Volume 322, number 1, 73–75 FEBS 12397 May 1993

Differanisole A, an inducer of the differentiation of Friend leukemic cells, induces stalk cell differentiation in *Dictyostelium discoideum*

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Received 9 March 1993

Differanisole A isolated from the conditioned medium of a soil microorganism, *Chaetomium* strain RB-001, is an inducer of the differentiation of the Friend leukemic cells (mouse leukemia cells). The chemical structure of this substance is very similar to that of stalk cell differentiation-inducing factor (DIF) isolated from the cellular slime mould, *Dictyostelium discoideum*. We examined the effects of differanisole A on *Dictyostelium* HM44 cells, a mutant strain which is defective in DIF production, and found this substance to be an inducer of stalk cell differentiation in *D. discoideum*.

Cellular slime mould; *Dictyostelium discoideum*; Differentiation inducing factor; Differanisole A; *Chaetomium*; Leukemia cell

1. INTRODUCTION

Cell differentiation is one of the most conspicuous phenomena which are seen during the course of development in multicellular organisms, and hence is a central subject in the field of developmental biology. Since cancer cells are thought to be dedifferentiated cells which have lost the properties of regulated development, the elucidation of the mechanisms of cell differentiation is thus expected to provide significant insights into cancer therapy.

Differanisole A was isolated from the conditioned medium of the soil microorganism *Chaetomium* (RB-001) as a factor which induces the differentiation of mouse erythroleukemia (B8) cells into hemoglobin-producing cells [1]. Since differanisole A has also been shown to induce the differentiation of some other tumor cells [2], this substance is expected to be utilized in medical treatment of cancer and also in the study of differentiation.

In the field of developmental biology, on the other hand, the cellular slime mould *Dictyostelium discoideum* has been widely used, because of its simple pattern of development and differentiation: this organism yields only two cell-types, spores and a multicellular stalk at the end of development. For stalk cell differentiation, cyclic AMP and DIF (differentiation inducing factor), both secreted by the cells, are required [3,4]. DIF-1, the most active form of DIFs, has been purified and identified to be 1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)-1-hexanone (Fig. 1A) [5], which is very similar to differanisole A, 3,5-dichloro-2-hydroxy-4-methoxy-6-*n*-propyl-benzoic acid (Fig. 1B) [6].

Because of a high degree of structural similarity of DIF-1 to differanisole A, we examined whether differanisole A mimics the effects of DIF-1 in *Dictyostelium* cells. We show here that differanisole A as well as DIF-1 induces stalk cell differentiation in the presence of cyclic AMP in *D. discoideum*.

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2. MATERIALS AND METHODS

2.1. *Chemicals and strains*

DIF-1 was purchased from Molecular Probes (UK). Differanisole A was synthesized as previously described [7]. DIF-deficient mutant HM44 of *D. discoideum* was kindly provided by Robert R. Kay (MRC Laboratory, UK).

2.2. *Cell culture and Western analysis*

HM44 cells were grown in association with *Klebsiella aerogenes* on modified SM agar plates [8,9]. Cells were collected with a salt solution (10 mM NaCl, 10 mM KCl) and allowed to develop in vitro at 21°C in 3.5-cm wells (10^5 cells/cm^2), each containing 2 ml of stalk salts (5 mM cAMP, 2 mM NaCl, 10 mM KCl, 1 mM $CaCl_2$, 200 μg/ml streptomycin sulfate, 10 mM MES adjusted to pH 6.2 by the addition of KOH) with or without various concentrations of DIF-1 or differanisole A. The stalk cell population was counted microscopically at 48 h.

For Western analysis, cells were collected at 48 h and lysed with SDS sample buffer solution (10^4 cells/μl). The cell proteins were analyzed by SDS-PAGE and the stalk-specific protein, wst34, of *D. discoideum* was detected with anti-*D. mucoroides* stalk (anti-Dmst) serum that recognizes wst34 [10–12].

3. RESULTS AND DISCUSSION

The effects of differanisole A (Fig. 1B) and DIF-1

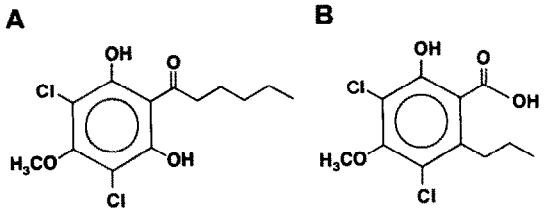

Fig. 1. Chemical structure of DIF-1 (A) and differanisole A (B).

(Fig. 1A) on HM44 in vitro development were examined as described in section 2. Since this strain is defective in DIF production, HM44 cells cannot differentiate into stalk cells without exogenous DIF (Fig. 2A). In the presence of added DIF-1, cells differentiated into fully vacuolated stalk cells (Fig. 2B) as reported previously [9,12,13]. Surprisingly, differanisole A at a comparatively high concentration induced stalk cell differentiation in *D. discoideum*, as judged by morphology (Fig. 2C). Fig. 3 shows DIF-1- and differanisole A-dose requirements for stalk cell differentiation.

