theoretically as a wave. In other words the presence or absence of radiation of a special frequency, wavelength, intensity, diffusion or polarization in the cell is the deciding factor in most reactions [1].

Chromotherapy, phototherapy and low level laser therapy are emerging techniques in the field of energy/vibrational medicine. Chromotherapy uses the visible spectrum (colors) of electromagnetic radiation to cure diseases. According to the doctrine of chromotherapy, visible range electromagnetic radiations (colors) generate electrical impulses and magnetic currents or a field of energy that are prime activators of the bio-chemical and hormonal processes in the human body, the stimulants or sedatives

*Corresponding author. Department of Physics, University of Balochistan, Quetta, Pakistan.
E-mail: saminatazayyen@yahoo.com

Table Dominant wavelength of monochromatic light measured by Hitachi U-2000 UV-Vis double beam spectrophotometer, spectral bandwidth 0.1 nm and ordinates selected 10

	Color	Dominant wavelength (nm)	Hue	Purity (%)	Transmission (%)
1	Violet	400	Dark violet	49	18
2	Purple	464	Violet	36	32
3	Green	538	Greenish yellow	15	37
4	Yellow	590	Reddish yellow	40	82
5	Red	644	Red	41	51

Source: Hardy, A.C, 1936. Handbook of colorimetry, Technology Press, Boston, Mass.

necessary to balance the entire system and its organs [2]. Chromotherapy suggests that "there are two ways of curing diseases, i.e., direct and indirect" [3]. During indirect treatment, chemical energy is provided to the cells of a body indirectly, in which food and drugs play an important role, while in direct treatment, the body tissues actually absorb the radiant energy. Chromotherapy is a system of treatment that directly induces vibrations [4].

According to Colicov, "Colored light has a particular ability to balance the autonomic nervous system, which is crucial in most chronic and functional disorders as it regulates all of the automatic processes of the human body: breathing, the beating of the heart, the functioning of the digestive tract and the stress response. Light as an environmental stimulant, is second only to food in its impact on controlling bodily functions. Interestingly, light through the eyes reaches not only to the visual centers of the brain, but also the hypothalamus. The hypothalamus is the brain's brain. It organizes information from our external and internal environments, initiates the stress response, regulates immune function, reproduction, thirst, hunger, temperature, emotions and sleep patterns" [5].

When radiant energy is absorbed, the energy levels are raised to a higher energy state (at least temporarily) and are able to act as catalysts for oxidation and for combining numerous compounds before returning to the normal energy state.

Here we discuss the method of absorption (*in vitro*) of a particular wavelength/color (visible region) within the enzymes glucose oxidase, cholesterol oxidase+, cholesterol esterase and lipase, in order to study the changes produced in our body as a result of chromotherapy.

2. Materials and Methods

To study the effect of light on enzymatic reactions, we used commercial test kits (Holzheim, Germany) [6] used in clinical laboratories for estimating the concentration of analytes of interest in the blood. These are calibrated kits and were used without any further pre-treatment.

Three different enzymes, glucose oxidase, cholesterol esterase+cholesterol oxidase and lipase, were chromatized (absorption of visible range wavelengths) in their solution form *in vitro* and their catalytic activity was studied as compared with non-chromatized controls, which were placed in a dark refrigerator. The enzymes were irradiated with selected color wavelengths (Table) by wrapping multipurpose cellophane filter sheets on glass tubes containing enzyme solution and placing them in front of full spectrum incandescent light bulbs of 100 watts, at a distance of 0.32 m. The selection of colors was made according to the theory of chromotherapy to cure particular ailments. In case of glucose oxidase, violet, yellow and green colors were selected and for cholesterol oxidase+esterase and lipase, dark violet, green and red colors were used.

Glucose oxidase (EC: 1.1.3.4; 10 KU/L) was part of a DiaSys kit [6], where one unit of glucose oxidase was the amount of enzyme that catalysed the transformation of 1.0 μM of glucose to gluconic acid per minute at 25°C. The assay was carried out as per the following reaction scheme. This enzyme was isolated from *Aspergillus*. The buffer used in the kit was the phosphate buffer (pH 7.5).

$$\text{Glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

The calorimetric indicator was the Quinoneimine generated from 4-aminoantipyrine and phenol by hydrogen peroxide, when catalyzed by peroxidase.

Measured spectrophotometrically ($\lambda = 546$ nm). Cholesterol ester is hydrolyzed into free cholesterol and fatty acids by cholesterol esterase (EC: 3.1.1.13; 300 U/L; 3.0 units per assay). The cholesterol is oxidized into cholesterol-3-one and H_2O_2 in the reaction catalyzed by cholesterol oxidase (EC: 1.1.3.6; 100 U/L stock; 1.0 units per assay).

The assay was carried out as per following reaction scheme,

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesterol-3-one} + H_2O_2$$

H_2O_2 is processed further as shown in the scheme above.

Lipase (EC: 3.1.1.3; 2 KU/L) catalyzes the hydrolysis of triglycerides into glycerol and fatty acids. The following assay procedure was adopted for this enzyme.

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol 3-Phosphate} + \text{ADP}$$

$$\text{Glycerol 3-p} + O_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{Dihydroxy acetone phosphate} + H_2O_2$$

H_2O_2 was treated as in the case of above two enzymatic schemes. The experiment was repeated to confirm the effect.

