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A new Belousov—Zhabotinsky reaction with fructose 1,6-bisphosphate as the organic substrate

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The effects of temperature, free radicals, chloride and bromide ions, and bromine on the Belousov—Zhabotinsky reaction with fructose 1,6-bisphosphate were studied. The apparent activation energies of the induction period, lifetime, and oscillation period were found to be 76.24, 101.29, and 62.93 kJ mol^{-1}, respectively.

Key words: D-fructose 1,6-bisphosphate, oscillation, glycolysis, Belousov—Zhabotinsky reaction.

Oscillating reactions have been of interest as a plausible way of explaining biological clocks and external rhythms. Among the chemical oscillating reactions, the classical Belousov—Zhabotinsky (B—Z) reaction is one of the best understood systems.[1] In biochemistry, the oscillation of glycolysis[2] occupies a similar position. In recent years, many intermediates of glycolysis such as fructose, glucose, and pyruvate have been investigated as the substrates of the B—Z reaction.[3—5] However, no B—Z oscillation of fructose 1,6-bisphosphate (FBP) has been reported.

Fructose 1,6-bisphosphate is an important intermediate of glycolysis. *In vivo*, FBP is a stimulator of phosphofructokinase and can be used as a high-energy substrate.[6] In recent years, FBP has been extensively used in medical application, particularly, for curing heart diseases. In the present work, a new B—Z oscillation that employs FBP as the substrate was studied, and its reaction mechanism was also discussed.

Experimental

Fructose 1,6-bisphosphate was provided by Guangdong Winnerway Pharmaceutical Co. The results of elemental analysis proved that FBP was D-fructose 1,6-bisphosphate trisodium salt octahydrate ($C_6H_{11}Na_3O_{12}P_2$, CAS: 38099-82-0). Other chemicals of AnalaR grade were used without further purification. Bidistilled water was used for preparing solutions.

The reaction was carried out in a cylindrical water jacketed vessel with magnetic stirring. Oscillations were monitored using a platinum electrode connected with a reference calomel electrode through a salt bridge filled with 0.1 *M* KNO_3. Data acquisition was made through a GPIB IEEE-4888 interface.

Results and Discussion

The order of addition of reagents was as follows: FBP, H_2SO_4, $(NH_4)_2Ce(NO_3)_6$, and finally $KBrO_3$. Oscillations were observed in the concentration interval given in Table 1.

The typical oscillation is shown in Fig. 1. The oscillation amplitude was ~100 mV. The oscillation period was in the range 33—41 s.

Temperature effect. The temperature range of oscillations was 303—320 K. Since the oscillations were irregular at 317—320 K, the apparent activation energy was measured in the interval of 303—316 K.

The apparent activation energy was calculated by the linear least-squares fit of the $\ln(1/t)$ *vs.* $1/T$ plot, where t_{in} is the induction period, t_l is the lifetime, and t_p is the oscillation period (Table 2).[7] The following activation energy values were found: E_{in} (for the induction period) = 76.24 kJ mol^{-1}, E_l (for the lifetime) = 101.29 kJ mol^{-1}, and E_p (for the oscillation period) = 62.93 kJ mol^{-1} (Fig. 2).

Table 1. Initial concentration ranges of the reagents where oscillations are observed

Entry	$[KBrO_3]_0$	$[FBP]_0$	$[H_2SO_4]_0$	$[Ce^{4+}]_0$
	mol L^{-1}			
1	0.11—0.27	0.07	1.5	1.45
2	0.15	0.03—0.175	1.5	1.45
3	0.15	0.07	1.2—4	1.45
4	0.15	0.07	1.5	1.00—1.75

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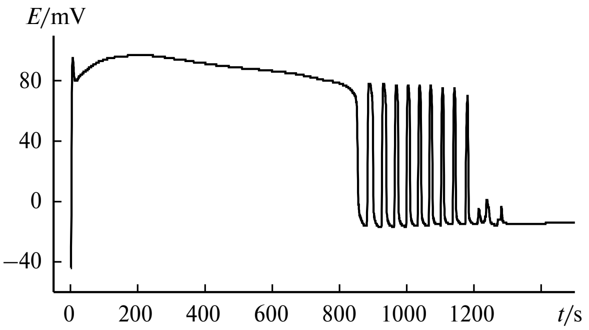

Fig. 1. Oscillation in the potential of the solution measured with a platinum electrode. Initial concentrations of the reactants: $[KBrO_3]_0$ = 0.15 mol L^{-1}, $[FBP]_0$ = 0.07 mol L^{-1}, $[Ce^{4+}]_0$ = $1.45 \cdot 10^{-3}$ mol L^{-1}, $[H_2SO_4]_0$ = 1.5 mol L^{-1}. Temperature 35±0.5 °C, total volume 20 mL.

