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Influence of Water Polarization Caused by Phonon Resonance on Catalytic Activity of Enolase

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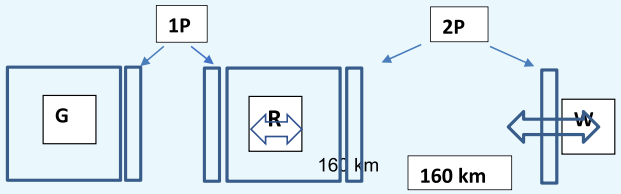
ABSTRACT: Enolase is a conservative protein. Its cellular enzymatic activity catalyzes the conversion of 2-phospho-D-glycerate (2-PGA) to a phosphoenolpyruvate (PEP) product in the glycolysis pathway. This enzyme also has a multifunctional nature participating in several biological processes. This work aims to determine the effect of water polarization on the catalytic activity of enolase. The experiments have been set based on the concept that water, a polar dielectric, may undergo the phenomenon of electric polarization, decreasing its configurational and vibrational entropy. Prior to the reaction, the 2-PGA substrate was incubated for 5 h in the glass cuvette with an attached chip-inductor. The latter device was designed to transfer quantum information about a given quantum state from the quantum state generator to water by a phonon resonance. Then, such substrate samples preincubated with the chip-inductor were removed every hour in a separate quartz cuvette with the enzyme to determine its catalytic activity. The influence of the chip-inductor on the preincubated substrate resulted in an increase in the catalytic activity of enolase by 30% compared to the control substrate, not preincubated with the chip-inductor. This suggests that the catalytic activity of the enzyme is augmented when the substrate was primed by chip-inductors. In another kind of experiment, wherein enolase was exposed to methylglyoxal modification, the catalytic activity of the enzyme dropped to 71.7%, while the same enzyme glycated with methylglyoxal primed by chip-inductors restored its activity by 8.4%. This shows the protective effect of chip-inductors on enolase activity despite the harmful effect of methylglyoxal on the protein.



Generator of quantum states (G), resonator of quantum states (R), the first pair (1P) and the second pair (2P) of chip inductors, with working chip inductor (W)

INTRODUCTION

Enolase (E.C. 4.2.1.11) acts as 2-phospho-D-glycerate hydrolase, catalyzing the conversion of 2-phospho-D-glycerate (2-PGA) to phosphoenolpyruvate (PEP) in the glycolysis pathway, and also in the reverse reaction, i.e., in the gluconeogenesis pathway as phosphoenolpyruvate hydratase.[1] It works in both catabolic and anabolic ways. This highly conserved protein preserves similar catalytic function both in prokaryotes and eukaryotes.[2] Enolase is a dimer, formed by three types of subunits, α, β, and γ, each encoded by a separate gene. Both homo- and heterodimers are formed. The α homodimer is found both in the human fetus and adult tissues such as lungs, liver, adipose tissue, pancreas, spleen, and kidneys. The β isoenzyme is found in tissues requiring high energy such as the heart and skeletal muscle ($\alpha\beta$ and $\beta\beta$ isoenzymes), while γ-enolase is present in neuronal and neuroendocrine cells ($\alpha\gamma$ and $\gamma\gamma$ isoforms). Enolase also performs multiple functions in the non-glycolytic pathway, and is therefore named the "moonlighting" protein, it is also involved in the regulation of the morphology of the cell and it interacts with the cytoskeleton system.[3] α-Enolase has been linked to numerous diseases including metastatic cancer, neurodegenerative diseases, autoimmune disorders, and bacterial infections.[2,4] Moreover, enolase plays a role in some allergies caused by pathogenic fungi. It is worth mentioning that α-enolase is a common autoantigen in various autoimmune diseases.[5] This multifunctional nature of enolase is determined based on preliminary studies on the effect of water electric polarization on the catalytic activity of the enzyme. The aim of this work is to investigate whether the catalytic activity of enolase can be affected by the electric polarization of water generated by the chip-inductor in the quantum state of suitably lower entropy than that of water, which may lead to a decrease in both configurational and vibrational entropy of water, and hence, also stabilize its hydrogen-bonding network,