To confirm biochemically that the differanisole A-induced cells are stalk cells, the cell proteins were analyzed by Western blot with anti-Dmst serum that recognizes the stalk-specific protein, wst34 [10]. As shown in Fig. 4, cells clearly accumulated wst34 in response to differanisole A.

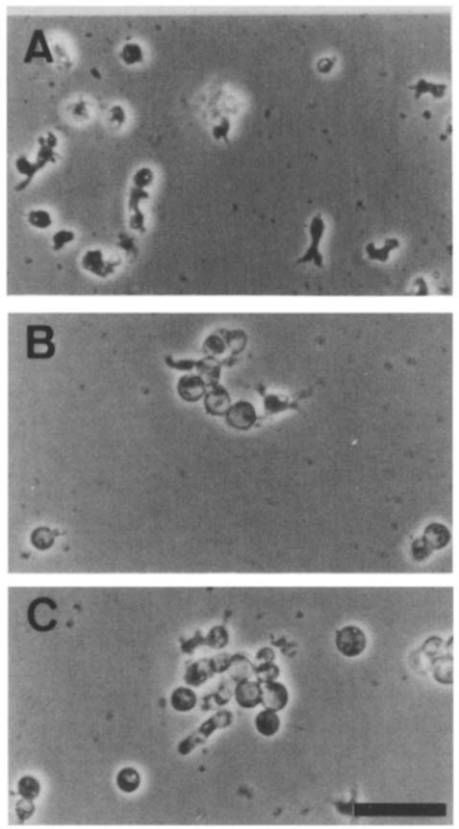

Fig. 2. Stalk cell induction by DIF-1 and differanisole A. HM44 cells were incubated in vitro without (A) or with 10 nM DIF-1 (B) or 1 mM differanisole A (C) for 3 days, and cell morphology was observed by a phase-contrast microscope. Stalk cells are fully vacuolated, covered with a smooth layer (cell wall). Bar = 30 μm.

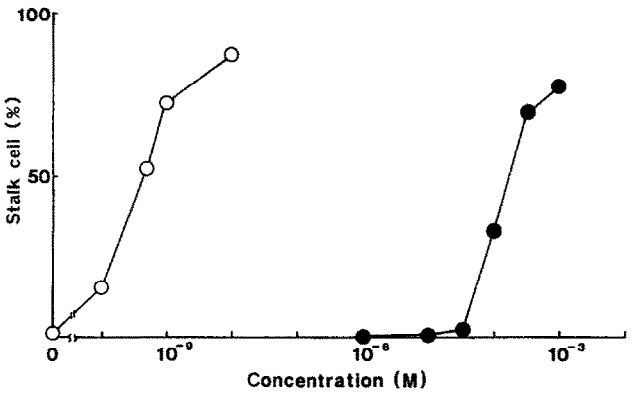

Fig. 3. Dose-requirement for DIF-1 and differanisole A for stalk cell induction. HM44 cells were incubated in vitro with various concentrations of DIF-1 (open circle) or differanisole A (closed circle), and stalk cell population was counted at 48 h.

It is of great interest to note that a naturally occurring substance such as differanisole A produced by a lower eukaryote can induce both the differentiation of tumor cells [1,2] and the stalk cell differentiation of *D. discoideum*. Differanisole A induces the differentiation of mouse leukemia cells at concentrations above ~ 20 μM [1,2], which are comparable to its active concentrations in stalk cell induction in *D. discoideum* (Fig. 3). It has been shown that differanisole A also causes the differentiation of some other tumor cells, e.g. mouse myeloid leukemia cells and mouse melanoma cells [2]. These results may be interpreted as suggesting that there exists

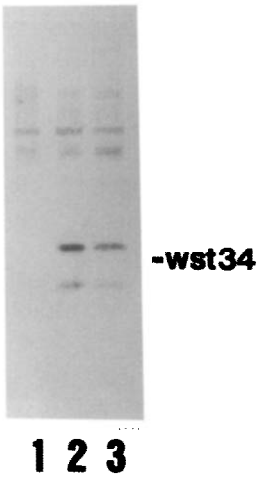

Fig. 4. Accumulation of wst34 induced by DIF-1 and differanisole A. HM44 cells were incubated in vitro without (lane 1) or with 10 nM DIF-1 (lane 2) or 1 mM differanisole A (lane 3) for 48 h, and the cell proteins (2.5×10^5 cells/lane) were analyzed by SDS-PAGE. The stalk-specific protein, wst34, was detected with anti-Dmst serum.

a common mechanism(s) in the induction processes of the redifferentiation of tumor cells and of stalk cell differentiation in the cellular slime moulds, and also that the chemical structure common to DIF-1 and differanisole A plays an important role in cell differentiation beyond species.

Acknowledgements: Y.K. is a Fellowship of the Japan Society for the Promotion of Science for Japanese Junior Scientists.

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