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Remote Control of Bovine Catalase Activity with Specific Frequencies of Human and Bovine Catalase with and without Bound NADP⁺

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Abstract

Remote control enzyme technology is widely used today through resonance. In this study, we showed that the use of frequencies of the catalase enzyme itself to increase enzymatic rate is successful not only in test tubes but also remotely. The present study also suggests that, under optimal temperature, the use of bovine catalase frequency (the specific frequency of that enzyme) has a superior rate promoting vibration than the human catalase frequency, and so increases very significantly the chemical rate of bovine catalase (about 120% at 40˚C). It also suggests that bovine catalase subjected to bovine and human frequencies with catalase bound NADP⁺ experienced more resonance weight towards NADP⁺ and so were more slowly reduced back to catalase bound NADPH, increasing compound II formation rate, and slowing down the catalase activity rate.

Keywords

Remote Control, Catalase, Frequency, Rate Promoting Vibration (RPV), NADP

1. Introduction

1.1. Use of Remote Control of Enzyme Activity through Resonance

1.1.1. Resonance

Remote-Controlled Enzyme Technologies

Many studies have been conducted to study the potential of resonance in enzyme-substrate systems. Wang *et al.* [1] summarized their review estimating that remote-controlled enzyme technologies have been widely used in biomedical and other industries [2].

Among those studies, some show a remarkable increase in enzymatic activity. Many techniques immobilize enzymes on nanoparticles, deactivating and deforming them when exposed to magnetic fields, increasing their activity by about 130% [3]-[5]. iNOS, CAT and Cyt P450 proteins were stimulated by extremely low frequency electromagnetic fields and their activities were changed significantly [6]. Another study shows that low frequency magnetic fields can induce a doubling in rate variations for Na, K-ATPase and Cyt. Oxidase [7]. All those studies, among many others, show that enzymatic rates can be remotely changed significantly through frequencies.

Successive Bond Shifts between Reactant and Product Bonding Patterns

The concept of enzyme resonance is based on the idea that specific frequencies can be influenced by external electromagnetic fields. The "resonance theory" has evolved into the "natural resonance theory" (NRT) that has acquired a strong theoretical basis, backed by more and more applications and research: NRT uses "convexity-based algorithms", dealing with chemical applications including resonance phenomena of organic chemistry and biochemistry [8].

Although traditional physical chemistry conceptions of reaction mechanism are formulated in terms of stationary points of an Arrhenius-style "energy profile" that differs sharply (in purpose and form) from the corresponding Robinson-style "arrow-pushing" mechanistic conceptions of organic chemistry, these diverse "mechanistic" conceptions can be reconciled in a unified computational protocol based on a NRT description of successive bond shifts between reactant and product bonding patterns [9]. Resonance weight can be calculated along tautomerization. As proton transfer begins, a resonance strengthens as the second structure contribution increases, then resonates in close proximity to the transition state [9] [10]. The transition state is then strongly delocalized [10]. As Tautomers are distinct chemical species that can be distinguished by their differing atomic connectivities, molecular geometries, and physicochemical and spectroscopic properties, this depiction might be useful for enzyme-substrate bonding patterns, but the unified theory between "energy profile" and "arrow-pushing" mechanistic conceptions might be more useful to research dealing with successive bond shifts between reactant and product bonding patterns, as well as enzyme-substrate bonding patterns. Thus, the resonance form with a specific energy profile can depict an enzyme state whose structure is a quantum superposition of frequencies from different resonance forms.

Water as an Essential Tool for Long-Range Communications with Supra-Molecular Changes

Neither classical nor standard quantum theory predicts quantum coherence for water, largely because they ignore quantum fluctuations and the interaction between matter and the vacuum electromagnetic field (VEMF), which are taken into account in Quantum Field Theory (QFT) [11].

Quantum coherence for water is ignored in classical and standard quantum theories. They do not take into account quantum fluctuations and matter-vacuum electromagnetic field interactions. The Quantum Field Theory does. Water that is structured/interfacial enables biological processes. That structure allows for absorption, storage, and emission of electromagnetic energy, and thus absorption, storage and emission of information [12].

Quantum Electrodynamic (QED) field theory has become an essential tool for long-distance communications thanks to its ability to change its supra-molecular organization in relation to its environment. Structured/Interfacial water has been shown to hold coherence domains and exclusion zones [11]. These structures are dynamic and can receive coherent signals (electromagnetically encoded) at low frequencies [11] [12]. They can sum those frequencies into another coherent frequency (negentropy) that may affect biological systems [11] [12]. In this study, we are using water-based frequency vials that will be placed in water baths for several minutes, allowing the water baths to organize themselves coherently (negentropy) while receiving electromagnetically encoded signals from the vials.

Macromolecular Activity Is Based on Electromagnetic Resonance

The "natural resonance theory" (NRT), although very powerful, could only be applied mathematically to relatively simple chemical compounds. Enzymes are very complex in comparison and the computational and algorithmic power needs to increase before being applied to such complex systems. The Resonant Recognition model (RRM) is a method that has been used successfully not only to modulate protein activity, but also to predict which frequency to use as the RRM treats the protein sequence as a discrete signal and identifies certain periodicities (frequencies) in this signal that characterizes protein biological function [13]. Proteins activity can be modified by electromagnetic radiation at a specific frequency that the RRM can predict. The RRM spectrum has characteristics that allow the calculation of its wavelength [13] [14]. Cosic *et al.* have used the RRM to predict successfully resonances in telomerase, DNA (telomere) and RNA (TERT mRNA), and proposed that RRM is a revolutionary new approach which is that macromolecular activity is based on electromagnetic resonances [13] [14].