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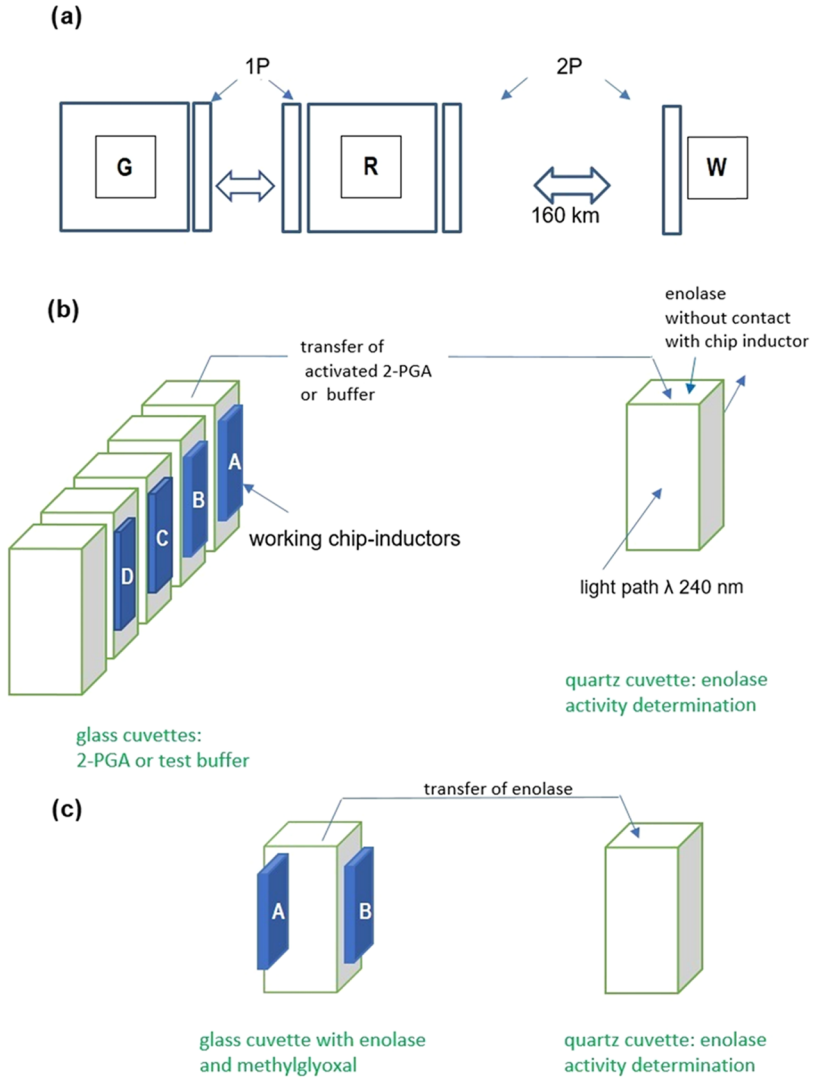

Figure 1. Schedule of (a) the system composed of a quantum state generator (G), quantum state resonator (R), the first pair (1P), and the second pair (2P) of chip-inductors, where the working chip-inductor (W) of the second pair of chip-inductors is in contact with glass cuvettes for the preincubated substrate or standard test buffer before enolase activity determination, as shown in (b). Glycation with methylglyoxal (c).

similar to the effect reported previously.[6] In turn, the so-called quantum tunneling is considered to play a crucial role during hydrogen transfer in enzymes.[7] The catalytic reaction can be very fast due to quantum effects (tunnel effect). The phenomenon of macroscopic quantum nonlocality was observed primarily for entangled pairs of photons or atoms.[8] However, recently, this has also been detected for entangled pairs of phonon modes in two macroscopic crystals of diamonds spatially separated at room temperature.[9] A similar phenomenon to the latter one also appears to be realized in our experiments, in which quantum entanglement with a fixed and coherent state is maintained by the generator and resonator of the system used.[10] Interestingly, transmission and reception of information at the subquantum level (the conversion of information into energy) and quantum level turns out to be a general feature of matter because matter shows a wide-range reception of electromagnetic information, especially in polar dielectrics and semiconductors.[11−13] In this context, the role of phonon or vibrational resonance that has been observed (e.g., in ref 14) is not well recognized. Our experiments were performed in the solution of the substrate used in the enolase reaction. As a control, the enzymatic reaction has been performed using samples either without the chip-inductor or with a nonactivated chip. We assume here that quantum information about the quantum states of the single-crystal silicon is transferred from the crystal by the phenomenon of quantum entanglement of multimolecular systems to the chip-inductor and, then, to the sample due to phonon resonance. The experiments revealed for the first time that the transferred quantum states affect the enzymatic reaction, most likely, by the phenomenon of water electric polarization decreasing its entropy.

MATERIALS AND METHODS

Materials and Buffers. 2-Phospho-D-glycerate was purchased from Fluka (Fluka Analytical, St. Gallen, Switzerland). Other reagents were purchased from Sigma-Aldrich (Sigma-Aldrich Poland, Poznan, Poland). All chemicals were of the analytical grade. Chip-inductors **A**, **B**, **C**, and **D**, aluminum plates with a surface area of 1 × 2 cm^2 and 0.8 mm thickness, were stored in polyethylene bags at room temperature (RT) and taken out and kept for 30 min before the experiment. Working chip-inductors W are marked in Figure 1a. Enolase

