The Structure and Function of Horseradish Peroxidase (HRP) under the Influence of Faradarmani Consciousness Field

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ABSTRACT

The study of biological enzymes with their unique properties, such as substrate interactions, conformational changes, and catalytic rates, is a key to basic and applied enzymology research. Among enzymes, horseradish peroxidase (HRP) has been widely used in studying the metabolism of biological systems. In this study, we measure changes in the activity and structure of horseradish peroxidase in both laboratory grade and isoforms extracted from the plant under the influence of Faradarmani Consciousness Field (FCF). Our objectives were first to test the effect of FCF on the structure and function of HRP and, secondly, to analyze whether or not changes in the maximum velocity and Michaelis constant were reproducible under the influence of FCF. We observed that the laboratory-grade HRP had a 5% decrease in maximum velocity and an 18% increase in Michaelis constant as a result of the FCF treatment when compared to the non-treated controls. On the other hand, the HRP extracted from the plant tissue showed a 6% and 40% increase in maximum velocity and Michaelis constant, respectively, under FCF. Additionally, changes in the fluorescence emission intensity spectrum suggest that both structural and functional changes in this enzyme result from the FCF treatment.

Keywords: Faradarmani Consciousness Fields (FCF); Taheri Consciousness Fields; Michaelis constant; maximum velocity; horseradish peroxidase

INTRODUCTION

Oxidoreductases are the superfamily of peroxidases (POXs) (EC:1,11,1,7) capable of catalyzing the oxidation of a wide variety of organic and inorganic substrates by using hydrogen peroxide (H_2O_2) (Conesa et al., 2002). HRP is an important heme-containing enzyme obtained from horseradish roots, which is absent from most mammalian cells. Our understanding of the structure and function of HRP has been achieved through X-ray crystallography (Gajhede et al., 1997), and recombinant enzymes (Smith et al., 1990). HRP has been the subject of much re-search in the fields of medicine, life sciences, bi o-technology, therapeutics, biosensor systems, etc. (Krainer et al., 2015). In plants, HRP isoenzymes contribute to indole-3-acetic acid metabolism,
lig-nification, cross-linking of cell wall polymers, s ub-erin formation, and resistance to infection (Nigel and Veitch, 2004).

Reactive Oxygen species (ROS) are typically produced as a product of cellular metabolism pathways, but their lev-els increase under environmental stresses. These free radicals destroy cellular structures and induce apoptosis or programmed cell death. It has been reported that enzymes protecting against oxidative stress could prevent apoptosis due to the interaction with peroxides (Khavari-Nejad et al., 2015). Additionally, POXs have been shown to increase ac-tivity upon pathogen infection like fungi (Harrison et al, 1995). Many recorded reports demonstrate that the in-creased levels of antioxidant enzyme activity in plants result in stronger resistance to environmental stresses (Demiral and Turkan, 2005). On the other hand, endogenous ROS, such as H_2O_2, has been regarded as a messenger molecule which contributes to various vital processes, including pro-liferation, differentiation, tissue repair, inflammation, aging, etc. (Sies, 2014). The Michaelis constant (Km) and the maximum veloci-ty (Vmax), which are the building blocks of the Michae-lis-Menten (MM) equation, are considered the functional constants of enzymes. These constants and other parameters obtained from different ratios (such as enzyme turnover and specificity constant) take specific concentrations of enzymes and substrates into account.

The MM equation has been measured in studies to investigate the structure and function of various enzymes in different environmental conditions, such as temperature and buffer types (Bisswanger, 1994), the various concentration of inhibitors and inducers (Lopina, 2017), and the comparison between kinetic properties of wild-type and mutant enzymes (Humer and Spadiut, 2019). One of the topics of interest during the 1970s and 1980s was the examination of the values of these constants and their changes during the evolution of enzymes. Some studies show the decrease and increase of Km in parallel with kcat and Vmax during evolution (Fersht, 1974), as well as their heritable changes due to the adaptation (Crowlfiy, 1975). It has been reported that in many organisms, kinetic mechanisms have evolved to aid survival in response to changing environmental factors (Ulusu, 2015). All the enzyme kinetics studies mentioned above investigate the effect of different physio-chemical and environmental factors on the constants of enzymes. In this regard, no study to date has examined the effectiveness of mind-body modalities or consciousness interactions on enzymatic activity and function. The nature of consciousness and its place in science has received much attention in the current century. Many philosophical and scientific theories have been proposed in this area. In the1980s, Mohammad Ali Taheri introduced novel fields with a non-material/non-energetic nature named Taheri Consciousness Fields (TCFs). In this perspective, T-Consciousness is one of the three existing elements of the universe apart from matter and energy. According to this theory, there are various TCFs with different functions, which are the subcategories of a networked universal internet called the Cosmic Consciousness Network (CCN). The major difference between the theory of TCFs and other theoretical concepts about consciousness is related to the practical application of the TCFs. These fields can be applied to all living and non-living creatures, including plants, animals, microorganisms, materials, etc. Mohammad Ali Taheri, the founder of Erfan Keyhani Halqeh, a school of thought, introduced a new science in 2020 as a branch of this school. He coined the term Sciencefact for this new science because it utilizes scientific investigations to prove