1.1.2. Rate Promoting Vibration Specific to the Active Site

In 2020, Yann Chalopin showed that, in the active site, the stiffest parts of the enzyme scaffold (the part which is conserved throughout evolution), fast vibrations take place spontaneously [15]. Those vibrations are localized and are directly related to the enzyme's biological function. Chapolin uses the term continuity/order to describe that area as it allows energy propagation, spreading and damping into the scaffold (noting that temperature is maintaining that function) [15]. Localized vibrations can be modulated by rate promoting vibrations (RPVs) to increase chemical rates [15]. The other areas that are mechanically coupled with more frequency motions are described as disorder/discontinuity and are more flexible than the scaffold [15].

Our *in vitro* experiment hypothesized that a rate promoting vibration (RPV) that is the specific frequency of an enzyme, recorded at its optimal functioning, would increase the chemical rate in suboptimal conditions (low substrate, non-optimal temperature, etc.). This is the preliminary part of our study, and it showed that the hypothesis was correct.

If the human catalase frequency increased the chemical rate of the catalase but not as much as the bovine catalase frequency, it would confirm that resonance can be achieved through different vibrations rates and patterns, and that some can be more optimal than others. We hypothesized that the closer the frequency (applied to the enzyme) is to the specific optimal frequency of the enzyme (or its optimal RPV) the more optimal the resonance and the chemical rate. To use the NRT paradigm again, we postulated that each specific resonance form/frequency used depicts its own specific energy profile. Each frequency applied to the enzyme-substrate complex would put different resonance weight on the system and might shift bonds differently between enzyme and substrate bonding patterns. It also implies that temperature might also shift those bonds differently. Specifically, we hypothesized that the use of human catalase frequency would have an inferior RPV than the bovine catalase frequency would, and so would have an inferior chemical rate effect on bovine catalase at optimal temperature (40˚C).

1.1.3. Catalase

Under stress conditions, catalase plays a major role by detoxifying hydrogen peroxide, mainly in peroxisomes and in the cytoplasm, to protect cellular components against oxidative damage [16]. Catalase deficiency needs to be considered as a complicating condition for aging and oxidative stress [17]. Catalase deficiency is mainly due to genetic heterogeneity, epigenetic, or environmental causes [18]. Catalase itself may be damaged by oxidative stress with the consequence of decreased enzyme activity and accumulation of hydrogen peroxide [18]. NADPH is likely protecting catalase from oxidative damage [19]. Catalase expression is also altered in cancer cells, most likely promoting genetic instability and activation of oncogenes [16]. Catalase might become a therapeutic target in cancer utilizing oxidative approaches [16].

1.1.4. Catalase Reaction

X-ray crystal structures of bovine liver catalase in its resting state [20] indicate that there is a single heme b group at the active site [21].

Catalase takes various states; Ferricatalase is the resting state of the enzyme:

$$\text{ferricatalase} + H_2O_2 - > \text{compound I} + H_2O$$

$$\text{compound I} + H_2O_2 - > \text{ferricatalase} + H_2O + O_2$$

A compound II, a product of compound I, is the inactive state, that takes shape when catalase is exposed to H_2O_2 [22]. Compound II will return to the ferricatalase state when compound II is not exposed to H_2O_2 anymore [23].

1.1.5. Bovine vs. Human Catalase

Although amino acid sequences do not have high identities between all typical catalases, the tridimensional structure is highly conserved. The tertiary structure of the β-barrel domain, the connection domain and the zone neighboring the distal histidine are highly conserved [16].

Bovine Catalase has almost 80% sequence similarity with human catalase, as well as the same conserved active-site residues, each subunit containing four distinct domains, each with one prosthetic heme group [24]-[26]. In contrast with the bovine catalase that binds with 4 NADPH molecules at the periphery of the enzyme, in the human catalase tetramer, only two monomers bind with a NADPH molecule [27].

1.1.6. Catalase with Bound NADPH

NADPH bounds to mammalian catalase (NADPH)b and counteracts the ability of its substrate to transform the enzyme to an inactive state (compound II) [28]. In so doing, (NADPH)b becomes $NADP^+$ and is replaced by another molecule of NADPH [29]. Electron tunneling between NADPH on the surface of the catalase and the heme group within the enzyme seems to be the process by which it can happen [28]. The idea that NADPH serves to prevent the formation and not increase the removal of compound II has been described [29]. However, the superoxide radical seems to avoid somehow compound II, the intermediate state, as NADPH has very little capability to prevent the superoxide radical from transforming the enzyme to compound II [29].

1.1.7. Prevention of Compound II Formation: The Role of $(NADP^+)$b

Kirkman *et al.* indicated that the action of NADPH is more one of prevention than of reversal of the formation of compound II by bovine liver or human catalase in the presence of H_2O_2 [29]. On the same paper, suggested that NADPH had very little ability to prevent the superoxide radical from converting catalase to compound II. Later, Gaetani *et al.* explained that (NADPH)b becomes oxidized in the process of preventing compound II formation, and the resulting $(NADP^+)$b is reduced back to (NADPH)b by free or transiently bound NADPH [30]. In that same paper, Gaetani *et al.* explained why their theory seems valid despite impediments.

Unbound NADPH reduces $(NADP^+)$b to (NADPH)b, and without such a reduction or some type or replacement, (NADPH)b would be an inadequate reservoir of NADPH for preventing compound II formation: the amount of (NADPH)b is small relative to the rate at which NADPH is needed. If this reduction of $(NADP^+)$b into (NADPH)b is slowed, NADPH cannot be an adequate enough reservoir for preventing compound II formation [30].