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Table 1. Influence of the Chip-Inductors on the 2-PGA Substrate in Water or on Buffer Used for Enolase Activity Determination (data in brackets) and the Effect on Catalytic Human Muscle Enolase Activity Not Subjected to Chip-Inductor Contact

sample							
time of incubation [min]		0	60	120	180	240	300
A	activity [IU/mg]	41,75	43,43	42,56	41,65	41,7	41,59
		(43,31)	(44,98)	(42,99)	(35,60)	(34,15)	(33,23)
	W_i/W_0 [%]	100	104	102	99.8	99.9	99.6
		(100)	(104)	(99,3)	(82,2)	(78,85)	(76,7)
	W_i/K_{0i} [%]	105.9	109.8	108.3	103.3	107.4	113.5
		(102,6)	(109,7)	(108,3)	(105,9)	(107,1)	(111)
B	activity [IU/mg]	45.85	47.43	46.20	46.50	46.95	46.08
		(49,56)	(51,22)	(50,80)	(46,37)	(41,54)	(39,0)
	W_i/W_0 [%]	100	103.4	100.8	101.4	102.4	102.5
		(100)	(103,3)	(102,5)	(93,6)	(83,8)	(78,7)
	W_i/K_{0i} [%]	114.8	119.8	117.6	115.3	122.9	125.7
		(117,4)	(125,6)	(136,5)	(137,4)	(130,3)	(97,8)
D	activity [IU/mg]	49.91	47.55	44.79	44.36	44.10	43.93
		(46,34)	(49,40)	(48,27)	(44,27)	(40,43)	(37,14)
	W_i/W_0 [%]	100	95.3	89.7	88.8	88.4	88.0
		(100)	(106,6)	(104,2)	(96,5)	(87,2)	(80,1)
	W_i/K_{0i} [%]	126.6	120.2	114	110	113.6	119.6
		(109,8)	(120,7)	(129,5)	(132,5)	(126,8)	(124,1)
control chip-inductor C	activity [IU/mg]	38.49	38.4	37.75	36.2	35.1	35.33
		(41,9)	(39,38)	(35,96)	(33,25)	(30,13)	(29,27)
	W_i/W_0 [%]	100	99.8	98.1	94.1	91.2	91.8
		(100)	(94)	(85,8)	(79,4)	(71,9)	(69,9)
	W_i/K_{0i} [%]	97.6	97.1	96.1	89.8	90.4	96.4
		(99,3)	(96,2)	(96,6)	(98,5)	(94,5)	(97,8)
control K_o (system without chip-inductor contact)	activity [IU/mg]	39.43	39.56	39.30	40.33	38.82	36.65
		(42,2)	(40,93)	(37,23)	(33,96)	(31,89)	(29,93)
	K_{0i}/K_0 [%]	100	100.3	99.7	109.8	98.5	93.0
		(100)	(97)	(88,2)	(80,5)	(75,6)	(70,9)
	K_{0i}/K_{0i} [%]	100	100	100	100	100	100
		(100)	(100)	(100)	(100)	(100)	(100)

isolated from human muscle tissue by the method of Bednarz-Misa et al. was used in the experiment.[15] Enzyme stock solution R_0 was prepared in stabilizing buffer 7.5 mM imidazole−HCl, pH 6.8, containing 2.5 mM $MgSO_4$, 50 mM NaCl, and 50% glycerol (kept at 4 °C). The concentration of enolase in this stock solution R_0 was 5 mg/ml, and its catalytic activity was 52 IU/mg, and the concentration of the working solution of the enzyme was 1 mg/ml (diluted from R_0 in test buffer pH 6.8). Standard test buffer contained 50 mM imidazole−HCl pH 6.8, 3 mM $MgSO_4$, and 0.4 M KCl and was used for enolase catalytic activity determination. The concentration of water stock solution R_0 of substrate 2-PGA (in the form of sodium salt) was 63 mM. The concentration of the protein was determined by measuring its absorbance at 280 nm. Quartz cuvettes were used for protein and catalytic activity determinations.

Chip-Inductors. In this study, the transmitter and receiver of quantum information are two pairs of quantum inductors. The first inductor of the first pair is connected to a quantum state generator in contact with a silicon crystal, as the source of quantum information (G in Figure 1a), and the second inductor is connected to a quantum phonon resonator (R in Figure 1a).[16] Note that the distance between the chip-inductor of the quantum resonator R and the working chip-inductor W (Figure 1a), both of the second pair of chip-inductors, is 160 km (Figure 1). The chips are made of previously crafted condensed matter of aluminum, for which each pair is separately quantum tangled in the superposition state.[16] The chip retrieves information upon direct contact. Activated chip-inductors transfer quantum information through interaction with silicon and about the entropy level of its crystalline network by a phonon resonance.

Medium Activation with the Chip-Inductor. Three series of experiments have been performed. In the first one (I), five 200 μL aliquots of the 2-PGA substrate stock solution were kept in 5 glass cuvettes for 5 h at RT, exposed to visual light with chip-inductors A, B, C, or D attached to cuvettes. Control Ko was without a chip-inductor. The distance between all five incubated samples was 3 m. Aliquots of 17 μL of 2-PGA were withdrawn from five vessels every hour to quartz cuvettes with 8 μL enzyme in 0.775 mL of buffer to perform the catalytic reaction. In the second experiment (II), five 4 ml samples of test buffer in separate glass cuvettes were interacted for 5 h at RT with the chip-inductors A, B, C, and D and Ko control sample without a chip-inductor attached to cuvettes. Then, 0.775 mL aliquots of treated test buffer were withdrawn every hour and placed in quartz cuvettes with 8 μg of enolase and 17 μL of 2-PGA for enzymatic activity measurement (Figure 1b). The third experiment (III) was set with both 2-PGA substrate and test buffer under the influence of the chip-inductor D, and then withdrawn to measure the catalytic activity of the enzyme. The chip-inductor labels were blinded,

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and their identity was revealed after finishing the experiments. All experiments were repeated in triplicates for statistical analysis.

