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Dependence of α-amylase activity on magnitude and direction of asymmetric gravity field*

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Abstract Asymmetry is often designated as right- and left-handed. The right- and left-handed helical hypergravity (HG) field can be artificially produced. In this paper, it is shown that the α-amylase activity is dependent on both the magnitude and direction of HG within the range between 1 g and 9 000 g. The right-handed HG has larger enhanced effect on enzyme activity than the left-handed HG. Considering the circadian rhythm of natural gravity on the earth, it is suggested that circadian rhythm of natural gravity may be an important mechanical factor for the origin of biological circadian rhythms.

Key words asymmetric gravity field; α-amylase; enzyme activity; biological circadian rhythms

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α-淀粉酶活性对不对称重力大小与方向的依赖性

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摘　要 手性有左右之分.对于重力,可人为产生左、右手螺旋力.通过光谱学手段,考察不对称重力大小及方向变化对 α-淀粉酶活性的影响.结果表明,在 1 ~ 9 000 g 范围内,超重力的增加能够逐渐提高 α-淀粉酶活性,并且右手螺旋力比左手螺旋力对于 α-淀粉酶活性具有更强的激活作用.考虑到地球天然重力昼夜周期性变化的事实和酶活性对重力大小与方向的依赖性,重力 24 h 的周期变化可能是昼夜生理节律产生的重要力学原因之一.

关键词 不对称重力场; α-淀粉酶; 酶活性; 生物节律

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Origin of life has aroused widespread attention. Since the origin of life, biological circadian rhythms have been ubiquitous on all kinds of organisms in nature. However, the origin of biological rhythms is still a scientific challenge.

In previous related studies, people have realized that the environment factors mignt result in their own biological effects in the life system. These environment factors including the nano-particles[1], light [2], magnetic field[3], force field [4-6], and so on. It is worth noting that the circadian rhythm of geophysical environments, which was caused by the earth motion in space, might play an important role in the origin of circadian rhythms in biological systems [7-8]. Based on the rhythms of geophysical environments, a variety of hypotheses on origin of circadian rhythms were put forward[9-10]. Among all kinds of the ubiquitous environmental cycles, light-dark cycle was considered as an important factor to result in biological circadian rhythm [11-13]. Remarkably, the effects of the tidal rhythm on the natural biological circadian rhythms in coastal organisms and marine organisms have been reported [14-18]. These studies led us to consider the hypothesis that the circadian rhythm of natural gravity may play an important role in the origin of biological circadian rhythms.

Many researchers studied the effect of magnitude change of force on enzymatic activity. For example, the expression of the gene could be affected by microgravity and hypergravity, respectively [5, 19-20]. The hypergravity can improve the activity of DNA polymerase α, and this may result from the fact that the hypergravity increases the affinity of enzyme for template DNA[4]. In previous studies, researchers studied only the effect of forces with the different magnitudes on enzymatic activity. However, the effect of forces with the different directions on enzymatic activity was ignored[21].

In this work, both the magnitude and direction effects of the asymmetric gravity field on the activity of α-amylase are studied. Our results show that the activity of α-amylase is dependent on both the magnitude and direction of hypergravity. Considering the fact of the circadian rhythm of natural gravity on earth, we suggest that the circadian rhythm of natural gravity may play an important role in the origin of biological circadian rhythms. To the test of our knowledge, this is the first study on the effect of asymmetric hypergravity with different magnitudes and directions on enzymatic activity.

1 Experiment

1.1 Materials and instrument

Soluble starch and α-amylase from *Bacillus licheniformis* were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Other chemicals were obtained from Beijing Chemical Company (Beijing, China) and used as received. All chemicals used are of analytical grade and prepared using high pure water with a resistance of 18.25 MΩ·cm.

The asymmetric hypergravity fields (right-handed, left-handed) were obtained by two high-speed tabletop refrigerated centrifuges with clockwise-rotation and anticlockwise-rotation rotors, respectively (Xiang instrument, China). The pH of the solution was measured with PB - 10 pH meter (Sartorius, Germany). UV-vis absorption spectra were acquired on a UV - 2550 spectrophotometer (Shimadzu, Japan), using 1 cm path length quartz cuvettes for measurements.