According to the NRT, if the $(NADP^+)$b catalase frequency were applied to the catalase-hydrogen peroxide system, the resonance weight of the system would be placed more on the $(NADP^+)$b than in the (NADPH)b state of the enzyme. In other words, if $(NADP^+)$b were more slowly reduced back to (NADPH)b, (NADPH)b would be relatively less present, oxidation would happen less, and compound II formation rate should be higher, slowing down the catalase activity rate. So, if the $(NADP^+)$b catalase frequency is applied to the catalase-hydrogen peroxide system, catalase activity might become slowed in comparison to putting the resonance weight on the catalase without $(NADP^+)b$.

Consequently, we hypothesized that a $(NADP^+)b$ catalase frequency would have a smaller RPV then the catalase frequency not bound to $NADP^+$, thus having a smaller chemical rate effect on bovine catalase at different temperatures.

1.1.8. Some Advantages of Catalase Remote Control

Catalase is used in the food industry for removing hydrogen peroxide from milk prior to cheese production, in food wrappers, in the textile industry by removing hydrogen peroxide from fabrics to make sure the material is peroxide-free, in bioremediation (as a provider of oxygen in aerobic bioremediation process) and in the removal of hydrogen peroxide from bleaching industry effluent [31]. Increasing the catalase rate remotely by applying the right frequency to that enzyme might reduce costs in those industries. In healthcare the remote-control advantage might become important both in increasing or reducing the catalase rate in human cells. As stated earlier, catalase plays a pivotal role in mitigating oxidative stress and preserving cellular homeostasis, and its rate *in vivo* might be increased by remote control using the human catalase frequency. Reducing catalase rate on established cancer sites to help reduce cancer growth might also be possible by applying remotely rate-reducing frequencies on cancerous tissue. Indeed, using a $(NADP^+)b$ catalase frequency would have, according to our hypothesis stated earlier, a smaller RPV then the catalase frequency without $(NADP^+)b$, and would consequently decrease the catalase rate of the bovine catalase, hypothetically slowing down established cancer cells growth.

2. Method

We used the Iwase *et al.* method to measure catalase activity by quantifying the trapped oxygen gas, which is visualized as foam [32]. We used that method for it is ease of reproducibility, not only for research but also for educational purposes. The height of foam developed in the tubes was measured. Iwase *et al.*, showed that the best linear fit was over a range of 20 - 300 units (U) of catalase activity (($y = 0.3794x - 2.0909$, $r^2 = 0.993$) [31]; hence, we chose the catalase concentrations in that range (100 U at 10˚C and 20˚C, and 50 U at 40˚C as too much foam was produced at 40˚C with 100 U for those test tubes). The generation of oxygen stops naturally within several minutes. Test tubes used were all new as suggested by the authors [32]. pH buffer is not used by the authors of this method as the pH of the solutions remains stable at around 7 and as the optimum pH for bovine catalase is approximately 7, and has a fairly broad maximum (the rate of reaction does not change appreciably at pHs between 6.8 and 7.5) [33] [34].

The Quantum Field Theory allows us to use frequency vials which are aqueous vials imprinted with a frequency signature. Two brands have been used: Ergopathics (Canada) and BioEnergy Assist (USA). The BioEnergy Assist brand adds 5% ethanol to distilled water. The Ergopathic brand adds an undisclosed amount of ethanol to water.

2.1. Enzyme Activity Increase in Suboptimal Conditions with Specific Enzyme Frequency

We tested the bovine catalase frequency applied on the bovine catalase *in vitro* at 20˚C (suboptimal temperature) in test tubes. We compared a bovine frequency vial placed in distilled water (from BioEnergy Assist, Worthington, USA) with another vial placed in distilled water but without frequency. The vials were placed in distilled water for 10 minutes (**Figure 1(a)**). 100 µl of those waters were poured in test tubes. Dissolved Catalase powder (20 U/mg, 100 µl), Triton X-100 (100 µl) and hydrogen peroxide (12%, 250 µl) were then added. The height of the foam for each test tube was measured.

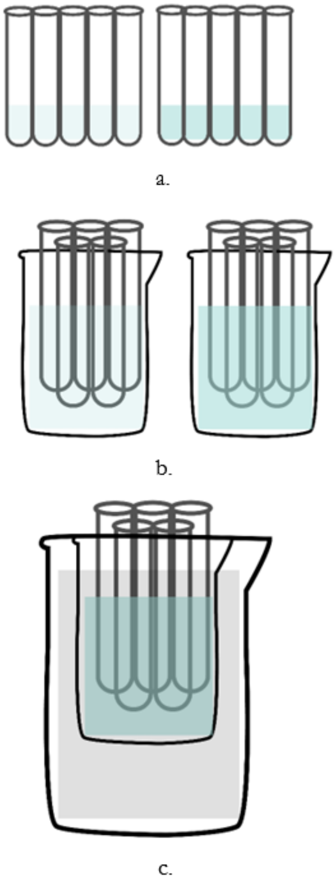


Figure 1. Graphic representation of assays without and with water baths for frequencies and temperature. a: Water without frequency in test tubes (left) and water with frequency in test tubes (right). b: Test tubes placed in water without frequency (left) and with frequency (right). c: Double beaker setting: The bigger beakers contained water bath at 10˚C, 20˚C or 40˚C. The smaller beakers contained test tubes placed in water baths with frequency.