Enolase Activity Assay. The enzymatic activity of enolase was determined as previously described.[15] Briefly, the substrate 2-PGA was added at 1 mM concentration to test buffer. The conversion of the 2-PGA to phosphoenolpyruvate (PEP) was followed with 8 μg of enolase. The absorbance of the PEP product was monitored at 240 nm at 25 °C for 1 min with a spectrophotometer, JASCO UV−vis V-530. One unit of enolase activity is defined as the amount of 1 μmole of PEP formed in 1 min by 1 mg of the enzyme under the reaction conditions. The molar absorption coefficient was taken as 1520 /(M^{-1} cm^{-1}) to calculate the amount of product formed from 2-PGA. The specific activity was expressed as international units per milligram of enzyme protein.

Glycation of Enolase by Methylglyoxal in the Presence of the Chip-Inductor. The stock solution of enolase, R_0 was used for modification of the enzyme. Methylglyoxal, also formed in the glycolytic pathway, is one of the most reactive compounds to protein glycation agents. The glycation has been performed using a large excess of methylglyoxal versus enolase (300:1 mol/mol ratio), as published previously.[17] A 175 μL sample of phosphate-buffered saline (PBS) was placed in a glass cuvette, then 20 μL of enolase stock solution and 5 μL of 0.8% aqueous solution of methylglyoxal were added. Glycation of enolase was performed for 3 h at RT in the presence of two chip-inductors, A and B, both attached to the external walls of the cuvette (Figure 1c). In the control K1 glycation experiment, the enolase was treated in a cuvette without the chip-inductor. In the second K2 control experiment, the enolase (20 μL) with 180 μL of PBS was neither modified with methylglyoxal nor exposed to a chip-inductor. During the enolase glycation, changes in catalytic activity were tested at every 1-h interval in 15 μL aliquots of enolase (12 μg of protein) transferred to 870 μL of test buffer and 15 μL of the 2-PGA substrate stock solution. The glycation of enolase was stopped by adding 15 μL of aqueous lysine solution (4 mg/mL) to bind the excess of unreacted methylglyoxal. To detect the presence of high molecular mass advanced glycation end-products (AGE), the samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), as discussed previously.[18] Samples of 12 μg of protein were added to the wells of the PAGE gel.

RESULTS AND DISCUSSION

Enolase Enzymatic Activity in the Presence of the Chip-Inductor. The experiments have been carried out to check the concept that transferred information on the quantum states might influence the enzymatic reaction. Only a small loss of enolase activity was observed during exposure of enzyme at RT in the Ko sample treated with the substrate not exposed to any chip-inductor. This indicates the stability of enolase kept at RT for 5 h (Table 1 and Figure 2). The changes in the activity of human enolase were observed only when substrate 2-PGA or test buffer were, prior to reaction, exposed to the chip-inductors. The effects of the chip-inductors on the 2-PGA substrate in water were beneficial for the catalytic activity of the enzyme (Figure 2a). The exposure of the substrate for a longer time to the chip-inductor resulted in an increase in the specific activity of the enzyme. After 5 h exposure of the substrate to the chip-inductor, the enzyme activity increased by