Table 2. Values of $\ln(1/t)$ ($t = t_{in}$, t_l, t_p) at different temperatures*

T/K	$1/T$	$\ln(1/t_{in})$	$\ln(1/t_l)$	$\ln(1/t_p)$
303	0.0033	−7.31721	−6.83195	−4.03424
308	0.00325	−6.80904	−6.09357	−3.6763
311.2	0.00321	−6.55108	−5.73979	−3.41773
313	0.00319	−6.33683	−5.53339	−3.28964
316	0.00316	−6.06379	−5.15906	−2.99573

* The initial concentrations of the reactants and the total reaction volume are presented in Fig. 1.

Effect of concentration of reagents $KBrO_3$, FBP, Ce^{4+}, and H_2SO_4 on t_{in}, t_p, and t_l. The following concentration relations were obtained from the dependence of ln(concentration) on $\ln(1/t)$, where $t = t_{in}$, t_l, and t_p, respectively:

$$1/t_{in} \propto [KBrO_3]_0^{-0.34}[FBP]_0^{0.62}[Ce^{4+}]_0^{0.27}[H_2SO_4]_0^{0.50}$$

$$1/t_l \propto [KBrO_3]_0^{-2.71}[FBP]_0^{1.76}[Ce^{4+}]_0^{-1.28}[H_2SO_4]_0^{-0.33}$$

$$1/t_p \propto [KBrO_3]_0^{0.29}[FBP]_0^{0.52}[Ce^{4+}]_0^{0.35}[H_2SO_4]_0^{1.44}$$

The reciprocal values $1/t$ correspond to the rates of certain reaction periods. As in the classical B—Z reaction,[8] the rate of oscillation ($1/t_p$) is proportional to $[KBrO_3]_0$, $[FBP]_0$, $[Ce^{4+}]_0$, and $[H_2SO_4]_0$. The $KBrO_3$ concentration is an important factor affecting oscillations: its increase considerably prolongs oscillations.

Chloride ion effect. Oscillations are inhibited by chloride ions at the concentration $\geq 5 \cdot 10^{-4}$ mol L^{-1} (Fig. 3). Therefore, $HBrO_2$ is the key of oscillations, because the chloride ion would successfully compete with both bromide and bromate ions for $HBrO_2$.[9]

Reaction mechanism. When acrylamide was added to the system, oscillations were suppressed (see Fig. 3). This indicates that free radicals are involved in the process.

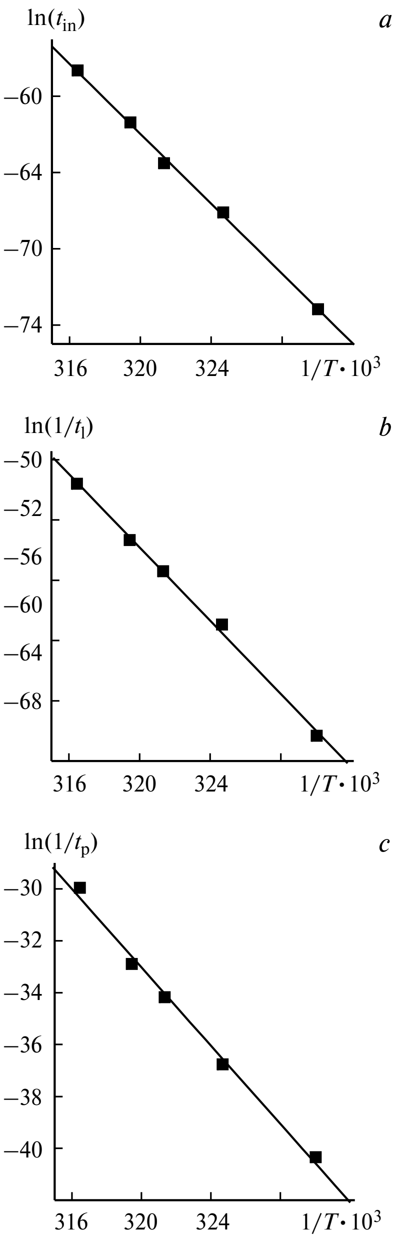

Fig. 2. Temperature dependences of the induction period t_{in} (*a*), lifetime t_l (*b*), and oscillation period t_p (*c*). The initial concentrations of the reactants and the total reaction volume are presented in Fig. 1.