2.2. Remote Effect of Enzyme Frequency

Tests were also conducted looking at a potential remote effect of enzyme frequency. The distance from the enzymatic solution was the thickness of the test tubes (about 1 mm). The setting of the experiment was the same as the preliminary experiment apart from the fact that frequency was applied from a water bath (vial placed in 10 ml of water in 50 ml beaker) at 20˚C (room temperature) in which test tubes were sitting (**Figure 1(b)**).

2.3. Human and Bovine Catalase Frequency Applied Remotely to Bovine Catalase at Different Temperatures

As those two previous experiments allowed us to conclude that bovine catalase frequency increases the enzyme activity at 20˚C remotely, we conducted the rest of the experiments remotely with water baths at different temperatures (**Figure 1(c)**).

The human catalase frequency vial (from Ergopathics, Canada) and the bovine catalase frequency vial (from BioEnergy Assist, USA) were each placed in distilled water for 10 minutes. 10 ml of that water was poured in small (50 ml) beakers for test tubes to sit in for the duration of the experiment. Those beakers were placed in a second water bath that were set at 10˚C, 20˚C and 40˚C. Dissolved Catalase powder (20 U/mg, 100 μl), Triton X-100 (250 μl) and hydrogen peroxide (12%, 250 μl) were added to test tubes.

2.4. Human and Bovine ($NADP^+$)-Bound Catalase Frequency Applied Remotely to Bovine Catalase at Different Temperatures

The human and bovine $NADP^+$ bound catalase frequency vials (from BioEnergy Assist, USA) were each placed in distilled water for 10 minutes. We used the same method as we did to compare human and bovine catalase frequencies remotely to bovine catalase.

2.5. Statistical Analysis

Comparisons were made between trapped oxygen height in test tubes with and without frequencies. The oxygen production rate after linearization and the difference between the height of total oxygen bubbles trapped were tested using the t-test method (2 samples assuming unequal variance). 10 test tubes were used for each experiment. We used the two-sample t-test with unequal variance (two-tailed) as we could assume that the samples were normally distributed, the standard deviation of both populations are unknown and assume to be unequal, and the sample is small. Alpha levels for all tests were set at 0.05.

3. Results

3.1. Frequencies in Test Tube and Remotely

At 20˚C, bovine frequency applied to bovine catalase in test tube (BTT) increases total oxygen production by 109% (NF1 = 6.15 +/− 0.01, BTT = 6.69 +/−0.01) (t(18) = 2.1, p = 4.32E−09) ($p < 0.01$) compared to bovine catalase without frequency application (NF1). The reaction stopped after 5 minutes. Remotely, bovine frequency (B) increased total oxygen production by 107% ($p < 0.01$) compared to bovine catalase without frequency application (NF) (NF = 6.18 +/− 0.01, B = 6.65 +/− 0.01) (t(18) = 2.1, p = 4.43E−09). The difference between applying frequencies in test tubes or remotely (1 mm) is not significant in terms of oxygen production (**Figure 2**).

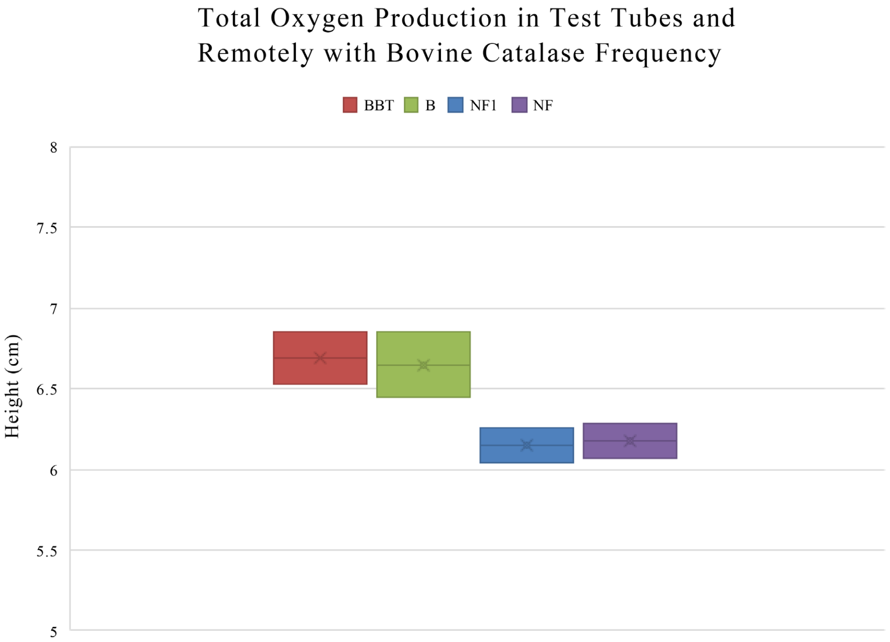


Figure 2. Total Oxygen production (oxygen bubbles height) in test tubes or remotely with frequency (BTT and B) or without frequency (NF and NF1). (Standard deviation at top and bottom of each box.) BTT = Bovine frequency in test tubes; B = Bovine frequency remotely; NF1 = No Frequency in test tubes; NF = No Frequency remotely.

3.2. Frequencies Applied Remotely (1 mm)

3.2.1. Oxygen Production at 10˚C

Total Oxygen production:

Compared to the catalase assays without any frequency application (NF), catalase assays applied with the human catalase frequency (H) and bovine catalase frequency (B^+) both very significantly produced more oxygen: 125% (NF: 5.23 +/− 0.23; H: 6.53 +/− 0.33) (t(11) = 2.03, p = 0.004) and 130% (NF: 5.23 +/− 0.23; B^+: 6.80 +/− 0.28) (t(11) = 2.3, p = 0.001) respectively.