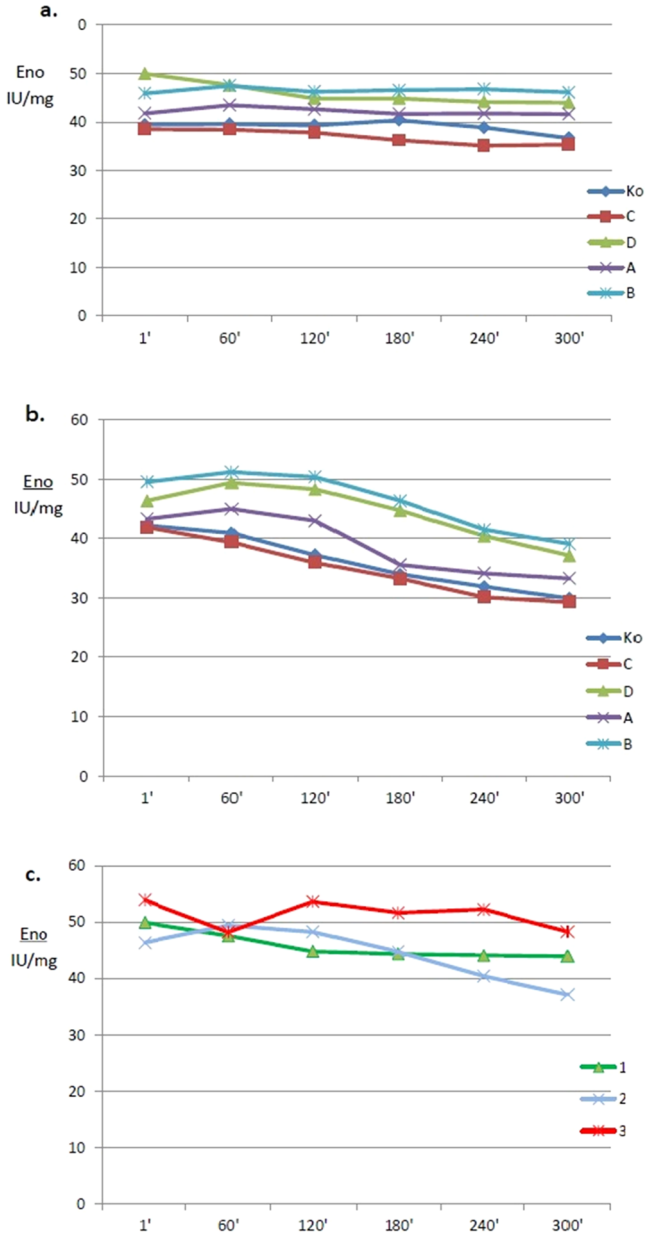

Figure 2. Enolase activity under various experimental conditions, determined in test assay containing: (a) substrate subjected to activated chip-inductors A, B, or D and nonactivated chip-inductor C as well as a control sample (Ko)—without contact of 2-PGA with the chip-inductor; (b) buffer exposed to activated chip-inductors A, B, or D and nonactivated chip-inductor C as well as the control sample (Ko)—without contact of the test buffer with the chip-inductor; and (c) substrate alone (line 1) or assay buffer alone (line 2) as well as substrate and assay buffer, both (line 3) exposed to chip-inductor D interactions and used for enolase activity determination, as described in the Materials and Methods section.

20−30% compared to the Ko control (Table 1). The observed phenomenon may possibly indicate the reduced energy needed to achieve proper orientation of the functional groups in 2-PGA (carboxyl and phosphate) toward the catalytic center of the enzyme. One may presume that substrate molecules ordered by the exposure to chip-inductors result in certain changes in the spatial structure of the enzyme catalytic pocket, facilitating reaction, 2-PGA → PEP. The catalytic activity of enolase, tested in the buffer exposed earlier to the chip-

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inductor D, was significantly higher compared to the control sample, where the buffer was not influenced by the chip-inductor (Figure 2b). In a subsequent experiment, we tested enolase activity on the 2-PGA substrate and the buffer both exposed to the chip-inductor D. As shown in Figure 2c, the enzyme tested under both conditions showed the highest activity. This indicates the favorable effect of chip-inductors on buffer or substrate and buffer on the 2-PGA → PEP + H_2O catalytic reaction. More effective participation of water molecules in inducing the changes in the spatial structure of the catalytic pocket is also highly probable, facilitating the transformation of the substrate to the reaction product. These experiments have been performed using β-enolase, the isoform studied in our laboratory, as a potential marker of heart muscle injury. $\beta\beta$ enolase accounts for 3% of the soluble protein in the human skeletal muscle, and more than 90% of the enzymatic activity is attributed to this enolase variant.[3,19] The catalytic center of enolase and the subunit interface in the native molecule are highly conserved and composed of identical amino acids, but there are short variable regions located on the surface of the molecule that are probably responsible for the interaction of enolase with other macromolecules.[20] The results of our experiment (I) indicate that the polar ordering of water molecules may be transferred with the substrate sample added to the test. The same phenomenon could be observed in experiment (II) where the buffer exposed to the chip-inductors is added to the enzymatic reaction. Hence, the conclusion may be drawn that during the determination of enolase activity, there is, most likely, a transfer of that ordered state induced by the activated chip-inductor in water molecules from buffer or substrate 2-PGA to structural water constitutively present in the active center of the enzyme. In the mechanism of the reaction of enolase, three water molecules and two magnesium ions are spatially oriented in the catalytic pocket. It has been well documented that the alteration of the solvation layer and hydrogen bond network can affect, among others, the protein dynamics.[21,22] Water is needed for stabilizing the position of magnesium ions—catalytic and conformational, necessary for the 2-PGA → PEP reaction to proceed, and through this transformation, there are dislocations of hydrogen bonds in the area of the active center.[23] The chip-inductor activated by the generation of the quantum state is efficient at ordering the water molecules. The investigated effects might also influence noncatalytic functions of enolase as a "moonlighting protein". In the cytoplasm of eukaryotic cells, enolase participates in the stabilization of the cytoskeleton for maintaining the proper structure of myotubules, in the stabilization of tRNA.[24] It acts as a chaperon protein under conditions of oxidative, thermic, and metabolic stress or protects the genome by blocking the nuclear c-myc oncogene.[25] In the formation of the complex with plasminogen in the plasma membranes, is involved in extracellular processes associated with pathogen invasiveness, tumor tissue growth, and myogenesis.[26] During the development of inflammation, the enolase−plasminogen system on the surface of immune cells is involved in the initiation of anti-inflammatory activities.[4] In the nervous system cells, the receptor of enolase plays a neuroprotective role by binding active plasmin, which allows the degradation of neurotoxic β-amyloid aggregates.[27]

