CELL-FREE BIOSYNTHESIS OF DISCADENINE, A SPORE GERMINATION INHIBITOR OF *DICTYOSTELIUM DISCOIDEUM*

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1. Introduction

The cellular slime mold *Dictyostelium discoideum* produces a factor which inhibits the germination of its own spore. This factor has been purified and shown to be an adenine derivative with an isopentenyl group in the N^6-position and a 3-amino-3-carboxypropyl group in the 3-position [1,2]. The factor, named discadenine, showed not only spore germination inhibitor activity, but also potent cytokinin activity, as expected from its structure [3].

Because of the unique structure and interesting physiological activity of discadenine, attempts have been made to elucidate the mechanism of its biosynthesis. Here we report the cell-free synthesis of discadenine by an extract of the cells of *D. discoideum.* Discadenine was synthesized from N^6-(Δ^2-isopentenyl)adenine (i^6Ade) by transfer of the 3-amino-3-carboxypropyl group from *S*-adenosylmethionine (SAM).

2. Materials and methods

2.1. *Materials*

[*carboxyl*-^{14}C]SAM was purchased from the Radiochemical Centre, Amersham. i^6Ade was obtained from Sigma. Discadenine was purified as in [2].

2.2. *Preparation of a crude extract of* D. discoideum

D. discoideum (NC-4) was cultured on agar plates as in [4] and cells were harvested in the late culmination stage. Cells, 1 g, were ground with 2 g alumina, and the homogenate was extracted with 5 ml buffer A (10 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate, 0.1 mM dithiothreitol, and 10% glycerol). The extract was centrifuged at 13 000 × *g* for 30 min and the resulting supernatant was recentrifuged at 156 000 × *g* for 60 min. The supernatant obtained was dialyzed overnight against 2 changes of buffer A (300 ml each). This dialyzed extract (S-100) was used as the enzyme preparation.

2.3. *Analysis of the reaction products*

The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.5), 5 mM magnesium chloride, 0.5 mM i^6Ade, 0.25 μCi [*carboxyl*-^{14}C]SAM (55 mCi/mmol) and 20 μl S-100 in final vol. 100 μl. After incubation for 2 h at 27°C, a 50 μl portion was directly spotted on Whatman 3MM paper and subjected to chromatography. Paper chromatograms were scanned with a radiochromatogram scanner (Packard Model 385).

2.4. *DEAE-cellulose column chromatography of S-100*

A 1.5 ml sample of S-100 was loaded on a DEAE-cellulose column (Whatman DE52, column size; 0.5 × 18 cm). Elution was carried out with a linear gradient of KCl (0–0.5 M, 30 ml each) and 1.2 ml fractions were collected at flow rate 10 ml/h.

3. Results and discussion

As shown in fig.1A, on paper chromatography of the reaction product with *n*-butanol–acetic acid–water (2:1:1) as solvent, a radioactive peak was separated that coincided with the spot of discadenine

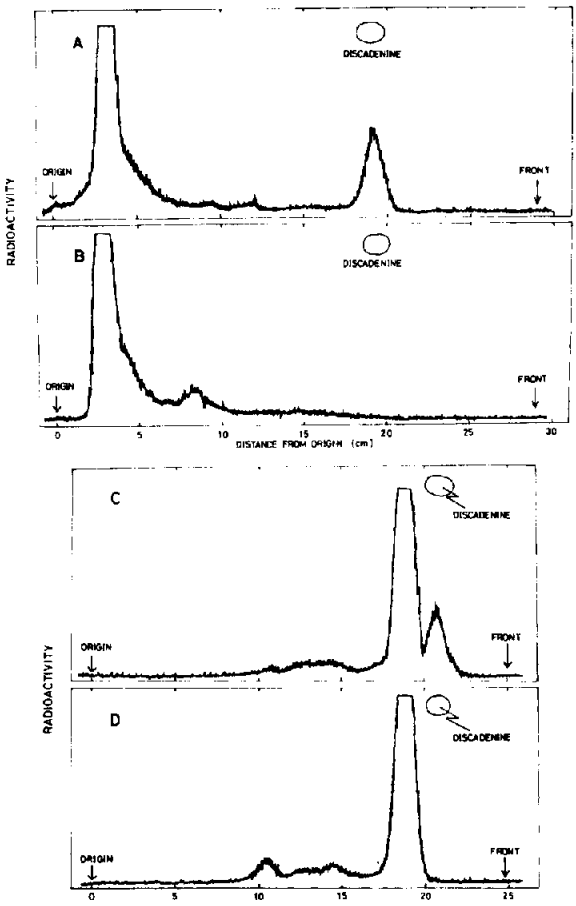

Fig.1. Detection of discadenine synthetase activity in vitro by paper chromatography. The reaction was carried out as specified in section 2: (A,C) in the presence of i^6Ade; (B,D) in the absence of i^6Ade. The solvent systems used were: (A,B) *n*-butanol–acetic acid–water (2:1:1); (C,D) isobutyric acid–0.5 N NH_4OH (5:3).