Catalase assays with Bovine catalase frequency without $NADP^+$ (B) produced significantly more oxygen: $p < 0.05$ significant differentiation with NF 121%; (NF: 5.23 +/− 0.23; B: 6.33 +/− 1.07) (t(11) = 1.9, p = 0.07).

Catalase assays with Human catalase frequency with $NADP^+$ (H^+) did not produce significantly more oxygen ($p > 0.05$) (**Figure 3**).

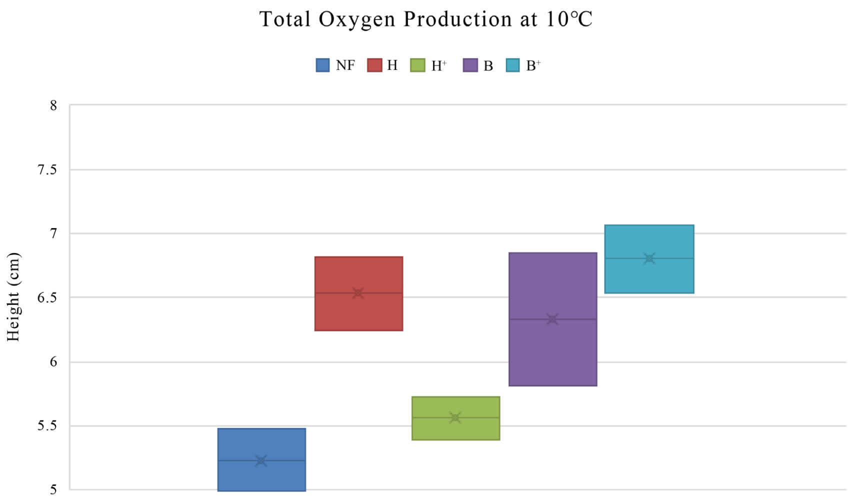
Catalase assays with Human catalase frequency with $NADP^+$ (H^+) did not produce significantly more oxygen ($p > 0.05$) (**Figure 3**).

Rate of Oxygen Production:

Compared to catalase assays with NF, the rate of oxygen production of the catalase assays with H was very significantly higher by 115% (NF: 0.42 +/− 0.03; H:

0.48 +/− 0.02) (t(136) = 0.04, p = 0.01), and the rate of oxygen production of the catalase assays with B were significantly higher by 107% (NF: 0.42 +/− 0.03; B: 0.45 +/− 0.12) (t(136) = 0.04, p = 0.04) (**Figure 4**).

The rates of oxygen production for both B^+ and H^+ were not significantly different from the rate of catalase assays with NF ($p > 0.05$).



$p < 0.05$ significant differentiation with NF 121%; (NF: 5.23 +/− 0.23; B: 6.33 +/− 1.07) (t(11) = 1.9, p = 0.07).

Figure 3. Total Oxygen production at 10˚C (oxygen bubbles height) remotely for NF, H, H^+, B, B^+ after 4.6 minutes (standard deviation at top and bottom of each box). NF = No Frequency; B = Bovine Frequency; B^+ = Bovine Frequency with bound $NADP^+$; H = Human Frequency; H^+ = Human Frequency with bound $NADP^+$.

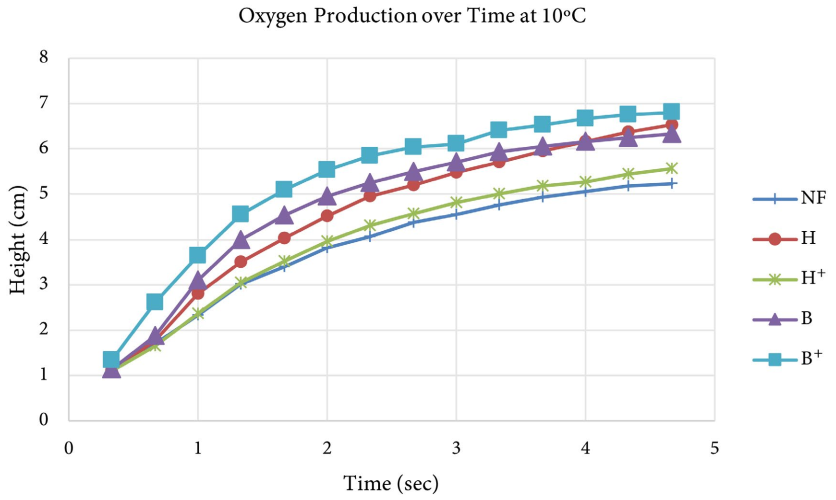


Figure 4. Trapped oxygen heights over time at 10˚C (averages) in test tubes with remote frequencies applied. NF = No Frequency; B = Bovine Frequency; H = Human Frequency; B^+ = Bovine Frequency with bound $NADP^+$; H^+ = Human Frequency with bound $NADP^+$.

3.2.2. Oxygen Production at 20˚C

Total Oxygen Production:

Compared to the catalase assays NF, catalase assays applied with H and B both very significantly produced more oxygen: 112% (NF = 6.19 +/− 0.03; H = 6.99 +/− 0.03) (t(11) = 2.3, p = 5.68E−05) and 109% (NF = 6.19 +/− 0.03; B = 6.71 +/− 0.12) (t(11) = 2.6, p = 0.01), respectively.

Catalase assays with H^+ and B^+ were not significantly different from NF ($p > 0.05$) (**Figure 5**).