Glycation of Human Enolase in the Presence of the Chip-Inductor. An enzyme exposed to room temperature partially loses its activity in a control sample (K2) containing a native enzyme not exposed to chip-inductors, while its specific activity after 3 h of incubation with chip-inductors decreased from 24.7 to 19.48 IU/mg and was more than 21% lower than the initial (Table 2 and Figure 3b). The modification of enolase with methylglyoxal inactivated the enzyme to a greater extent, after 3 h of glycation, the specific activity of the K1 sample decreased from 24.6 to 17.64 IU/mg, i.e., 28.3%. In the

Table 2. Activity (Marked in Bold) of Human Muscle Enolase Glycated with Methylglyoxal in the Presence of Chip-Inductors (A + B)[a]

	sample				
	time of incubation [h]	0	1	2	3
W	activity [IU/mg]	**29.22**	**25.78**	**24.12**	**23.4**
	W_i/W_0 [%]	100	88.2	82.5	80.1
	$W_i/K_{(2)I}$ [%]	118.3	113.2	117.3	120.1
K1	activity [IU/mg]	**24.60**	**21.37**	**19.15**	**17.64**
	$K_{(1)}/K_{(1)0}$ [%]	100	86.9	77.85	71.7
	$K_{(1)i}/K_{(2)I}$ [%]	99.6	93.8	88.8	90.6
K2	activity [IU/mg]	**24.70**	**22.78**	**20.56**	**19.48**
	$K_{(2)i}/K_{(2)0}$ [%]	100	92.2	83.2	78.9

[a]W: analyzed sample (enolase + methylglyoxal + chips A + B); K1: control sample 1 (enolase + methylglyoxal, without contact with chips A and B); K2: control sample 2 (enolase without methylglyoxal, without contact with chips A, B).

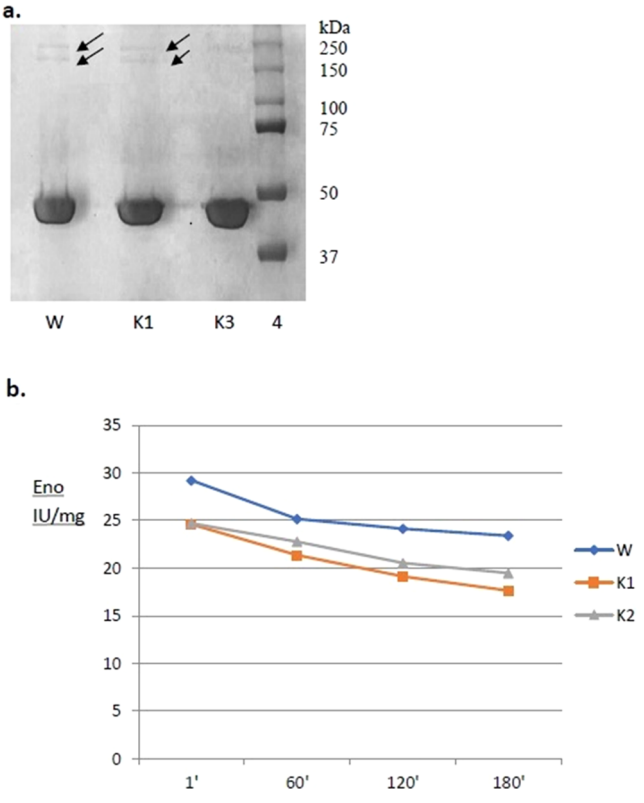

Figure 3. Glycation of human muscle enolase with excess methylglyoxal (1:300 mol/mol) in pH 7.4 PBS at room temperature for 3 h. Picture of SDS/PAGE using 10% resolving gel (a) and changes of enolase activity (b) in the analyzed sample *W* (enzyme modified with MG in the presence of 2 chip-inductor A and B, (1 × 2 cm^2)) and control K1 (glycation of the enzyme without the influence of chip-inductors) and control K2 (enolase unmodified with MG). Protein standards of molecular mass are shown in lane 4.