the scientific approach in proving TCFs. The influence of the TCFs begins with the Connection between CCN as the Whole Taheri Consciousness of the universe and the subjects of study as a part. This Connection called "Ette-sal" is established by a Faradarmangar's mind (a certified and trained individual who has been entrusted with the TCFs). The human mind has an intermediary role (Announcer) which plays a part by fleeting attention to the subject of study and then the main achievement obtained as a result of the effects of the TCFs. These fields cannot be directly measured by science, but it is possible to investigate their effects on various subjects through reproducible laboratory experiments (Taheri, 2013). The research methodology in the study of T-Consciousness has been founded on the process of Assumption, Argument, and Proof, in which the basic Assumption is: The Cosmos was formed by a third element called T-Consciousness that is different from matter and energy. The Argument: The existence of TCFs can be demonstrated by their effects on matter and energy (e.g., humans, animals, plants, microorganisms, cells, materials, etc.) The Proof is the scientific verification of the effects of TCFs on matter and energy (according to the Argument) through various reproducible scientific experiments.

Accordingly, to investigate and verify the existence, effects and mechanisms of TCFs, the following five research phases (Phases 0 through 4), and the aims of each phase are outlined below. Phase-0 studies aim to prove the existence of TCFs by observ-ing their effects. The nature of T-Consciousness and what it is will not be addressed in this phase. Phase-1 explores the varied effects of different TCFs. Phase-2 examines the reason behind the varied effects of these fields. Phase-3 investigates the mechanism of TCFs effects on matter and energy. Finally, Phase-4 draws significant conclusions, particularly with regard to the mind and memory of matter and their relation to the T-Consciousness, etc.

In previous studies on wheat plants, we observed that Far-adarmani CF minimized the negative effects of salt stress by inducing antioxidant enzyme activity (Torabi et al., 2020). Additional studies have demonstrated the perceptible and measurable effect of FCF on the electrical activity of the brain during connection to the CCN (Taheri et al., 2020b), as well as the effects of TCFs on the MCF7 cancer cell line (Taheri et al., 2020a), Alzheimer's disease (Taheri et al., 2021b; Taheri et al., 2021c), bacterial (Taheri et al., 2021d) and viral growth (Taheri et al., 2021a). In order to obtain a more accurate look at the molecular dimensions and evidence for effects and types of functioning of FCF, this study investigates the effect of FCF on HRP in both pure laboratory grade and extracted forms of HRP. Enzymatic function and activities are analyzed based on the principles of enzymology and under the influence of FCF compared to the untreated control.

MATERIALS AND METHODS

In this study, we used HRP obtained from two sources. The first is the laboratory HRP, manufactured and sold under the brand [Sigma], and the second is HRP extracted from the plant (horseradish). This diversity in the sources gives us the possibility of reproducibility of the FCF treatment at more times and stages. Respective Materials and Methods sections for each source are provided separately.

THE FCF APPLICATION

TCFs were applied to the samples according to the protocols regulated by the COSMOintel research center (www.COS-MOintel.com). A request for Connection to the CCN to uti-lize TCFs can be placed through the COSMOintel website in the "Assign Announcement" section. This access is available for everyone at no cost. In order to study and experience this Connection, the researchers can register on the website at any time and in order to report the experiment to the COSMOintel research center. Certain details of the experiment must be pro-vided to the center; for example, the characteristics or num-ber and name of samples and controls must be specified. This entire experiment was carried out as a double-blind method where lab technicians were completely unaware of TCFs theo-ry, and the Faradarmangar at the COSMOintel research center who established the Connection was unaware of the details of the study. Double-blind is a gold standard that is common in science experiments in the field of medicine and psychology, involving theoretical and practical testing

THE LABORATORY SCALE HRP

Materials: HRP (donor: HRP, oxidoreductase, EC 1.11.1.7) and 3,3',5,5'-Tetramethylbenzidine (TMB) were purchased from Sigma Co. Ltd. Other reagents and chemicals were analytical reagent grade. Double-distilled water was used throughout the experiments.

