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Cytisine inhibition of informosome release from wheat embryo nuclei

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We have studied the action of the alkaloid cytisine on protein synthesis of plants. The alkaloid has been shown to have no effect on mRNA translation. At the same time it considerably decreases the release of mRNP particles from nuclei.

Protein synthesis; mRNA transport; Cytisine

1. INTRODUCTION

Inhibition analysis is widely applied for the elucidation of mechanisms of macromolecular synthesis. For this purpose antibiotics are often used. In recent years, a number of new inhibitors have been discovered from a group of biologically active compounds – the alkaloids harringtonine, lycorine, vinblastine, in particular [1–3]. Here, an attempt has been made to examine the action of a quinolizidine alkaloid on protein biosynthesis in plant cells. It has been established that cytisine is an effective inhibitor of mRNA release from the nucleus of wheat embryos. The in vivo inhibition of protein synthesis that we have observed is a consequence of limiting transport to the cytoplasm of mRNA capable of translation.

2. MATERIALS AND METHODS

Experiments were performed using wheat embryos isolated as suggested by Johnston and Stern [4]. Wheat embryos were separately incubated with [^{3}H]thymidine, [^{3}H]uridine (0.5 mCi/mmol) or [^{14}C]leucine (136 Ci/mmol) at 28°C in buffer (0.01 M Tris, 0.02 M KCl, pH 7.0) for 3 h, 20 and 40 min, respectively. Cytisine was added to embryos for 15 min before labeled precursors at the concentrations indicated in the figures.

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After incubation embryos were washed off from unincorporated label, homogenised, and incorporation of radioactive precursors into acid-insoluble material was then assayed in controls and samples pretreated with alkaloid. Translation of tobacco mosaic virus (TMV) RNA, poly(A)$^+$ RNA from wheat embryos was carried out according to [5]. Experiments on release of nuclear RNP particles were performed in a cell-free system containing nuclei (4×10^6 cpm), 25 mM KCl, 4 mM $MgCl_2$, 2 mM $CaCl_2$, 3 mM 2-mercaptoethanol, 2.5% Ficoll, 20 mM triethanolamine, pH 7.6, and 6 mM ATP as in [6]. Incubation was carried out for 20 min at 25°C and followed by centrifugation on sucrose and CsCl density gradients. To control the effect of alkaloid on RNA processing, embryos were labeled with [^{3}H]uridine in the presence of actinomycin D to inhibit nucleolar preribosomal RNA synthesis. Isolated nuclei were incubated with 0.1 mM cytisine at 0°C for 20 min for the binding of alkaloid to nuclei. RNA release from control (without alkaloid) and experimental samples was performed in the above-described cell-free system which supports RNA processing for 20 min at 25°C. Nuclei were suspended and homogenized in buffer containing 0.14 M NaCl, 0.5 M sodium acetate, pH 5.1, 0.5% SDS. RNA was isolated under standard conditions and analysed on a 10–30% sucrose gradient. Poly(A) containing RNA was revealed by oligo(dT)-cellulose chromatography according to [7].

3. RESULTS

3.1. *Effect of cytisine on DNA, RNA and protein synthesis*

In order to study the effect of the alkaloid on syntheses of macromolecules, data on the incorporation of radioactive precursors into DNA, RNA and protein in controls (100%) were com-

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pared with those obtained in the presence of different concentrations of the test preparation in the incubation medium. As shown in fig.1 incorporation of [^{3}H]thymidine and [^{3}H]uridine in controls and samples pretreated with alkaloid did not differ significantly. Cytisine had no inhibitory effect on the incorporation of [^{32}P]dCTP and [^{32}P]CTP into DNA and RNA in a cell-free system catalyzed by DNA polymerase and RNA polymerase II from calf thymus (not shown), thus pointing to the absence of the effect of cytisine on DNA and RNA syntheses. At the same time protein synthesis was inhibited noticeably at alkaloid concentrations of 10^{-6}–10^{-5} M. Further attempts were made to establish which stage of protein synthesis was sensitive to cytisine.

3.2. *Effect of alkaloid on mRNA translation and stability of polysomes*

Translation of different mRNAs was achieved using the S-30 fraction of wheat embryos, the incubation being performed within 60–90 min. The results of these experiments are illustrated in fig.2. One can observe that the alkaloid did not markedly inhibit the translation of TMV RNA (curve 1), poly(A)$^+$ RNA from wheat embryos (curve 2) and poly(U). Fig.3 shows that addition of the alkaloid does not change the distribution profile of polysomes in the sucrose density gradient. Its action was compared with that of pyrocatechol violet

