Changes in ATP Concentration Triggered by Chemoreception in the Plasmodia of the Myxomycete *Physarum polycephalum*

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(Received 13 May 1980; revised 23 June 1980)

The intracellular ATP concentration in plasmodia of the myxomycete *Physarum polycephalum* was determined by the luciferin–luciferase method after stimulation with various chemotactic chemicals. Repellent salts induced an ATP increase. The change in ATP concentration paralleled changes in contraction and in electrical potential with respect to both time course and concentration dependence. Other repellents (certain organic chemicals and high osmolarity) did not induce appreciable change in ATP concentration within 15 min, but prolonged treatment with hydrophobic repellents and D₂O led to diminished ATP. None of the attractants examined (e.g. glucose, alanine, KH₂PO₄ and 2-deoxyglucose) changed the ATP concentration. A possible role of ATP as a mediator in response to salts is discussed.

INTRODUCTION

Plasmodia of *Physarum polycephalum* react to various chemical stimuli and exhibit positive or negative chemotaxis (Carlile, 1970; Knowles & Carlile, 1978; Chet *et al.*, 1977; Kincaid & Mansour, 1978, 1979; Ueda *et al.*, 1975; Ueda & Kobatake, 1977*a, b*; Ataka *et al.*, 1978). External chemical stimuli are first recognized at the surface membrane. Conformational change of the receptive membrane in the recognition processes has been demonstrated by studying changes in zeta potential (Hato *et al.*, 1976*a*; Ueda & Kobatake, 1977*a*), adhesiveness (Ishida *et al.*, 1977), reactivity with chemical reagents (Ishida *et al.*, 1978) and fluorescence signals (Ueda & Kobatake, 1979). Positive and negative taxes are found to be correlated with relaxation and contraction in the plasmodial strand, respectively (Ueda *et al.*, 1976; Hato *et al.*, 1976*b*). However, the mechanisms or molecules which mediate the passage of the information from the surface membrane to the contractile system are little understood. Involvement of Ca²⁺ (Mito *et al.*, 1980) or cyclic AMP phosphodiesterase (Kincaid & Mansour, 1979) has been suggested, but the evidence was indirect. We have compared the intracellular ATP content in the plasmodium before and after chemotactic stimulation; these and other results suggest that the intracellular ATP works as a mediator in the transduction mechanism in the plasmodium.

METHODS

Plasmodia. Except where otherwise indicated plasmodia of an American strain of *Physarum polycephalum* provided by Professor N. Kamiya, National Institute for Basic Biology, Okazaki, Japan, was used. A second strain, an albino (LU647 × LU861) differing in chemotactic sensitivity, was kindly supplied by Dr M. Carlile, Imperial College, London. The plasmodia were cultured by the method of Camp (1936) and allowed to crawl overnight on wet filter paper without feeding.

Chemicals. Firefly Lantern Extract (FLE-50) which contained luciferin and luciferase was purchased from Sigma. All chemicals used as stimuli were of analytical grade.

Fig. 1. (a) Advancing edge of a plasmodium; numbers indicate samples removed for experiment. (b) Procedures for measuring the intracellular ATP concentration of the samples.

Measurement of intracellular ATP concentration. A single large plasmodium was collected on a filter paper. The frontal region of the advancing plasmodium was cut into several pieces (0.5×1 cm) together with the filter paper (Fig. 1a). After standing for 15 to 30 min to allow recovery from possible damage, each piece was immersed in a test or control solution of 1 cm depth. The ATP assay procedures are indicated in Fig. 1(b) and are based on the method of Beutler & Baluda (1964). The chemiluminescence emitted in the ATP assay was measured by a photometer (American Instrument Co., J4-7441). The maximum chemiluminescence was proportional to ATP concentration when the luciferin + luciferase level was kept constant. The amount of protein in each sample was determined by the Lowry method. The ATP concentration in the plasmodium was determined for each sample by dividing the amount of ATP observed by the protein content. The water and protein contents of plasmodia were 83 % and 7.8 %, respectively. Experiments were performed four times for each concentration of chemical. Standard deviations ranged from 5 to 10 %. All experiments were performed at room temperature (19 to 20 °C).

