ALKALINE PHOSPHATASE OF *DICTYOSTELIUM DISCOIDEUM*: CELL SURFACE LOCATION AND COLCHICINE EFFECT ON INTERNALIZATION DURING PHAGOCYTOSIS

Roger W. PARISH and Claudia PELLI

Cytologisches Laboratorium, Institut für allgemeine Botanik, Universität Zürich, Birchstrasse 95, 8050 Zürich, Switzerland

Received 11 October 1974

1. Introduction

The mobility of proteins in plasma membranes and their distribution on the inner and outer surfaces of these membranes are currently attracting attention in cell biology [1–5]. We report evidence that the alkaline phosphatase of *Dictyostelium discoideum* is, in part, located on the cell surface and its internalization during phagocytosis is prevented by colchicine binding proteins.

The alkaline phosphatase of *D. discoideum* is a 5′-nucleotidase attacking adenosine monophosphate and deoxyadenosine monophosphate [6]. There is evidence that the 5′-nucleotidases of eukaryotic cells are associated, at least partly, with the plasma membrane [4,7,8]. Furthermore, DePierre and Karnovsky [7], treating guinea pig polymorphonuclear leukocytes with the diazonium salt of sulphanilic acid, and Trams and Lauter [8], comparing whole cell and homogenate activities of several cultured cell lines, conclude that 5′-nucleotidase is an 'ectoenzyme'.

Alkaline phosphatase of *D. discoideum* may also be associated with the plasma membrane as (i) the enzyme cosediments in density gradients with a membrane fraction containing cyclic AMP phosphodiesterase (Hintermann and Parish, unpublished) and (ii) when cell surfaces are labelled with ^{131}I some alkaline phosphatase activity is found associated with radioactive membrane fragments [9].

2. Materials and methods

2.1. *Measurement of alkaline phosphatase activity*

D. discoideum cells (Ax 3, from W. F. Loomis Jr.) grown in HL5 medium 12 were washed in PDF solution [13] and resuspended in 0.05 M Tris–HCl (pH 8.5) containing 50 mM $MgCl_2$ and *p*-nitrophenylphosphate (0.25 mg/ml, a substrate of the alkaline phosphatase [6]). The suspension (5 ml; 8×10^6 cells/ml) was shaken in Erlenmeyer flasks (25 ml) at 23°C in a Clim-o-shake (Kühner Ag, Basel; 90 cmp). At intervals 1.0 ml was withdrawn, the cells sedimented and 2.0 ml of 0.2 M Na_2CO_3 added to the supernatant. The extinction at 400 nm was measured.

2.2. *Isolation of phagocytic vacuoles*

Cells were grown (see fig. 1) in 500 ml HL5 medium to a concentration of 9×10^6 cells/ml. The culture was divided and colchicine (2.5 mM) added to one half. The cultures were incubated for a further 60 min and 50 μl of latex beads (0.794 μm diam, 3.7×10^{11}/ml Dow Latex) added. After 30 min the cultures were centrifuged and the cells washed three times with 2 mM phosphate buffer (pH 6.5). The cells were resuspended in 5.0ml of 70% (w/v) sucrose in 0.2 M Tris–HCl (pH 7.2) and homogenized with a Teflon-glass homogenizer attached to an electric drill (14 strokes). Two ml of the homogenate was overlayered with 30% (1.0ml), 20% (1.5 ml) and 10% (0.1 ml) sucrose (w/v) in buffer, and spun for 60 min at 45 000 rev/