In other experiments, the bromide ion was added to the reaction mixture (see Fig. 3). When NaBr was added at the concentration $>3.4 \cdot 10^{-3}$ mol L^{-1}, oscillations became irregular and disappeared at $5.0 \cdot 10^{-3}$ mol L^{-1} NaBr.

In the system under study, oscillations were observed without any bromine scavenger. It is well known that in the B—Z reaction an N_2 flow or acetone are necessary to remove Br_2 that formed and prevent substrate bromination. However, two oscillation systems breaking the principle have been found recently.[3,11] In one of them, fruc-

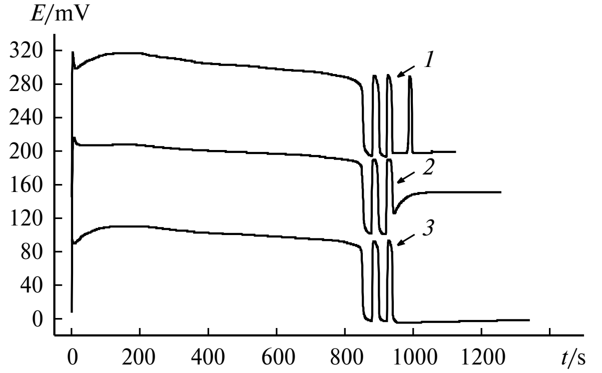

Fig. 3. Inhibition of oscillations by adding NaBr ($3.4 \cdot 10^{-6}$ mol L^{-1}) (*1*), KCl ($5.0 \cdot 10^{-7}$ mol L^{-1}) (*2*), and acrylamide ($2.0 \cdot 10^{-6}$ mol L^{-1}) (*3*).

tose was the organic substrate. FBP used in our system is the fructose derivative. Comparing conditions of oscillations for the systems with FBP and fructose showed that the initial concentrations of FBP and fructose, as well as those of Ce^{4+} and H_2SO_4, were close but $[KBrO_3]_0$ in the B—Z oscillation with FBP was higher than that for fructose. In further studies of the mechanism, we replaced FBP by fructose and Na_2HPO_4 in an equimolar ratio. In this experiment, the color of the solution changed to dark brown quickly, the induction period was very short, and only one oscillation was observed. These phenomena implied that a great deal of Br_2 was eliminated in the initial step. At the same time, in the B—Z oscillation with FBP the color changed to brown slowly. This indicates that the reaction rate with FBP is slower, so that the Br_2 concentration remained at a low level. However, when 2 mL of CCl_4 were added to remove Br_2, oscillations in the B—Z reaction were stopped. Therefore, the concentration of Br_2 in the B—Z oscillation with FBD is controlled specifically.

Fructose 1,6-bisphosphate is an important intermediate in glycolytic oscillation. During metabolism, fructose 6-phosphate is transformed into FBP under the action of phosphofructokinase. This reaction is one of three crossover points of the glycolytic oscillation and generates the glycolytic oscillations primarily.[12] Comparing oscillations of the B—Z reaction in the presence of FBP and glycolytic oscillations, it should be mentioned that they have close periods (33—41 and 37 s in the B—Z reaction and glycolytic oscillation, respectively[13]). We presume that in the B—Z oscillation with FBP the Ce^{4+} ion makes the same work as the enzyme (such as phosphofructoaldolase) does in the glycolytic oscillation. The high concentration of H_2SO_4 helps the Ce^{4+} ion to oxidize and decompose FBP.

Thus, the B—Z oscillation with FBP is controlled by steps involving Br^-, $HBrO_2$, and free radicals. A decrease in the rate of Br_2 formation results in the appearance of oscillations.

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