Changes in Intracellular pH Accompanying Chemoreception in the Plasmodia of *Physarum polycephalum*

By TATSUMI HIROSE,* TETSUO UEDA AND YONOSUKE KOBATAKE

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

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A new method for measuring intracellular pH, employing the intrinsic fluorescent pigments of the plasmodia of the myxomycete *Physarum polycephalum*, was used to study the role of pH in chemotactic transduction in the plasmodia. The cell became acidified following stimulation with the attractants alanine, glucose, galactose and maltose when their concentrations exceeded the respective thresholds of chemoreception and taxis. The degree of cell acidification paralleled the relaxing tendency in tension generation. A non-metabolizable attractant, 2-deoxyglucose, also acidified the cell. However, the repellent salts NaCl, KCl and CaCl₂ did not change the intracellular pH. Our results suggest that the effects of attractants are mediated by intracellular pH, while the effects of repellents are transduced by ATP as reported previously.

INTRODUCTION

Plasmodia of the myxomycete *Physarum polycephalum* react to certain chemicals by 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). Studies on the primary process occurring at the receptive membrane have revealed a conformational change in the membrane, as indicated by changes in membrane fluidity (Ueda & Kobatake, 1979), zeta potential (Hato *et al.*, 1976*a*; Ueda & Kobatake, 1977*a*), adhesiveness (Ishida *et al.*, 1977) and chemical reactivity (Ishida *et al.*, 1978).

Negative and positive taxes in the plasmodia correlate with contraction and relaxation, respectively (Ueda *et al.*, 1976; Hato *et al.*, 1976*b*). Thus the problem of chemotactic transduction is simplified to that of finding modulators of contractile activity in the plasmodia. Recently we have shown that intracellular ATP can be such a mediator for repellent salts, but not for attractants (Hirose *et al.*, 1980).

In the present paper we show that attractant information is transmitted to the motile system through changes in the intracellular pH. The results were obtained using a new method for measuring the intracellular pH, which utilizes the intrinsic fluorescence of pigments in the plasmodia.

METHODS

Plasmodia. The true slime mould *Physarum polycephalum* was cultured by the method of Camp (1936). Before use, the plasmodia were allowed to differentiate into tips and strands by migrating on 0.9% water agar overnight without feeding.

Preparation of pigments from plasmodia. Plasmodial pigments were extracted by dipping plasmodia in five times their weight of hot water (95 °C) for 10 min, and the residues were removed by ultracentrifugation (50000 g, 30 min). The pigments were kept in a measuring solution containing 30 mM-KCl, 3 mM-MgCl₂, 10 mM-Ca/EGTA buffer and 10 mM-MES buffer. The free Ca²⁺ concentration and pH of the test solution were varied between 10⁻⁸ and 10⁻⁶ M and 6.8–7.6, respectively. Other chemicals were added to this solution.

Measurements of fluorescence in vitro and in vivo. Fluorescence spectra of the intrinsic pigment and their second derivatives were measured by a fluorescence spectrophotometer (Hitachi, Model MPF-2A and Shimadzu, unit DES-2). Fluorescence spectra of the pigments were measured at the emission peak of 480 nm by scanning the excitation spectrum from 250 to 360 nm. Measurements of *in vivo* fluorescence of the plasmodia were performed as

Fig. 1. Fluorescence properties of intrinsic pigments extracted from the plasmodia of *Physarum polycephalum*. (a) Excitation and the second-derivative spectra monitored at the emission peak of 480 nm. The concentration of pigments was 10% of that in the intact plasmodia. (b), (c), (d) Fluorescence ratio R_f as a function of pH, and ATP and Ca^{2+} concentrations, respectively. R_f is defined as the ratio of fluorescence intensity excited at 325 nm to that excited at 310 nm.

previously described (Ueda & Kobatake, 1979) with the minor alteration that quartz plates were used throughout. All experiments were done at room temperature (22 °C) and repeated four or five times for each experimental condition.

RESULTS

Intrinsic pigments of the plasmodia as pH indicators

The excitation spectrum of the fluorescent pigments extracted from the plasmodia is shown in Fig. 1(a). The second derivative spectrum indicates that there are two components, at 310 and 325 nm. Thus, the fluorescence intensity at 480 nm excited by 325 nm light, relative to that excited by 310 nm light, $R_f = I_{325}/I_{310}$, was measured as a function of the pH and of the ATP and Ca^{2+} concentrations. The value of R_f decrease linearly with increasing pH (Fig. 1b) according to the equation:

$$R_f - 0.612 = -0.062 (\text{pH} - 7.0) \quad (1)$$

However, R_f was not affected by ATP and Ca^{2+} in their physiological concentration ranges (Fig. 1c, d), and was also independent of the amount of the pigments. Therefore, we concluded that the plasmodial pigment is a useful pH indicator for measuring cytoplasmic pH in an intact plasmodium.