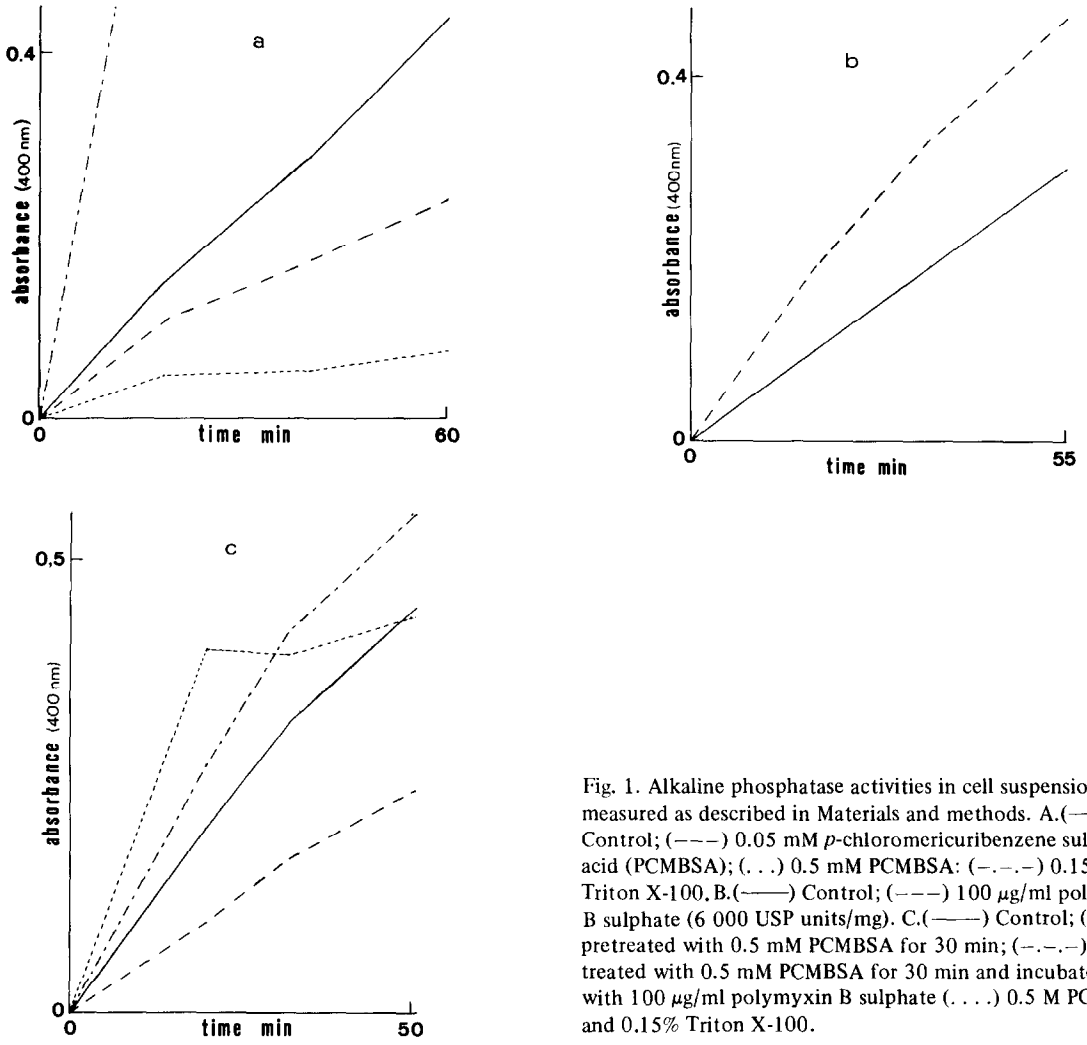

Fig. 1. Alkaline phosphatase activities in cell suspensions, measured as described in Materials and methods. A.(——) Control; (– – –) 0.05 mM *p*-chloromericuribenzene sulphonic acid (PCMBSA); (. . .) 0.5 mM PCMBSA: (–.–.–) 0.15% Triton X-100. B.(——) Control; (– – –) 100 μg/ml polymyxin B sulphate (6 000 USP units/mg). C.(——) Control; (– – –) pretreated with 0.5 mM PCMBSA for 30 min; (–.–.–) pretreated with 0.5 mM PCMBSA for 30 min and incubated with 100 μg/ml polymyxin B sulphate (. . . .) 0.5 M PCMBSA and 0.15% Triton X-100.

min in a Spinco L2 65b ultracentrifuge (SW 50 rotor). Latex beads were visible at the 10–20% and 20–30% sucrose interfaces. Triton X-100 (20 μl of 7%) was added to each fraction collected and fractions containing latex were filtered under vacuum through Metricel OA-8 filters (25 mm, pore size 0.2 μm). Alkaline and acid phosphatase were measured at pH 8.5 and 3.5 respectively using *p*-nitrophenylphosphate.