RESULTS

Changes in ATP concentration resulting from chemical stimulation

The ATP concentration in plasmodia immersed in water decreased and attained a constant level within 8 min. This was probably caused by anaerobic conditions. In the subsequent analysis, the ATP concentration 15 min after immersion in water was taken as standard; this ranged from 1.8 to 2.3 mM. The effect of immersion in various solutions on ATP concentrations relative to those in water is shown in Fig. 2. There was an increase in ATP concentration soon after immersion in CaCl_2 and KCl, but little change in glucose, KH_2PO_4 and sucrose. A slow decrease in ATP concentration occurred in ethanol or heavy water, and this was often accompanied by a release of yellow pigments into the medium. Hence, ethanol and heavy water may have toxic effects.

We have shown that contraction or relaxation in the plasmodium takes place about 15 min after chemical stimulation (Ueda *et al.*, 1976) and we will therefore concentrate on the ATP concentration at this time.

Chemically induced changes in ATP concentration in relation to contraction and chemotaxis

Table 1 summarizes the effects of various chemicals on the ATP concentration 15 min after stimulation, along with references to their effects on chemotaxis and contraction. Repellent electrolytes induced an increase in ATP (CaCl_2 , MgCl_2 , NaCl and KCl). Hydrophobic repellents, such as ethanol, butyric acid, picrate and quinine, did not result in any marked change in ATP content. High concentrations of sucrose induced a large transient contraction and repulsion presumably due to their osmotic effect, but did not affect the ATP content.

Fig. 2. Time course of the change in ATP concentration after chemical stimulation, relative to that in control plasmodia immersed in water for 15 min (100). ○, CaCl_2 (3 mM); △, KCl (20 mM); ⊙, H_2O ; ●, glucose (3 mM); ▲, alanine (3 mM); ■, KH_2PO_4 (10 mM); ▽, sucrose (300 mM); □, ethanol (400 mM); ◇, D_2O (50 % v/v).

Table 1. ATP concentrations in plasmodia after 15 min treatment with various chemicals

Class	Chemical	Concn (mM)	Relative ATP concn*	Reference(s)†
Repellents				
(protoplasmic contraction)				
(a) Electrolytes				
	KCl	20	154 ± 16	1, 2
	NaCl	20	132 ± 10	
	CaCl_2	3	151 ± 10	
	MgCl_2	3	130 ± 8	
(b) Hydrophobic substances				
	Ethanol	400	96 ± 12	3, 4, 5
	Acetaldehyde	10	100 ± 3	
	Picrate	3	108 ± 9	
	Butyric acid	1	113 ± 5	
	Quinine	1	74 ± 3	
(c) High osmotic pressure				
	Sucrose	300	85 ± 5	2
(d) Heavy water				
	D_2O	20 % (v/v)	97 ± 4	6
		50 % (v/v)	72 ± 10	
Attractants				
(protoplasmic relaxation)				
	Glucose	3	97 ± 8	1, 2, 7, 8, 9
	2-Deoxyglucose	3	90 ± 5	
	Alanine	3	113 ± 16	
	Maltose	10	109 ± 4	
	Phenylalanine	10	86 ± 10	
	KH_2PO_4	10	111 ± 6	

* Relative to the value in water at 15 min which is taken as 100.

† References to the effects of these chemicals on protoplasmic contraction or relaxation: 1, Ueda *et al.* (1975); 2, Ueda *et al.* (1976); 3, Ueda & Kobatake (1977a); 4, Ueda & Kobatake (1977b); 5, Ataka *et al.* (1978); 6, Götz von Olenhusen & Wohlfarth-Bottermann (1979); 7, Knowles & Carlile (1978); 8, Kincaid *et al.* (1978); 9, Chet *et al.* (1977).