METHODS

The UV/Vis spectra were recorded on a Varian Cary Bio 100 spectrophotometer. Intrinsic fluorescence spectra of the pro-tein were measured at 25° C using a Varian Cary Eclipse fluo-rescence spectrophotometer with the excitation and emission

slit widths of 5 nm. Fluorescence emission from Trp was mea-sured by using excitation at 295 nm to avoid the contribution of tyrosine. In intrinsic fluorescence studies, the concentration of protein was 90.4 μM in 0.2 M potassium phosphate buffer, pH 7.0.

Enzyme assay: Freshly prepared enzyme solutions were used throughout the experiments. The enzyme stock solution was prepared by dissolving HRP (1.5 mg) in PBS (3 mL, 0.01 M, pH 7) and kept on ice. The stock solution of H_2O_2 (0.1 M) was prepared by diluting H_2O_2 (0.5 mL, 30 wt%) to 50 mL in PBS (0.01 M, pH 7). The HRP enzymatic reactions were carried out in PBS (0.01 M, pH 7) at 20°C. All enzymatic reactions were conducted in conventional quartz UV-visible cuvettes in sets of 3 replicates. The total volume of the reaction mixture was 1.5 mL. Experiments were carried out in the presence of a constant concentration of HRP (50 μL) and H_2O_2 (10 μL) with different concentrations of TMB (1.23, 1.85, 3.7, 7.4, 11.1, and 14.8 mM). The colorimetric changes were determined by recording the absorbance at 652 nm.

Kinetics parameters: kinetics constants Vmax and Km have been calculated by fitting the reaction velocity values and the substrate concentrations to the Michaelis–Menten equation as follows:

$$V = (Vmax \times [S]) / (Km + [S])$$

Where V is the initial reaction velocity and Vmax is the max-imal reaction rate that is observed at saturating substrate con-centrations. [S] is the concentration of the substrate and Km isthe Michaelisconstant. Inallthesteps, two containerswere considered blank and samples with different labels. Extracted HRP

Materials: 3,3',5,5'-Tetramethylbenzidine (TMB) were pur-chased from Sigma Co. Ltd. (UK). Horseradish roots were obtained from local markets. Other reagents and chemicals were analytical reagent grade. Double-distilled water was used throughout the experiments.

METHODS

Enzyme extraction: About 100 g of horseradish was thor-oughly washed with water and was cut down into small pieces and homogenized in a blender with 500 ml distilled water. It was centrifuged at 10,000 rpm for 5 min at 4°C. The filtrate was heated in the water bath at 65°C for 3 min to inactivate catalases and cooled quickly in iced water.

The enzyme was precipitated by ammonium sulphate for partial purification. Solid ammonium sulphate was added to 100 ml of peroxidase at the concentration of 30% (w/v). The suspension was stirred for half an hour at 4°C. After sufficient shaking, the precipitates were collected by centrifugation at 10,000 rpm for 30 min. Enzyme activity was determined for each concentration and precipitates were collected for further purification.

Dialysis: Before the dialysis procedure, the dialysis bag was boiled with 0.1 M sodium carbonate solution for 1 h and then retained overnight. This procedure causes the dialysis bag to become open. The precipitates obtained by ammonium sul-phate precipitate were dialyzed in a dialysis bag with 0.2 M phosphate buffer (pH 6.5) with constant stirring on a magnetic stirrer for 2 h. The precipitates were subjected to enzyme assay and protein estimation.

Protein estimation: Proteins were estimated according to the method of Moss and Lowry (1951). The reagents used are as follows: solution A = sodium carbonate in 500 ml distilled wa-ter; solution B = 0.38 g $CuSO_4 \cdot 5H_2O$ and 0.6 g potassium-sodium tartrate in 500 ml of distilled water; solution C = 1-part Folin reagent; solution D = 1 N NaOH.40 g.

The **UV/Vis spectra:** were recorded on a Varian Cary Bio 100 spectrophotometer. Intrinsic fluorescence spectra of the protein were measured at 25°C using a Varian Cary Eclipse fluorescence spectrophotometer with the excitation and emis-sion slit widths of 5 nm. Fluorescence emission from Trp was measured using excitation at 295 nm to avoid the contribution of tyrosine. In intrinsic fluorescence studies, the concentration of protein was 45.2 μM in 0.2 M potassium phosphate buffer, pH 7.0.