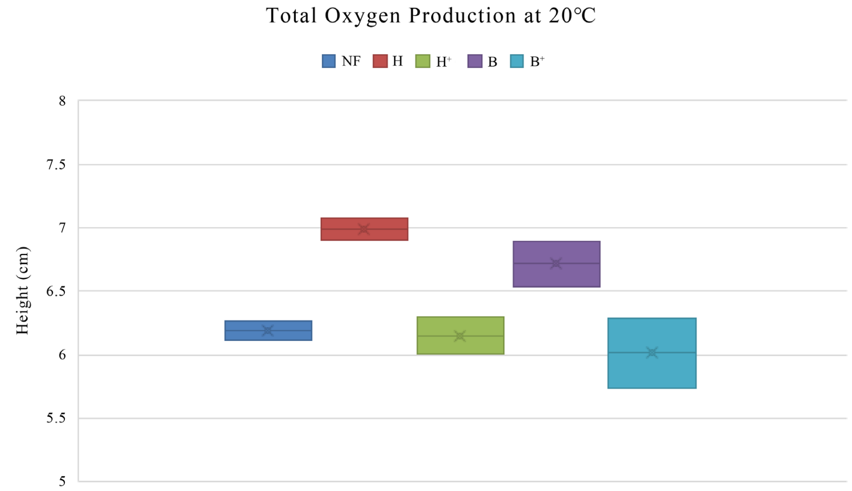


Figure 5. Total Oxygen production at 20˚C (oxygen bubbles height) remotely for NF, H, H^+, B, B^+ after 3.6 minutes (standard deviation at top and bottom of each box). NF = No Frequency; B = Bovine Frequency; B^+ = Bovine Frequency with bound $NADP^+$; H = Human Frequency; H^+ = Human Frequency with bound $NADP^+$.

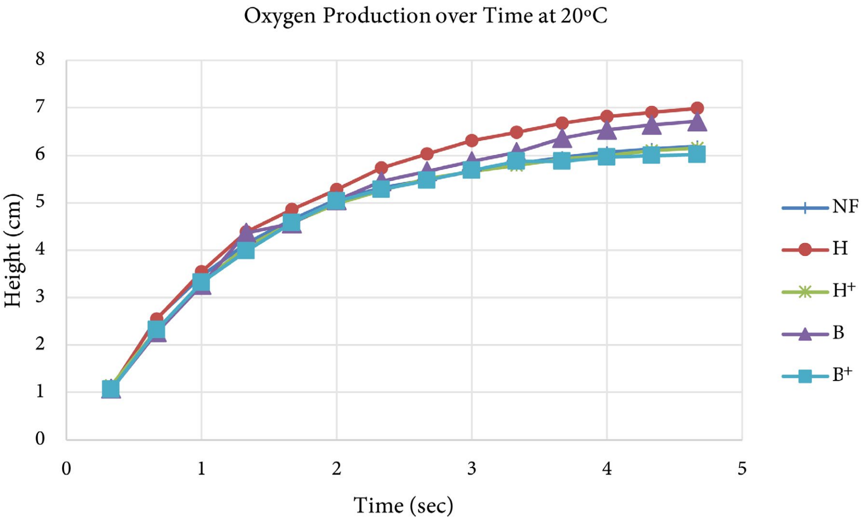


Figure 6. Trapped oxygen heights over time at 20˚C (averages) in test tubes with remote frequencies applied. NF = No Frequency; B = Bovine Frequency; H = Human Frequency; B^+ = Bovine Frequency with bound $NADP^+$; H^+ = Human Frequency with bound $NADP^+$.

Rate of Oxygen Production:

Compared to catalase assays with NF, the rate of oxygen production of the catalase assays with H and B were significantly higher: 109% (NF = 0.42 +/− 0.02; H = 0.46 +/− 0.03) (t(136) = 0.04, p = 0.04) and 109% (NF = 0.42 +/− 0.02; B = 0.46

+/− 0.01) (t(136) = 0.02, p = 0.02), but those with H^+ and B^+ were not significantly different ($p > 0.05$) (**Figure 6**).

3.2.3. Oxygen Production at 40˚C:

Total Oxygen Production:

Compared to the catalase assays with NF, catalase assays applied with H and B both significantly produced more oxygen: 106% (NF = 5.2 +/− 0.02; H = 5.51 +/− 0.04) (t(11) = 2.36, p = 0.02) and 119% (NF = 5.2 +/− 0.02; B = 6.17 +/− 0.36) (t(11) = 2.77, p = 0.02) respectively, whereas those with B^+ and H^+ were not significantly different from NF ($p > 0.05$) (**Figure 7**).