3. Results and discussion

We found that cells in incubation medium remained intact and impermeable to Evans Blue. The cell suspension exhibited alkaline phosphatase activity, on average 10–15% of the total activity determined after cells had been ruptured with Triton X-100 (fig. 1A). The majority of alkaline phosphatase activity in cell homogenates is sedimentable and enzyme is not released into the medium when cells are being incubated.

In order to ascertain whether the enzyme is associated with the outer surface of the plasma membrane we added *p*-chloromercuribenzene sulphonic acid (PCMBSA; Sigma) to the reaction mixture. The compound binds to the sulfhydryl groups of proteins on the cell surface and inhibits, for example, certain

transport systems in lymphocytes, but does not penetrate these cells [10]. PCMBSA inhibited the alkaline phosphatase activity (fig. 1A). When cells were incubated in 0.5 mM PCMBSA for 30 min, then washed and resuspended in the assay medium, activity was reduced (fig. 1C). After addition of Triton X-100 to cells pretreated with PCMBSA, enzyme activities resembling those of lysed untreated cells were found.

Polymyxin B renders the cells permeable to Evans Blue. When the antibiotic was present in the incubation medium or when cells pre-treated with PCMBSA were incubated with polymyxin B, increased alkaline phosphatase activities were found (fig. 1B,C).

Finally, cells incubated with Triton X-100 and PCMBSA showed no activity after the first few minutes, Indicating that intracellular and soluble enzyme are inhibited (fig. 1C).

We conclude that the cells are normally impermeable to *p*- nitrophenylphosphate and PCMBSA and, hence, the alkaline phosphatase activity measured resides on the cell surface. However, enzyme is also present within the cells, although whether associated with the inner side of the plasma membrane or other membranes is not known. The relative amount of total enzyme activity associated with the cell surface is difficult to estimate since detergents and homogenization may activate the enzyme. The experiments with polymyxin B suggest that no more than 40–50% of activity is at the cell surface.

The amoebae of *D. discoideum* internalize part of their plasma membrane during phagocytosis and we wished to discover whether alkaline phosphatase activity was associated with the phagocytic vacuoles. We have used latex beads to isolate and characterize these vacuoles (paper in preparation). Acid phosphatase is used as a marker for vacuole membranes and fig. 2A shows that alkaline phosphatase does not 'peak' with acid phosphatase in density gradients. Some alkaline phosphatase activity was present fairly evenly distributed among the fractions (fig. 2A) although in many gradients, we have found no activity whatever.

When phagocytosis occured in the presence of colchicine, however, two peaks of alkaline phosphatase, coinciding with the vacuole membranes, were present in the density gradient (fig. 2B).