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presence of chip-inductors, the pattern of changes of specific activity was lowered to 19.9%, but higher specific activity values were observed from 29.22 IU/mg initially to 23.4 IU/mg in the third hour of the glycation experiment. Comparing the relative changes in enzyme specific activity in the analyzed sample (W) compared to the values obtained for enolase in the unmodified control (K2), the beneficial effect could be observed in the electric polarization on the catalytic efficiency of the enzyme. Throughout the experiment, the glycated enzyme maintained high activity in the presence of chip-inductors—it represented 113−120% of the specific activity of the unmodified control sample (Table 2). At the same time, contact with the chip-inductors did not affect the formation of AGE during the glycation of enolase by methylglyoxal. The profile of the high molecular mass products of advanced glycation is visible in Figure 3a. Both in the analyzed sample treated with chip-inductors (lane 1) and in the control sample, where glycation occurred in the absence of chip-inductors (lane 2), AGE was observed at M_w of 180 and 230 kDa (marked with stars), while lane 3 shows the protein profile of the native enolase, not glycated. Glycation that occurs in an organism throughout its life is an unfavorable process, as it reduces the metabolic efficiency of the body. Accumulation of AGE in cells can lead to the activation of intracellular signal transduction pathways, causing a number of adverse metabolic changes during aging.[28] In previous reports, we have shown the susceptibility of muscle enolase to glycation by several reactive glycation agents, especially methylglyoxal among others.[17,18] Methylglyoxal (H_3C-CO-CHO) is formed in the body mainly as a product of defective glycolysis or in the process of lipid peroxidation, as well as in the disordered defense systems during carbonyl and oxidative stress. It is interesting that despite the formation of AGE, the enzyme maintained good catalytic activity (Table 2). Compared to the control K1 (enzyme glycation without contact with the chip-inductor), the observed relative increase in enzyme activity ranged from about 19% to approx. 33% (Table 2). Thus, the effects of the chip-inductor on the enzymatic reaction indicate the possibility of a protective effect on enolase, despite conditions promoting glycation in cells (hyperglycemia, carbonyl stress, oxidative stress). This could ensure that the effective glycolysis process is maintained. The positive effect of electric polarization indicates a possible therapeutic potential in diabetic complications and neurodegenerative diseases.

CONCLUSIONS

The electric polarization phenomenon generated by the chip-inductors has a beneficial effect on the catalytic activity of the enzyme. This indicates the arrangement of substrate molecules. This phenomenon can reduce the energy effort needed to achieve the correct orientation of 2-PGA functional groups toward the catalytic enzyme center. In addition, it is possible to induce some changes in the spatial structure of the catalytic pocket by thus ordered substrate molecules, facilitating the 2-PGA → PEP reaction. It is advisable to further study the effect of polarization on the enzyme to check this effect on the kinetic parameters of enolase. It is expedient to examine how the maximum reaction speed (V_{max}) and affinity (Michaelis−Menten K_M constant) change in relation to substrates in both direct (2-PGA → PEP) and inverted (PEP → 2-PGA) reactions. It is important to investigate the effect of polarization on the sensitivity of the enzyme to fluoride and phosphates—its natural inhibitors. It will be important to check *in vitro* the ability of enolase to bind plasminogen after the polarization phenomenon is triggered. It is also important to investigate the effect of these phenomena on the susceptibility of enolase on glycation and the creation of high molecular mass products AGE—typical in the chronic state of hyperglycemia, which causes pathologies associated with diabetes and neurodegenerative diseases.

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Notes

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REFERENCES

(1) Wold, F. Enolase. In *The enzymes*, 3rd ed.; Boyer, P. D., Ed. Academic Press: New York, 1971; *5*, pp 499−538.

(2) Pancholi, V. Multifunctional alpha-enolase: its role in diseases. *Cell. Mol. Life Sci.* **2001**, *58*, 902−920.

(3) Kim, J. W.; Dang, C. V. Multifaceted roles of glycolytic enzymes. *Trends Biochem. Sci.* **2005**, *30*, 142−150.

(4) Díaz-Ramos, A.; Roig-Borrellas, A.; García-Melero, A.; López-Alemany, R. α-Enolase, a multifunctional protein: its role on pathophysiological situations. *J. Biomed Biotechnol.* **2012**, *2012*, No. 156795.

(5) Montes, A.; Dieguez-Gonzalez, R.; Perez-Pampin, E.; Calaza, M.; Mera-Varela, A.; Gomez-Reino, J. J.; Gonzalez, A. Particular association of clinical and genetic features with autoimmunity to citrullinated α-enolase in rheumatoid arthritis. *Arthritis Rheum.* **2011**, *63*, 654−661.