Enzyme assay: Freshly extracted enzyme solutions were used in this work. The enzyme stock solution was prepared by dis-solving HRP (1.5 mg) in PBS (3 mL, 0.01 M, pH 7) and kept on ice. The stock solution of H_2O_2 (0.1 M) was prepared by diluting H_2O_2 (0.5 mL, 30 wt%) to 50 mL in PBS (0.01 M, pH 7). The HRP enzymatic reactions were carried out in PBS (0.01 M, pH 7) at 20 °C. All enzymatic reactions were conducted in conventional quartz UV-visible cuvettes in sets of 3 replicates. The total volume of the reaction mixture was 1.5 mL. Experiments were carried out in the presence of a constant concentration of HRP (50 μL) and H_2O_2 (10 μL) with different concentrations of TMB (1.23, 1.85, 3.7, 7.4, 11.1, and 14.8 mM). The colorimetric changes were determined by recording

the absorbance at 652 nm.

Kinetics parameters: All steps and kinetic constant types and calculations have been done similarly to the details mentioned in the previous section.

RESULTS

The results of the FCF treatment on the Lineweaver–Burk plot of laboratory grade HRP are shown in Figure 1.

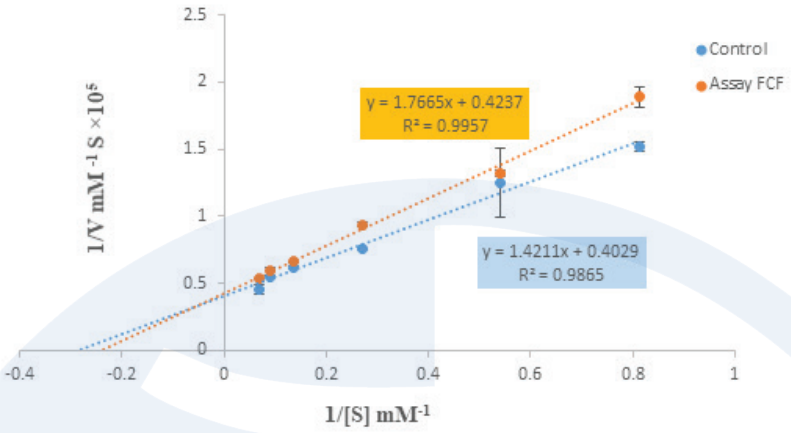


Figure 1. Influence of Faradarmani Consciousness Field (FCF) on Lineweaver–Burk plot of activity of laboratory HRP in H_2O_2 catalysis in comparison with the control.

The kinetic constants of the laboratory scale HRP are shown in Table 1 based on the data in Figure 1. According to Table 1, FCF treatment of laboratory HRP during kinetic assay causes 4.8% decrease and 18.2% increase in Vmax and Km of the enzyme, respectively. Moreover, the turnover and specificity constants of FCF treated laboratory HRP decrease about 5% and 19%, respectively.

Table 1 . Calculated kinetic constants for Laboratory HRP sample. FCF: Faradarmani Consciousness Field

	Vmax (mM/s) × 10^{-5}	Km (mM)	kcat (s-1)	kcat/Km (M-1s-1)	% Δ Vmax	% Δ Km	% Δ kcat	% Δ kcat/Km
Control (None)	2.48	3.52	0.0620	17.6	-	-	-	-
Assay FCF	2.36	4.16	0.0589	14.2	-4.8%	18.2%	-5.0%	-19.3%