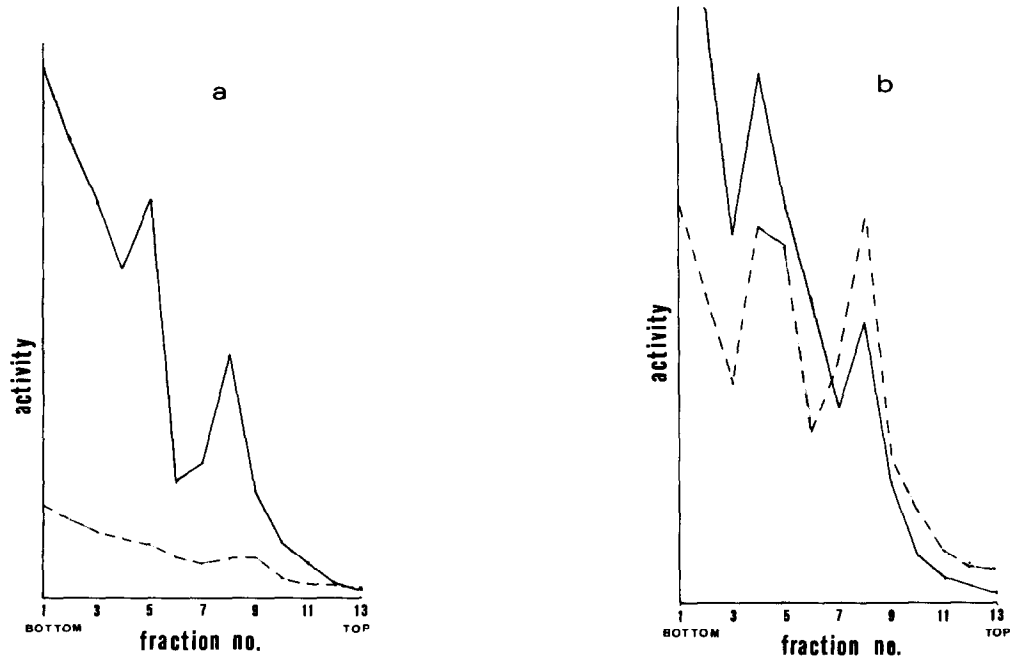

Fig. 2. Effects of colchicine on the appearance of alkaline phosphatase in phagocytic vacuoles, A. Control. B. Cochicine (2.5 mM). (———) Acid phosphatase; (– – –) alkaline phosphatase.

4. Conclusions

1) The alkaline phosphatase of *D. discoideum* cells is, in part, associated with the cell surface.
2) The enzyme is not normally present in the membranes of phagocytic vacuoles. Since the latter derive from plasma membrane, either distinctive areas of this membrane are phagocytosed or the alkaline phosphatase is laterally redistributed during phagocytosis.
3) Colchicine binding proteins are involved in preventing the appearance of alkaline phosphatase in phagocytic vacuoles. They may maintain the topographic distribution or direct lateral redistribution of alkaline phosphatase. Similarly, concentrative movements of lectin binding sites and transport carriers of lymphocytes, thought to be induced by phagocytosis, are inhibited by colchicine [3,11].

This work was supported by the 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung' (Grant Nr. 3.8950.72).

References

[1] Singer, S. J. and Nicolson, G. L. (1972) Science N.Y. 175, 720–731.
[2] Edelman, G. M., Yagara, I. and Wang, J. L. (1973) Proc. Natl. Acad. Sci. USA 70, 1442–1446.
[3] Berlin, R. D., Oliver, J. M. Ukena, T. E. and Yin, H. H. (1974) Nature 247, 45–46.
[4] Solyom, A. and Trams, E. G. (1973) Enzyme 13, 329–372.
[5] DePierre, J. W. and Karnovsky, M. J. (1973) J. Cell Biol. 56, 275–303.
[6] Gezelius, K. and Wright, B. E. (1965) J. Gen Microbiol 308, 309–328.
[7] DePierre, J. W. and Karnovsky, M. J. (1974) Science N.Y. 183, 1096–1098.
[8] Trams, E. G. and Lauter, C. J. (1974) Biochim. Biophys. Acta, 345, 180–197.
[9] Green, A. A. and Newell, P. C. (1974) Biochem. J. 140, 313–322.
[10] Tsan, M. F. and Berlin, R. D. (1971) J. Exp. Med. 134, 1016–1034.
[11] Ukena, T. E. and Berlin, R. D. (1972) J. Exp. Med. 136, 1–7.
[12] Cocucci, S. M. and Sussman, M. (1970) J. Cell Biol. 45, 399–407.
[13] Newell, P. C. and Sussman, M. J. (1969) Biol. Chem. 244, 2990–2995.