1.2 Influence of α-amylase concentration and temperature on enzymatic degradation

0.5 mL 0.4 g/L phosphate buffer was added into 0.5 mL 1×10^{-3} mol/L iodine solution, and it was diluted to 4 mL with water. 3, 4, and 5 g/L α-amylase solutions were prepared, respectively. The 0.1 mL three α-amylase solutions were added into starch-iodine solution and completely mixed, respectively. Their absorbencies at 660 nm were immediately determined along with time.

2.5 mL 0.4 g/L phosphate buffer was added into 2.5 mL 1×10^{-3} mol/L iodine solution and the soltution was diluted to 20 mL with water. 0.5 mL 4 g/L α-amylase solution was added into the above starch-iodine solution. The sample absorbance at 660 nm was determined at 20, 25, 30, 35, 40 ℃, respectively, and it was repeated for at least three times.

1.3 Effects of magnitude and direction of asymmetric gravity field on activity of α-amylase

According to the definition of asymmetric force[22], the asymmetric gravity field was simulated by the external circular rotations in clockwise (L-rotation) and anticlockwise (R-rotation) centrifuges (facing to viewer) to obtain two types of the helical chiral force (radius 3 cm, RCF 0 ~ 11 400 × g). Rotation speed (r/min) was used to control the intensity of the asymmetric gravity field.

1.5 mL 0.4 g/L phosphate buffer was added into 1.5 mL 1×10^{-3} mol/L iodine solution, and the solution was diluted to 12 mL. 0.3 mL 4 g/L α-amylase solution was added into the above starch-iodine solution and completely mixed. The solution was immediately equally distributed into three samples. Then they were put in a clockwise rotation centrifuge (L-rotation), anticlockwise rotation centrifuge (R-rotation), and natural condition as a control at 30 ℃, respectively. In the process of rotation, the sample time scanning at 660 nm was determined at an interval of about 6 min until the experiment was over. Similar experiments were conducted with different rotation speeds at 1 000, 2 000, 3 000, 4 000, 5 000, 7 000, and 9 000 g, respectively.

2 Results and discussion

Figure 1 shows the linear relation between starch-iodine solution concentration and absorbance at 660 nm, and it shows a good linearity with the concentration of starch solution ranging from 0 to 0.4 g/L. The linear regression equation is $y = 0.803\,47x + 0.037\,21$ ($R^2 = 0.998\,62$). This experiment was repeated three times at least, and the relative standard deviation of the same concentration is 0.011 5, which indicated that the method had good repeatability.

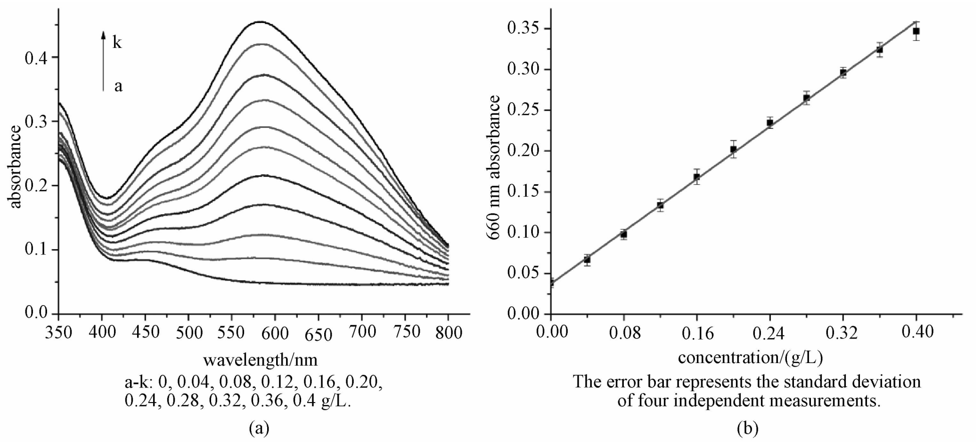

Fig. 1 (a) UV-VIS spectra of starch-iodine solution with various concentrations of starch solution ranging from 0 g/L to 0.4 g/L and (b) linear relation between starch-iodine solution concentration and absorbance at 660 nm