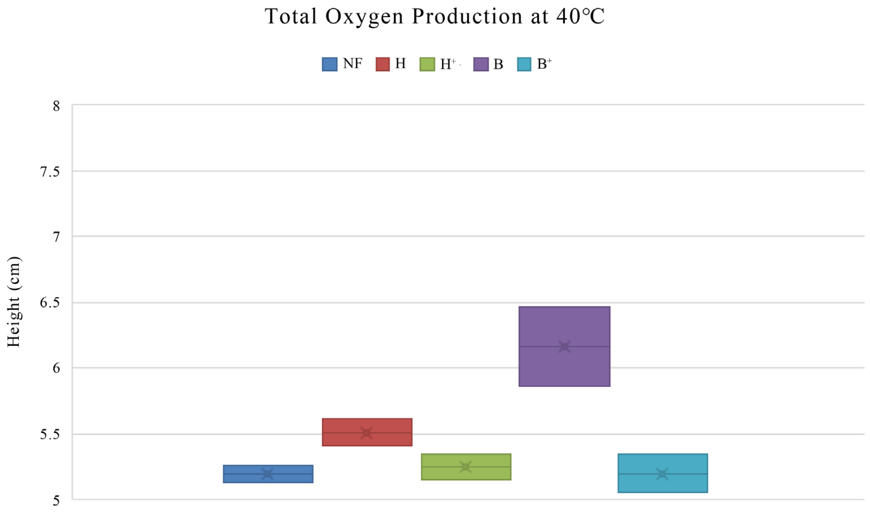


Figure 7. Total Oxygen production at 40˚C (oxygen bubbles height) remotely for NF, H, H^+, B, B^+ after 3 minutes (standard deviation at top and bottom of each box). NF = No Frequency; B = Bovine Frequency; B^+ = Bovine Frequency with bound $NADP^+$; H = Human Frequency; H^+ = Human Frequency with bound $NADP^+$.

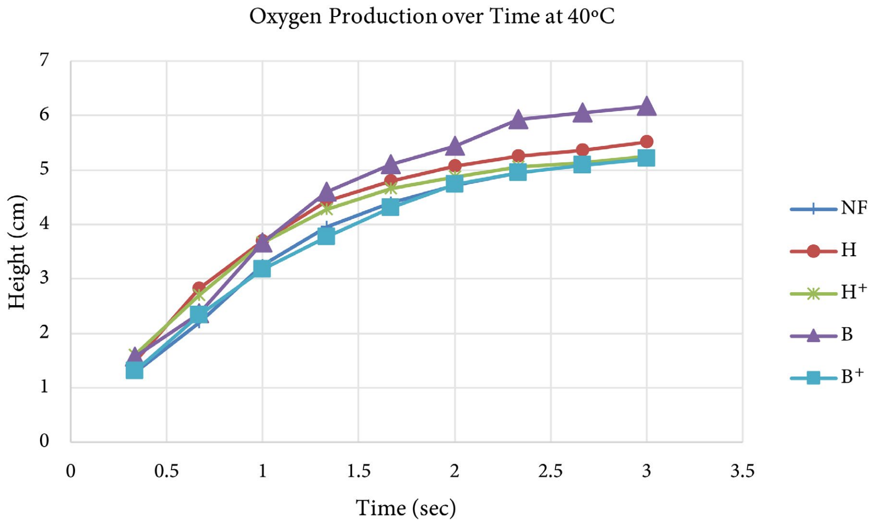


Figure 8. Trapped oxygen heights over time at 40˚C (averages) in test tubes with remote frequencies applied. NF = No Frequency; B = Bovine Frequency; H = Human Frequency; B^+ = Bovine Frequency with bound $NADP^+$; H^+ = Human Frequency with bound $NADP^+$.

Rate of Oxygen Production:

Compared to catalase assays with NF, the rate of oxygen production of the catalase assays with H and H^+ were significantly lower: 90% (NF = 0.42 +/− 0.03; H = 0.38 +/− 0.02) (t(86) = 0.04, p = 0.03) and 83% (NF = 0.42 +/− 0.03; H^+ = 0.35 +/− 0.03) (t(86) = 0.04, p = 0.02), respectively, whereas those with B was significantly higher by 110% (NF = 0.42 +/− 0.03; B = 0.46 +/− 0.04 (t(86) = 0.06), p = 0.04) and those with B^+ were not significantly different ($p > 0.05$) (**Figure 8**).

4. Discussion

The present study showed that frequencies coming from vials that contain water and ethanol could be applied in test tubes or remotely (1 mm distance) with the same result. The second purpose of this study was to assess if specific frequencies of an enzyme could increase or decrease that enzyme's chemical rate or production in test tubes and remotely. Our *in vitro* experiment showed that an RPV, which is the specific frequency of an enzyme, recorded at its optimal functioning, increased the enzyme's chemical rate in suboptimal conditions (low substrate, non-optimal temperature, etc.) up to 130%.

The interpretation of our results is based on the NRT and RRM concepts. Those were validated by Chapolin in 2020. They provide a paradigm that Cosic *et al.* called, relating to RRM, "a revolutionary new approach which is that macromolecular activity is based on electromagnetic resonances" [11]. They allow us to think about resonance weights that can shift bonds differently between enzyme and substrate bonding patterns, potentially strengthening (or weakening) a site. Among the three features of enzyme catalysis that Chalopin exposed, one of them is of particular importance to this study: in enzymes, most of the energy coming from the backbone (conserved part in evolution) vibrations are confined within the most rigid parts of the structure. "Active sites in enzymes, because they are usually stiff, are subjected to rate promoting vibrations (RPVs) playing a key role in increasing the chemical rate in enzymes. This area is the area of order/continuity that allows energy to propagate (…) waves spreading and damping within the scaffold, an action constantly maintained by the environment temperature (…)" [13]. Human and bovine catalase share the same order/continuity active site and should be therefore subjected to the same promoting vibrations. Although their active sites are subjected to the same RPVs, their frequencies or resonance forms are different. Even though the human catalase has an 80% sequence similarity with the bovine catalase, their 3D configuration is quite similar, but the discontinuity/disorder parts of the enzymes are very likely fed differently with different density frequency motions. Consequently, the optimal frequency of the human catalase would be different than that of the bovine catalase.