Changes in intracellular pH induced by chemical stimulation

Time courses of the fluorescence ratio R_f in the intact plasmodia before and after chemical stimulation are shown in Fig. 2. The variation of R_f had a period of 2–3 min, which is the same

Fig. 2. Time course of changes in intracellular pH of the plasmodia in response to chemical stimuli. Addition of chemicals is indicated by the arrows. The bars represent 5 min in each case. The distance between the pairs of broken lines in (b), (c) and (d) represents ΔR_i .

Fig. 3. Dependence of the changes in intracellular pH (a) and tension generation (b) on the concentration of the attractant sugars glucose (○), galactose (□) and maltose (Δ). (b) is taken from Ueda *et al.* (1976); the value of S is defined as an integral of isometric tension with respect to time during a period of oscillation, and S/S_0 is a ratio of S before and after the addition of chemicals.

Table 1. Comparison of changes in intracellular pH and ATP concentration induced by chemical stimulation

The concentrations of the stimulants indicated in the table are 10–30 times higher than the thresholds for the respective chemicals. The changes in pH were calculated from the observed R_i using equation (1); negative values indicate acidification. The data for ATP concentrations are taken from Hirose *et al.* (1980). Numbers in parentheses represent S.D.

Class	Chemical	Concn (mM)	pH change	ATP concn (% of control)
Attractants	Alanine	3	$-0.42(\pm 0.08)$	$113(\pm 16)$
	2-Deoxyglucose	3	$-0.74(\pm 0.16)$	$90(\pm 5)$
	Glucose	3	$-0.57(\pm 0.08)$	$97(\pm 8)$
	Galactose	3	$-0.37(\pm 0.06)$	
	Maltose	10	$-0.72(\pm 0.14)$	$109(\pm 4)$
Repellents	NaCl	30	$0.08(\pm 0.03)$	$132(\pm 10)$
	KCl	30	$0.14(\pm 0.03)$	$154(\pm 16)$
	CaCl ₂	5	$0.15(\pm 0.05)$	$151(\pm 10)$

as the contraction rhythm of the plasmodia. This suggests that intracellular pH oscillates with the contraction rhythm. This is consistent with the report of Yoshimoto *et al.* (1981), that the contraction rhythm parallels the oscillation in pH of the surrounding medium as monitored with

an H^+ -sensitive Sb electrode. Stimulation with attractant sugars such as glucose, galactose, maltose, and also 2-deoxyglucose, increased the R_f value, on average (i.e. the cell was acidified by reception of attractant sugars) whereas the repellent salts NaCl and $CaCl_2$ caused no appreciable variation of R_f .

This acidification of the cell induced by attractants cannot be attributed solely to possible enhanced glycolysis inside the cell, because 2-deoxyglucose cannot be metabolized in this organism (Knowles & Carlile, 1978).

The averaged changes in intracellular pH in response to different sugar concentrations are shown in Fig. 3(a). The intracellular pH was calculated from the averaged change in the relative fluorescence ΔR_f , using equation (1). The acidification of the cell did not occur until the stimulus chemicals reached their respective thresholds, but thereafter increased with increasing sugar concentration. The results in Fig. 3(b) show that the sugars caused a parallel decrease in tension of the plasmodia (Ueda *et al.*, 1976). The thresholds for cell acidification, tension generation and chemoreception were similar.

The changes in intracellular pH induced by chemical stimulation are summarized in Table 1. All the attractants examined, namely alanine, glucose, 2-deoxyglucose, galactose and maltose, acidified the cell, whereas the repellent salts NaCl, KCl and $CaCl_2$ did not change the intracellular pH.

These results indicate that the acidification of the cell is triggered by attractant chemoreception and acts to relax the tension in the plasmodia.

DISCUSSION

All the attractants examined, whether amino acid, or metabolizable or non-metabolizable carbohydrate, acidified the cell, whereas the repellent salts did not change the intracellular pH (Table 1). On the other hand, the repellent salts increase the ATP concentration, leading to plasmodial contraction, whilst the attractants do not affect the ATP level (Hirose *et al.*, 1980). Thus, attractant and repellent salts affect the motile system by different pathways, involving pH and ATP, respectively.

Evidence that the intracellular pH can influence the actomyosin system is accumulating: pH changes produce contraction in Triton-extracted muscle (Taylor, 1976), solation and contraction of gel in amoebae (Hellewell & Taylor, 1979), actin polymerization in echinoderm sperm (Tilney *et al.*, 1978), and elongation of actin-filled microvilli in sea urchin eggs (Johnson *et al.*, 1976; Shen & Steinhardt, 1978; Begg & Rebhum, 1979). All these results indicate that an increase in pH causes contraction and actin polymerization. The present results, together with those of Yoshimoto *et al.* (1981), are compatible with these effects of pH on the actomyosin system.

Some, if not all, of the intrinsic fluorescent pigments have been found as cytoplasmic particles (Daniel, 1966), hence it is plausible that the present method of intracellular pH measurement may reflect values of pH in the cytoplasm. In fact, a large change in the pH of the surrounding medium, which might be expected to alter the pH of the cytoplasm, induced a corresponding change in intracellular pH as measured by the present method.

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