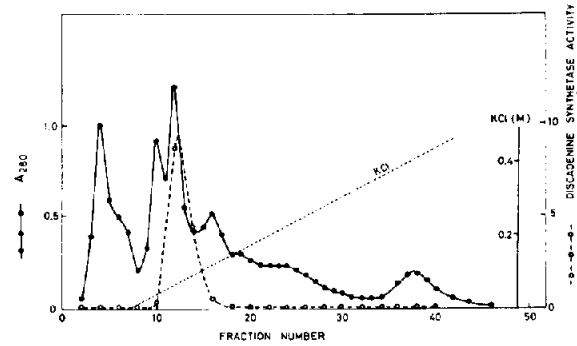

Fig.2. Fractionation of discadenine synthetase by DEAE-cellulose column chromatography. Discadenine synthetase activity was assayed as in section 2 using 20 μl column effluent as enzyme source. After incubation for 6 h at 27°C, a 60 μl portion was spotted on Whatman 3MM paper and subjected to chromatography with *n*-butanol–acetic acid–water (2:1:1) as solvent. The paper was dried and the area coinciding with that of marker discadenine was cut out and its radioactivity was measured in toluene-based scintillator using a liquid scintillation counter.

marker. This peak was not detected when the reaction was carried out in the absence of i^6 Ade (fig.1 A,B). Essentially the same result was obtained with another solvent system (fig.1C,D), confirming that radioactive spot detected was discadenine. The yield of radioactive discadenine increased linearly with increase in the time of incubation up to 5 h, and after incubation for 10 h, more than 90% SAM added had been used for synthesis of discadenine. Use of unlabeled SAM (final conc. 1 mM) instead of [^{14}C]SAM resulted in the appearance of a spot with ultraviolet absorption in the same position as marker discadenine (data not shown). This spot was not detected in the absence of either i^6Ade or SAM in the reaction mixture.

The enzyme, discadenine synthetase, was extensively purified by DEAE-cellulose column chromatography, as shown in fig.2. The discadenine-synthesizing activity was eluted as a single peak, suggesting that a single enzyme is involved in the synthesis of discadenine from i^6Ade. *S*-Adenosylhomocysteine, which is a known methylase inhibitor, strongly inhibited discadenine synthesis by the purified enzyme (table 1). The pH optimum for discadenine synthesis

Table 1
Inhibition of discadenine synthetase activity by *S*-adenosylhomocysteine (SAH)

	Discadenine synthetase activity (cpm)
– SAH	15 395
+ SAH (2 mM)	5616

Fractions 12–14 of fig.2 were combined and used as the enzyme source. Assay was carried out as in fig.2 legend. Enzyme, 32 μg, was added to each reaction mixture and incubation was for 4 h at 27°C

was found to be 7.5, and no ATP seemed to be required for the synthesis.

These results indicate that the 3-amino-3-carboxypropyl moiety of SAM was transferred to i^6Ade, forming discadenine. SAM is a methyl donor for a variety of methylation reactions. In polyamine biosynthesis, the 3-amino-3-carboxypropyl group of SAM is first decarboxylated, and the resulting 3-aminopropyl group is then utilized [5–8]. Unlike in these reactions, in the biosynthesis of discadenine, the 3-amino-3-carboxypropyl group of SAM is transferred directly to isopentenyladenine without prior decarboxylation. Direct transfer of the 3-amino-3-carboxypropyl group of SAM occurred in the biosynthesis of 3-(3-amino-3-carboxypropyl) uridine in the tRNA molecule [9]. The modified nucleoside 1-methyl-3-(3-amino-3-carboxypropyl) pseudouridine in 18 S RNA is synthesized in a similar fashion [10]. In vivo studies on the biosynthesis of Y_{o}base in tRNA have also shown that the 3-amino-3-carboxypropyl group is used for its synthesis [11]. It is very likely that nicotianine, namely *N*-(3-amino-3-carboxypropyl)-β-carboxypyridinium betain isolated from tobacco leaves [12], is synthesized through transfer of the 3-amino-3-carboxypropyl group of SAM. The present results clearly show that this unique side chain transfer reaction of SAM proceeds at the level of a low molecular weight compound as well as at the polynucleotide level.

We have recently shown that i^6Ade, a precursor of discadenine, is synthesized from 5′-AMP through transfer of the isopentenyl group in *D. discoideum* [13]; the isopentenyl AMP formed is then converted to i^6Ade. Discadenine appears in the cells of *D. discoideum* in a specific stage of cell differentiation (unpublished data). An interesting problem is how the appearance of discadeninesynthetase, and of i^6Ade-synthesizing enzymes, and other enzymes involved in metabolism of adenine nucleotides are correlated with cell differentiation. Studies on this problem are now in progress.

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