3. Results

The results show that each enzyme responded to a particular wavelength/color. Glucose oxidase, responded to purple (464 nm) as shown in Figure 1, while cholesterol esterase+oxidase and lipase responded to dark violet (400 nm). The response of lipase and cholesterol esterase+oxidase is shown in Figures 2 and 3, respectively. This shows that each part of the body has its own frequency response; different color wavelengths are required for different organisms. Therefore, these enzymes responded differently, and the mechanism of absorption of specific wavelengths in the visible region (induced by chromotherapy) influences the enzymatic activity by enhancing or reducing enzyme function. The other interesting aspect of these observations is that the findings correlate with conventional chromotherapy which has been in use for centuries. For example, for diabetes purple is used, as supported in our case by the experimental observation in which purple enhanced the activity of the enzyme responsible for oxidation of glucose. Cholesterol esterase+oxidase and lipase may assist in curing hyperlipidemia and hyperlipoproteinemia. Enzymes use binding energy from the binding of substrates to assist in catalysis because the enzyme is flexible. The framework may

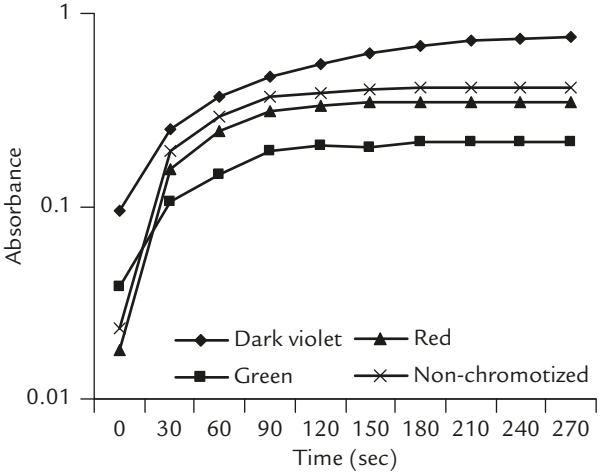

Figure 2 Effect of irradiation of different wavelengths on cholesterol (oxidase+esterase) (*in vitro*). Dark violet accelerated the reaction while red and green showed the opposite effect.

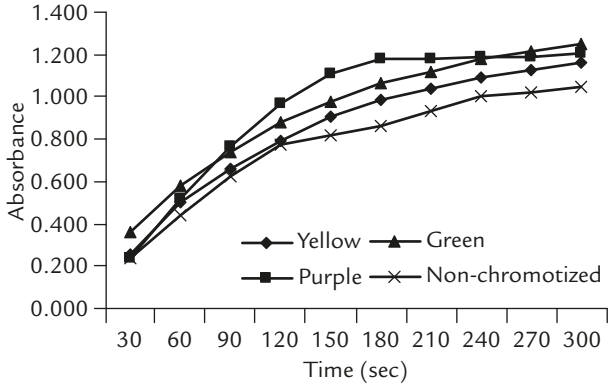

Figure 1 Effect of irradiation of different wavelengths on glucose oxidase (*in vitro*) with purple being the most effective color.

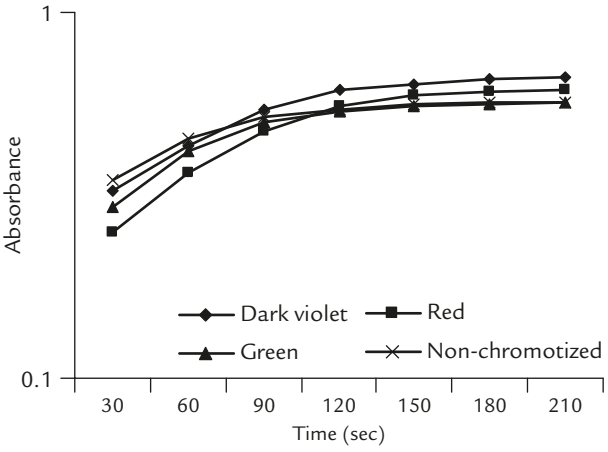

Figure 3 Effect of irradiation of different wavelengths on lipase (*in vitro*) with dark violet being the most effective color.

absorb energy in a very efficient way and put this energy to use or assist with catalysis.

4. Discussion

Phototherapy is being used successfully to cure various pathological conditions like neonatal jaundice, psoriasis, leukoderma and other skin diseases. Primary interaction of light with a biological object is of a purely photochemical nature. Photobiomodulation is characterized by its ability to induce photo-biological processes in cells. The relationship between these biological responses and the radiation wavelength suggests the existence of a photo-acceptor. It has been found that there are photo-receptors at the molecular-cellular level which, when triggered, activate a number of biological reactions: DNA/RNA synthesis, increased cAMP levels, protein and collagen synthesis and cellular proliferation [7]. Exact action spectra are needed for determination of photo-acceptors as well as for further investigations into cellular mechanisms of light therapy. The results suggest that the effect of chromotherapy is to create a photobiomodulation effect which activates the enzymatic process in cells to promote metabolism. Most enzymes need light (energy) for proper functioning. This study shows that different wavelengths affect different enzymatic reactions. This particular frequency when provided to the enzymes (*in vitro*) leads to the changes which might be occurring *in vivo* as well. This suggests that specific wavelengths of light may regulate living processes by acting as promoters to enzyme activity.

We may draw the following conclusions from the present study:

- Enzymes respond to specific visible range radiations to accelerate biochemical reactions.
- The behavior of different enzymes is quite different; different enzymes responded to different wavelengths differently i.e., glucose oxidase to a purple color, cholesterol esterase+cholesterol oxidase and lipase to a dark violet color.
- Differential response of specific enzymes to various wavelengths of light may explain the colors therapeutic use.

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