We postulated that each specific resonance form/frequency used in the present experiment depicts its own specific energy profile and that each frequency applied to the enzyme-substrate complex would put different resonance weight on the system and might shift bonds differently between enzyme and substrate bonding patterns. Specifically, we hypothesized that the use of bovine catalase frequency, the specific resonance of that enzyme, would have a superior RPV than the human catalase frequency would, and so would increase the chemical rate of bovine catalase more than the human catalase would at a given temperature. Our study shows that it is the case at 40˚C, the optimal temperature of bovine catalase, as oxygen production increased by 119% with the application of B ($p < 0.01$), and with a 110% rate increase ($p < 0.05$), whereas the application of H increased oxygen production by 106% ($p < 0.05$) (with a 90% rate decrease ($p < 0.05$)—this 90% rate decrease is explained by the fact that the enzymatic rate was higher earlier (before and around 1 minute) and slowed down more than NF after 1 minute. It is therefore not significant to our interpretation.)

The NRT, RRM and Chapolin's theories also imply that temperature might also shift those bonds differently and that temperature and our frequencies could work in a synergistic way to produce resonance, potentially bringing higher or lower enzyme activity. As human and bovine frequencies (H and B) applied to catalase at 10˚C and 20˚C increase oxygen production by about 125 - 130% and about 110% respectively, and an enzymatic rate increase of 115% and 107% respectively, and as there is no significant difference between the oxygen production for H and B at 10˚C and 20˚C, we could speculate here that temperature vibration in synergy with each specific enzyme vibration would bring a similar total RPV to the system and particularly to the active site for each temperature.

In the second part of this experiment, we hypothesized that the frequencies with $(NADP^+)b$ would reduce the oxygen production more than the frequencies without $(NADP^+)b$.

At 10˚C, bovine catalase had a very significant oxygen production increase when applied to any bovine catalase frequency, but the human catalase frequency with $(NADP^+)b$ did not significantly increase production. It is not clear to us why B^+ produced more oxygen than any other frequency at 10˚C. The NRT, RRM and Chapolin's ideas allow us to speculate that a 10˚C temperature might shift bonds between enzyme and substrate bounding patterns differently, possibly stiffening the heme, so that a 10˚C temperature and our B^+ frequency might work in a synergistic way to produce a resonance that brings a higher enzyme activity. That synergetic vibration might bring more resonance weight towards strengthening (stiffening) the heme site than the other temperatures.

At 20˚C, both the bovine and the human catalase frequencies without $(NADP^+)b$ increased production by about 110%, and their enzymatic rate by about 109%, likely putting the resonance weight on the heme part, whereas both catalase frequencies with $(NADP^+)b$ neither increased oxygen production nor enzymatic rates. Compared to the frequencies without $(NADP^+)b$, catalase subjected to frequencies with $(NADP^+)b$ likely experienced more resonance weight towards $(NADP^+)b$ and so were more slowly reduced back to (NADPH)b, increasing compound II formation rate, and slowing down the catalase activity rate.

Our data also show that, at optimal temperature (40˚C), as it was the case at

20˚C, $(NADP^+)b$ frequencies reduced the oxygen production more than the frequencies without $(NADP^+)b$. Our interpretation is that, compared to the frequencies without $(NADP^+)b$, catalase subjected to frequencies with $(NADP^+)b$ likely experienced more resonance weight towards $(NADP^+)b$ and so were more slowly reduced back to (NADPH)b, increasing compound II formation rate, and slowing down the catalase activity rate. At 40˚C, comparing the applications of B^+ and H^+, total oxygen production did not change although the enzymatic rate changed when H^+ was applied (83%) as the oxygen production curve started to flatten earlier in that case. This data alone is not enough to demonstrate a higher affinity of the enzyme when catalase is subjected to human frequencies with $(NADP^+)b$ compared to bovine frequencies with $(NADP^+)b$.

5. Conclusion

Remote control enzyme technology is widely used today through resonance. Increasing catalase rate remotely might be economically beneficial to several industries (dairy, textile and agriculture to name a few) and be beneficial to livestock farming and healthcare, for example, where increasing catalase rate remotely can protect cellular components against oxidative damage, and decreasing its rate remotely could become a tool against established cancer cells. In this study, we showed that the use of frequencies of the catalase enzyme itself to increase enzymatic rate is successful not only in test tubes but also remotely. The present study also suggests that, under optimal temperature, the use of bovine catalase frequency (the specific frequency of that enzyme) has a superior rate promoting vibration than the human catalase frequency, and so increases very significantly the chemical rate of bovine catalase (about 120% at 40˚C). It also suggests that bovine catalase subjected to bovine and human frequencies with catalase bound $NADP^+$ experienced more resonance weight towards $NADP^+$ and so were more slowly reduced back to catalase bound NADPH, increasing compound II formation rate, and slowing down the catalase activity rate. Consequently, we could assume that established bovine cancer cells might be fought by applying remotely bovine catalase frequency with bound $NADP^+$, and that healthy bovine tissues could benefit from the bovine catalase frequency applied remotely to combat oxidative damage. The same idea might be applicable to humans, other animals and plants although, obviously, more research needs to be conducted to implement it.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

The vials used are available at bioenergyassist.com and at ergopathics.com.

Conflicts of Interest

Although Dr. Thibaud d'Oultremont is the owner of BioEnergy Assist, one of the companies that provided electromagnetic frequency vials for this work, the reproducibility of this work is so easy, simple and inexpensive that it could be reproduced by most high school students. Moreover, he has also used another company's vials with whom he has no conflict of interest whatsoever, with the same main results. No financial support has been provided (again, this is an inexpensive and simple experiment).

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