https://dx.doi.org/10.1021/acsomega.0c05199
ACS Omega 2021, 6, 4255−4261

(6) Elton, D. C.; Fernández-Serra, M. The hydrogen-bond network of water supports propagating optical phonon-like modes. *Nat. Commun.* **2016**, *7*, No. 10193.
(7) Brookes, J. C. Quantum effects in biology: golden rule in enzymes, olfaction, photosynthesis and magnetodetection. *Proc. R. Soc. A* **2017**, *473*, No. 20160822.
(8) Wasak, T.; Chwedeńczuk, J. Bell Inequality, Einstein-Podolski-Rosen Steering, and Quantum Metrology with Spinor Bose-Einstein Condensates. *Phys. Rev. Lett.* **2018**, *120*, No. 140406.
(9) Lee, K. C.; Sprague, M. R.; Sussman, B. J.; Nunn, J.; Langford, N. K.; Jin, X.-M.; Champion, T.; Michelberger, P.; Reim, K. F.; England, D.; Jaksch, D.; Walmsley, I. A. Entangling macroscopic diamonds at room temperature. *Science* **2011**, *334*, 1253−1256.
(10) Gorska, K.; Horzela, A.; Szafraniec, F. H. In *Coherence, Squeezing and Entanglement: An example of peaceful coexistence*, Antoine, J.-P.; Antoine, J.-P.; Bagarello, F.; Gazeau, J. P., Eds., Coherent States and their Applications: A Contemporary Panorama, in Proceedings of the CIRM Workshop, Nov 13-18, 2016; Springer Proceedings in Physics (SPPHY), 2018; pp 89−117.
(11) Bao, Xiao-Hui.; Xu, Xiao-Fan.; Li, Che-Ming.; Yuan, Zhen-Sheng.; Lu, Chao-Yang.; Pan, Jian-Wei. Quantum teleportation between remote atomic-ensemble quantum memories. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 20347−20351.
(12) Smith, Cyril. W. Quantum and coherence effects in water and living systems. *J. Altern. Complement. Med.* **2004**, *10*, 69−78.
(13) Trixler, F. Quantum tunneling to the origin and evolution of life. *Curr. Org. Chem.* **2013**, *17*, 1758−1770.
(14) Fletcher, J. R.; Naftaly, M.; Molloy, J. F.; Andreev, Y. M.; Kokh, K. A.; Lanskii, G. V. Measurement of a phonon resonance in a GaSe crystal using THz free induction decay. *Vib. Spectrosc.* **2017**, 169−172.
(15) Bednarz-Misa, I.; Pietkiewicz, J.; Banaś, T.; Gamian, A. Enolase from *Klebsiella pneumoniae* and human muscle cells. I. Purification and comparative molecular studies. *Adv. Clin. Exp. Med.* **2009**, *18*, 71−78.
(16) Beznosiuk, J.; Wandzel, Cz. System and way of transferring information about quantum states, especially macromolecular systems. Patent Application No. P4328522020.
(17) Pietkiewicz, J.; Gamian, A.; Staniszewska, M.; Danielewicz, R. Inhibition of human muscle-specific enolase by methylglyoxal and irreversible formation of advanced glycation end products. *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 356−364.
(18) Pietkiewicz, J.; Bronowicka-Szydełko, A.; Dzierzba, K.; Danielewicz, R.; Gamian, A. Glycation of the muscle-specific enolase by reactive carbonyls: effect of temperature and the protection role of carnosine, pirydoxamine and phosphatidylserine. *Protein J.* **2011**, *30*, 149−158.
(19) Chai, G.; Brewer, J. M.; Lovelace, L. L.; Aoki, T.; Minor, W.; Lebioda, L. Expression, purification and the 1.8 angstroms resolution crystal structure of human neuron specific enolase. *J. Mol. Biol.* **2004**, *20*, 1015−1021.
(20) Lebioda, L.; Stec, B. Mapping of isozymic differences in enolase. *Int. J. Biol. Macromol.* **1991**, *13*, 97−100.
(21) Zhang, L.; Yang, Y.; Kao, Y. T.; Wang, L.; Zhong, D. Protein hydration dynamics and molecular mechanism of coupled water-protein fluctuations. *J. Am. Chem. Soc.* **2009**, *131*, 10677−10691.
(22) Kuffel, A.; Zielkiewicz, J. Why the Solvation Water around Proteins Is More Dense than Bulk Water. *J. Phys. Chem. B.* **2012**, *116*, 12113−12124.
(23) Qin, J.; Chai, G.; Brewer, J. M.; Lovelace, L. L.; Lebioda, L. Structures of asymmetric complexes of human neuron specific enolase with resolved substrate and product and an analogous complex with two inhibitors indicate subunit interaction and inhibitor cooperativity. *J. Inorg. Biochem.* **2012**, *111*, 187−194.
(24) Didiasova, M.; Schaefer, L.; Wygrecka, M. When Place Matters: Shuttling of Enolase-1 Across Cellular Compartments. *Front. Cell Dev. Biol.* **2019**, *7*, No. 61.
(25) Seweryn, E.; Pietkiewicz, J.; Szamborska, A.; Gamian, A. Enolase on the surface of prokaryotic and eukaryotic cells is a receptor for human plasminogen. *Postepy Hig. Med. Dosw. (Online)*, **2007**, *61*, 672−682.
(26) Ji, H.; Wang, J.; Guo, J.; Li, Y.; Lian, S.; Guo, W.; Yang, H.; Kong, F.; Zhen, L.; Guo, L.; Liu, Y. Progress in the biological function of alpha-enolase. *Anim. Nutr.* **2016**, *2*, 12−17.
(27) Butterfield, D. A.; Lange, M. L. Multifunctional roles of enolase in Alzheimer's disease brain: beyond altered glucose metabolism. *J. Neurochem.* **2009**, *111*, 915−933.
(28) Fournet, M.; Bonté, F.; Desmoulière, A. Glycation Damage: A Possible Hub for Major Pathophysiological Disorders and Aging. *Aging Dis.* **2018**, *9*, 880−900.

https://dx.doi.org/10.1021/acsomega.0c05199
ACS Omega 2021, 6, 4255−4261