2.1 Influence of α-amylase concentration on enzymatic degradation

The concentration of α-amylase is an important factor to affect the enzymatic degradation of starch-iodine complex. Figure 2 (a) shows that the reaction time to reach the end point increases from 640 s, 1 480 s to 2 850 s with the decrease of the α-amylase concentration from 5 g/L, 4 g/L to 3 g/L at room temperature (25 ℃). Considering that about 30 min of reaction time is relatively suitable to explore the effect of external force on the enzymatic activity, 4 g/L was selected as the optimal α-amylase solution concentration for further experiments in this work.

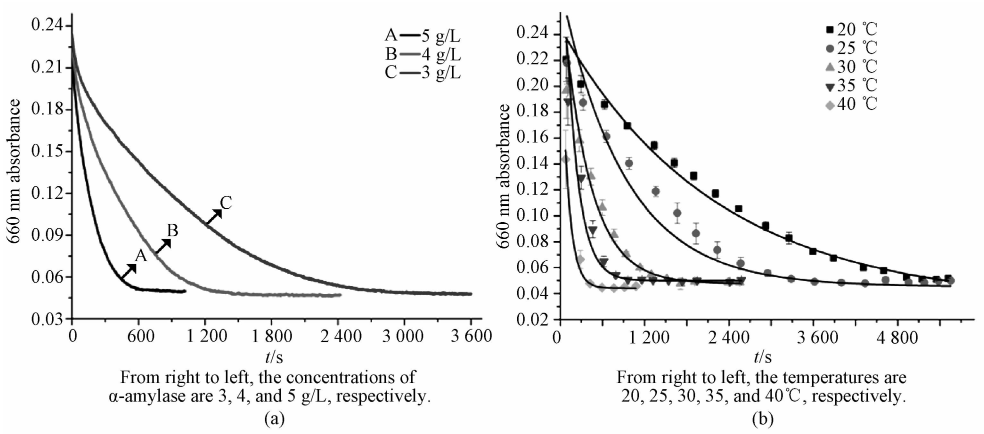

Fig. 2　Influence of reaction conditions on the enzymatic degradation: (a) the effect of concentration of α-amylase on enzymatic degradation and (b) the effect of temperature on enzymatic degradation

2.2　Influence of temperature on the enzymatic degradation

Temperature obviously affects the enzymatic reaction rate via changing protein conformation. Figure 2 (b) shows that the reaction time increases from 450 s (7.5 min) to 5 700 s (95 min) with the decline of temperature from 40 ℃ to 20 ℃, which indicates that the enzyme activity of α-amylase decreases. This shows that the enzyme activity of α-amylase significantly depends on the temperature of reaction. As mentioned above, considering about 30 min of reaction time is relatively suitable to explore the effect of external force on the enzymatic activity, 30 ℃ was selected as the optimal temperature for further experiments in this work.

2.3　Dependence of α-amylase activity on both the magnitude and direction of gravity

The fresh starch-iodine solution with α-amylase was immediately treated under the different hypergravity field with L-rotation and R-rotation. Figure 3 shows that the activity of α-amylase increases with the hypergravity from 1 g to 9 000 g. Obviously there were positive correlations between the activity of α-amylase and magnitude of hypergravity with either L-rotation or R-rotation. This is similar to the effects of hypergravity on other enzymes [4-5, 19-20, 23].

Interestingly, according to Fig. 3, L-rotation and R-rotation had different hypergravity effects when gravity was greater than 1 g. The hypergravity field with R-rotation had a bigger effect on improving the activity of α-amylase than that with L-rotation. These novel results showed that the α-amylase activity is not only dependent on the magnitude of hypergravity but also on the direction of hypergravity.