There are thus two types of repellent, both of which act through causing contraction, but which differ in their effect on ATP concentration. This implies that there is more than one pathway by which the information sensed at the surface membrane is transmitted to cause the contraction.

The attractants examined (glucose, galactose, alanine, 2-deoxyglucose and KH_2PO_4) did not greatly affect the ATP concentration, whether they were electrolytes or non-electrolytes.

Fig. 3

Fig. 4

Fig. 3. Dependence of the increase in ATP concentration and the membrane potential ($\Delta\psi$) for the plasmodium of the American strain on the concentration of stimulant salt: $\bullet$, MgCl₂; $\square$, CaCl₂; $\circ$, KCl; $\triangle$, NaCl.

Fig. 4. Dependence of the increase in ATP concentration and the membrane potential ($\Delta\psi$) for the plasmodium of the albino strain on the concentration of stimulant salt: $\bullet$, MgCl₂; $\square$, CaCl₂; $\circ$, KCl; $\triangle$, NaCl.

ATP increase as a function of the concentration of stimulant salt

Figure 3 shows the dependence of the ATP increase on the stimulant salt concentration for the plasmodium of the American strain. For comparison, corresponding data for the membrane potential (Ueda *et al.*, 1975) are reproduced in the lower trace. The ATP concentration stayed constant until the concentration of each salt reached its respective threshold (C_{th}). Above C_{th} , the intracellular ATP increased gradually with increase in salt concentration. The ATP increase reached 20 to 40 % above the control at a concentration 10 times the respective C_{th} . These changes paralleled those in membrane potential response as shown in the lower trace.

Figure 4 shows similar plots for the albino strain (LU647 $\times$ LU861), which had a threshold for membrane potential (Ueda & Kobatake, 1979) and ATP increase at about one-tenth of the concentration effective for the American strain.

These results indicate that the ATP increase described above originates from the chemoreceptive process. Metabolizable chemicals which support the growth of the plasmodia, such as glucose, did not appreciably affect the ATP content.

DISCUSSION

Stimulation of the receptive membrane should alter a postulated mediator concentration, and changes in mediator concentration should modify the contractile activity. ATP has been shown, by an injection method, to cause contraction (Ueda & Götz von Olenhusen, 1978; Ueda *et al.*, 1978). Our present results show that stimulation causes changes in ATP concentrations. We therefore suggest that ATP is a mediator in the response to repellent salts.

Recently, Yoshimoto *et al.* (1980) found that the amount of ATP varied with rhythmic contraction and that ATP concentrations increased during the contraction phase. Since they monitored the ATP concentration indirectly, by measuring the ATP which leaked from the cell interior with chemical modification of the surface membrane, the variation in ATP in their experiment can be attributed to changes in either the permeability of the membrane or the intracellular concentration of ATP. If the latter is the explanation for their results, then their data and ours indicate that contraction and ATP increase coincide.

Plasmodia contrast with skeletal muscle where the intracellular ATP concentration is kept constant by the regulating action of phosphocreatine systems (Curtin & Davies, 1973). However, the lack of ATP-buffering system in the plasmodium is also found in other cells. For example, in nerve electroplaque synapses of *Torpedo*, ATP concentration paralleled the change in acetylcholine concentration (Israel *et al.*, 1977). Thus, there is a possibility that ATP itself may work as a key substance in regulation.

We found the ATP concentration to be about 2 mM in the frontal region of the plasmodium, but a value of about 1 mM was reported by Hatano & Takeuchi (1960). These values are much higher than that of 0.2 mM which was shown by injection studies to bring about the optimal contraction in the plasmodial strand (Ueda & Götz von Olenhusen, 1978). This discrepancy might be due to differences between the plasmodial front and strands removed from the rear of a plasmodium. Sakai & Kamiya (1976) found that the ATP concentration was highest in the frontal region and decreased gradually towards the rear.

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