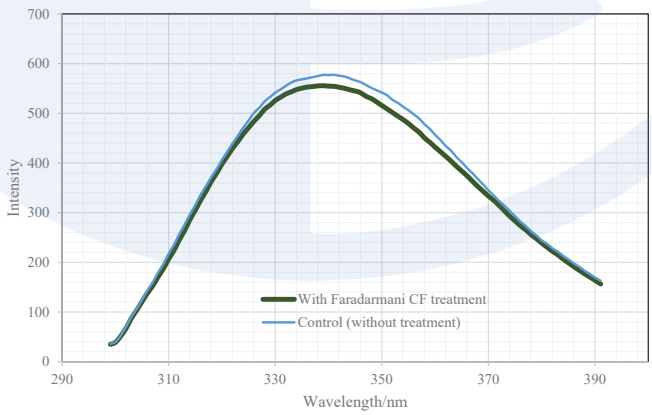
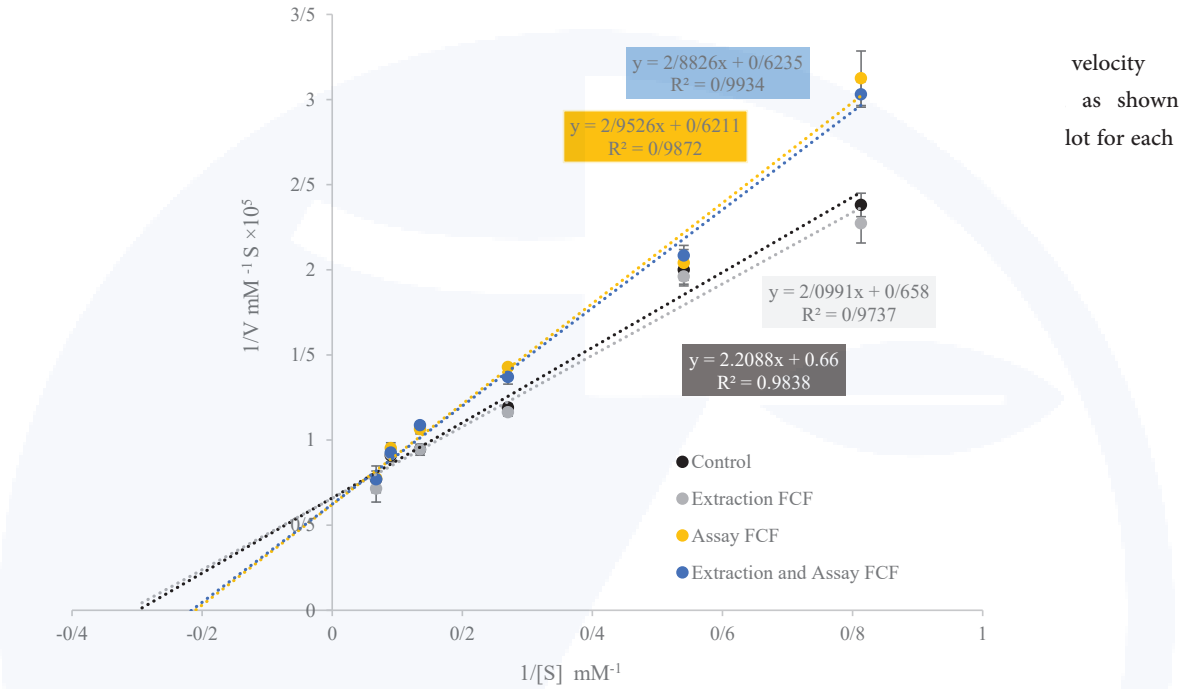


Figure 2. Fluorescence emission of Faradarmani Consciousness Field (FCF) treated sample of laboratory HRP in comparison with the control.

The change in the fluorescence emission intensity of the FCF-treated laboratory grade of HRP in comparison with control (without treatment) is shown in Figure 2. As suggested by this figure, FCF treatment causes 4% decrease in the fluorescence emission intensity as a conformational change in HRP.

Extracted HRP

Since the treatment was performed in two stages of extraction and kinetic measurements, four types of measurements (tests 1-4) were performed (Control with no FCF; FCF during extraction; FCF during assay; FCF during both extraction and assay)



velocity as shown lot for each

Figure 3. Influence of Faradarmani Consciousness Field (FCF) on the Lineweaver–Burk plot of H2O2 catalysis activity of extracted HRP in 2, 3 and 4 tests mentioned in Table 2 in comparison with the control (test No. 1).

Figure 3 shows the Lineweaver-Burk Plot of all tests with extracted HRP, for calculating the kinetic constants of the enzyme in each test (mentioned in Table 2), which can be compared to that of the laboratory HRP (Figure 1).

Table 2 . Calculated kinetic constants for Extracted HRP samples based on the Lineweaver–Burk plots. FCF: Faradarmani Consciousness Field

Test No.	FCF Treatment	Vmax = (mM/s)×10⁻⁵	Km (mM)	kcat	kcat/Km	% Δ Vmax	% Δ Km	% Δ kcat	% Δ kcat/Km
1	Control (None)	1.51	3.34	0.0377	11.3	-	-	-	-
2	Extraction FCF	1.52	3.19	0.0379	11.9	0.7%	-4.5%	0.5%	5.3%
3	Assay FCF	1.61	4.76	0.0403	8.45	6.6%	42.5%	6.9%	-25.2%
4	Extraction & Assay FCF	1.60	4.62	0.0400	8.65	6.0%	38.3%	6.1%	-23.5%

Table 2 indicates the values of constants obtained in different test modes and their comparisons to the control. Figure 4 shows the intensity of fluorescence emission of the extracted enzyme with the FCF treatment in comparison with control (extracted enzyme without the FCF treatment), indicating a 7% increase in fluorescence emission.