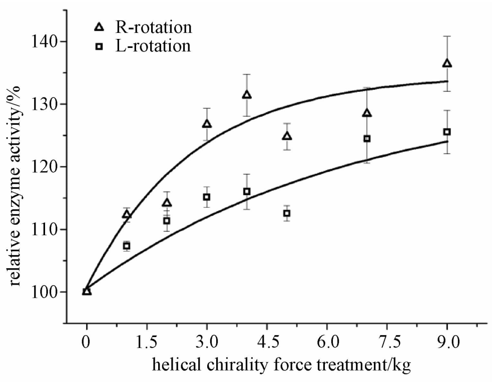

Fig. 3　Increases in activity of α-amylase with hypergravity from 1 g to 9 000 g with both L-rotation and R-rotation

2.4　Possible mechanical mechanisms for effect of asymmetric gravity on α-amylase activity

According to the concept of helix, chiral helix is produced by both axial vector and polar vector. In laboratory, axial vector can be delined by the circular rotation and the natural polar vector can be provided by the Earth's moving along the Sun's peculiar motion to Hercules[10, 21-22]. As a result, the clockwise and anticlockwise circular rotations on Northern Hemisphere will produce the artificial left- and right-handed helical motions and their corresponding helical force fields [10, 21-22].

In addition, the chiral helical force field may result in an intrinsic energy difference between helical enantiomers, and the right-handed helical force stabilizes the right-handed conformations and destabilizes the left-handed conformations [22]. The α-amylases (EC 3. 2. 1. 1) distribute widely among various organisms, and it is reported that there are eight α-helices in its structure [24]. As α-helix is right-handed conformation, the right-handed helical hypergravity field may have a bigger effect on improving the activity of α-amylase than the left-handed helical hypergravity field. The experimental data shown in Fig. 3 demonstrated that the α-amylase activity is not only dependent on the magnitude of hypergravity but also dependent on the direction of hypergravity.

2. 5 Possible relationship between circadian rhythm of natural gravity and circadian rhythms

In this study, we found that there is a positive correlation between the activity of α-amylase and the magnitude of the external force (Fig. 3). This is similar to the effects of hypergravity on other enzymes[4-5, 19-20, 23]. It is suggested that the enzyme activity would have the corresponding circadian rhythm, when the external force is circadian rhythm, and then would result in the corresponding circadian rhythm in physiological change.

The circadian rhythmic change of natural gravity is mainly caused by the earth tide, which has been generally reported and accepted[17, 25-26]. Figure 4 shows the curve of theoretical value of earth tide fluctuation in Beijing (north latitude 39. 9° east longitude 116. 3°) within 120 h since 1 April 2011, which was calculated by using the GOTIC2 program[26]. The change of gravitational acceleration is about 200 μGal [17, 25]. According to the dependence of enzyme activity on gravity, we believe that the circadian rhythmic change of natural gravity would be able to be transfer to the enzyme activity, then to result in the origin of circadian rhythm in biological systems. In other words, the circadian rhythm of natural gravity may play an important role in the origin of biological circadian rhythms.

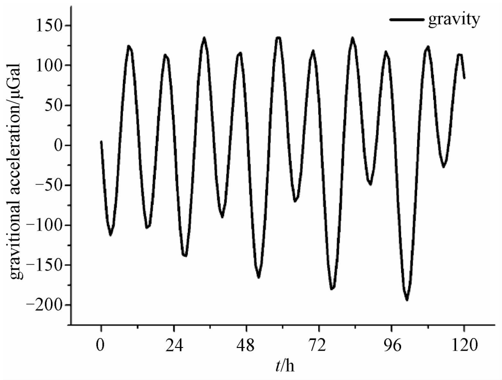

Fig. 4 Theoretical value of earth tide fluctuation in Beijing within 120 h since 1 April 2011

3 Conclusions

In the present work a degradation reaction of starch hydrolysis by α-amylase was used as model system to assess that the enzymatic activity depends on the magnitude and direction of hypergravity. Our data show that the activity of α-amylase is dependent on both the magnitude and chiral direction of hypergravity. Considering the circadian rhythm of natural gravity on earth, we suggest that the circadian rhythm of natural gravity may play an important role in the origin of biological circadian rhythms.

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