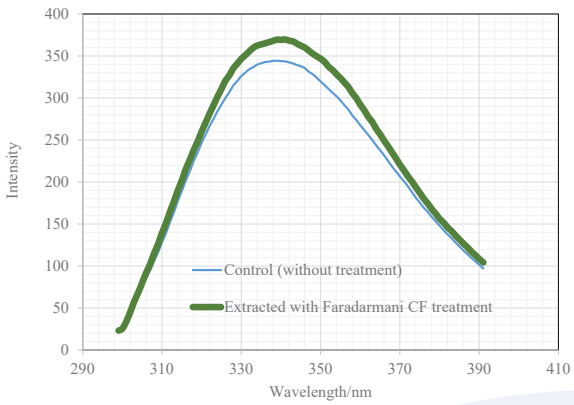


Figure 4. Fluorescence emission of Faradarmani Conscious-ness Field (FCF) treated sample of extracted HRP in compar-ison with control.

DISCUSSION

In this study, the effect of FCF is studied on the structure and function of HRP in both pure laboratory and plant extracts. The results of the FCF treatment suggest an indication of the ef-fect of this non-material and non-energetic field on the struc-ture and function of HRP under the same physical conditions in the samples that received FCF compared to the untreated control. Secondly, the increase in the value of Km of the en-zyme in the FCF treated suggests that a higher concentration of the substrate under influence of FCF is required. Finally, the change in enzyme function along with the structural changes, as measured by fluorescence technique, indicates the effect of FCF on the subject under the study. Previous studies related to the evolution of enzymes have shown an increase in Km and its kcat in relation to the changes in the functional pathways of enzymes during their evolution (Pettersson, 1989). Since the measurement of constants in the same environmental conditions yield limited changes in the range of fluctuations of laboratory errors, the results, especial-ly in the case of the Km value, indicate that FCF seems to have acted as a mechanism for the evolutionary changes of the enzyme in the short duration of the study.

The increasing value of Km in an environment with a higher concentration of the substrate (as in a kinetic assay situation) demonstrates a characteristic reduction in enzymatic behav-ior. It prepares the enzyme to accept more diverse substrates. On the other hand, the increased kcat in the extracted en-zyme under the FCF treatment, during both extraction and kinetic assay, shows significant accordance with HRP general function. It is noteworthy that the ~4% decrease in Km value between the control (test 1) and the extraction + FCF (test 2) is mirrored by the ~ 4% decrease in Km value between test 3 and test 4 (where the only difference is the application of FCF during extraction) which suggests reproducibility of behavior under FCF. In this study, the laboratory HRP sample demonstrated a mod-est increase (~18%) in Kmand a decrease in Vmax (~5%), and kcat (~5%), while the extracted HRP sample demonstrated a significant increase (~40%) in Km, and increases in Vmax (~6%), and kcat (~6%). In both samples, there was a notable effect on the enzyme kinetics when FCF was applied during the assay. The changes in Vmax and kcat also correlate with the changes in fluorescence emission intensity, as shown in Figures 2 and 4. When Vmax and kcat decrease, the emission intensity decreases, and when Vmax and kcat increase, the fluorescence emission intensity increases. These results indi-cate that the application of FCF to HRP creates a measurable change in the performance of the enzyme, substantiating the presence of FCF. The differences observed between the laboratory and extract-ed samples, considering Taheri's theory of TCFs, may be due to the proximity of the influence of the consciousness of the natural environment as the consciousness of the Parts (horse-radish plant) on the HRP enzyme. In the freshly extracted HRP sample, we saw an increase in catalytic activity under FCF, while in the FCF treatment of lyophilized laboratory en-zyme, a decrease in catalytic activity was observed that seems to be a result of the long-time separation of the subject of the study (laboratory HRP) from the generating organism (horse-radish plant), and the industrial stages of enzyme preparation. These demonstrations of the effects of FC F on HRP, by the remarkable changes observed in HRP kinetics, suggest it is the existence of such a field, which requires further investigation in other fields of biological, chemical, and physical sciences. Additional studies on the influence of FCF on other types of vital enzymes and proteins are currently being pursued by the authors of this study.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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