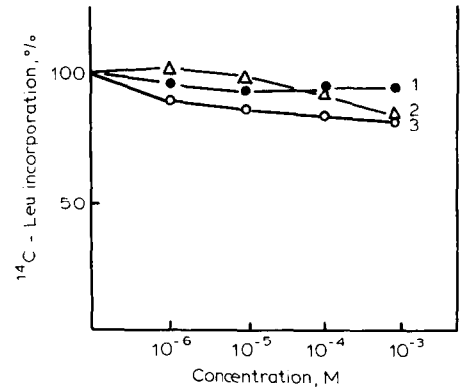

Fig.2. Effect of alkaloid on the translation of TMV RNA (1), poly(A)$^+$ RNA from wheat embryos (2) and poly(U) (3). The reaction mixture (50 μl) contained the following components: 20 μl preincubated wheat embryo S-30 fraction, 20 mM Hepes (pH 7.6), 2.5 mM magnesium acetate, 100 mM KCl, 1 mM ATP, 20 μM GTP, 10 mM creatine phosphate, 2 μg creatine phosphokinase, 1 mM dithiothreitol, 0.1 mM each of 19 unlabeled amino acids, 3 μCi/ml [^{14}C]leucine (Amersham, 136 Ci/mmol), alkaloid at the concentration tested, 5 μg RNA. Incubation was for 90 min at 25°C.

that causes accumulation of free 80 S ribosomes [8]. In contrast to this compound, cytisine did not induce formation of free ribosomes. The conclusion was drawn that the alkaloid does not influence the structure of polysomes. However, the amount of polysomes involved in protein synthesis in the

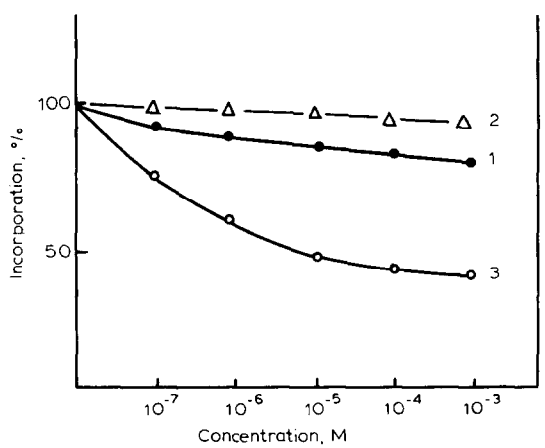

Fig.1. Incorporation of [^{3}H]uridine (1), [^{3}H]thymidine (2) and [^{14}C]leucine (3) into wheat embryos in the presence of different concentrations of cytisine.

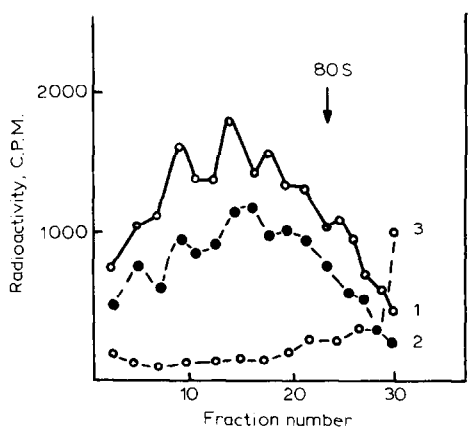

Fig.3. Distribution of polysomes isolated from control embryos (1) and processed by alkaloid (2) in a 10–30% sucrose gradient. The results are compared with the action of pyrocatechol violet (3). Centrifugation was carried out at 4°C at 38000 rpm in an SW-50 rotor for 3 h.

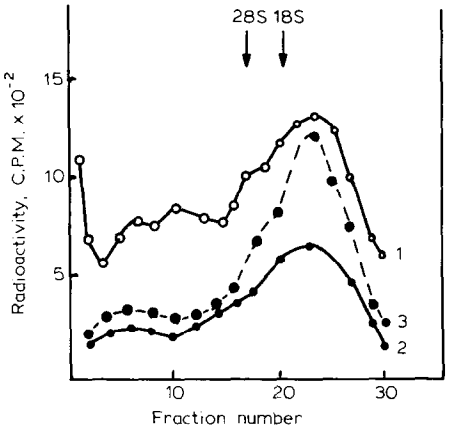

Fig.4. Sedimentation distribution in a 10–30% sucrose gradient of RNA isolated from nuclei after incubation at 0°C in the presence and absence of 0.1 mM cytisine (1) and release of RNA in a cell-free system at 25°C for 20 min in the absence (2) and presence of alkaloid (3).

cytisine-treated embryos was lower than in controls. Such an effect was not due to the inhibition of nuclear pre-mRNA processing and reduction of mRNA formation by cytisine. The conversion of labeled, newly synthesized RNA to 14–18 S RNA in both control and alkaloid-treated nuclei was practically unaffected, since only one form of sedimentation behavior is demonstrated (fig.4, curve 1). The decrease in amount of 14–18 S RNA on prolonged incubation in a cell-free system reflected the transfer of mRNA from control nuclei (fig.4, curve 3) and confirmed the previous finding that the alkaloid inhibited the release of RNA. These results are comparable to those of Schumm and Webb [9] obtained using colchicine on cells of rat liver. Most of the processed 14–18 S RNA was shown to be associated with poly(A).

Table 1

Effect of cytisine on the release of mRNP particles from wheat embryos nuclei

	mRNP yield		Inhibition (%)
	cpm	%	
Control	48500	100	–
Cytisine 0.1 mM	15656	32.3	67.7
Cytisine 1 mM	8196	16.9	83.1

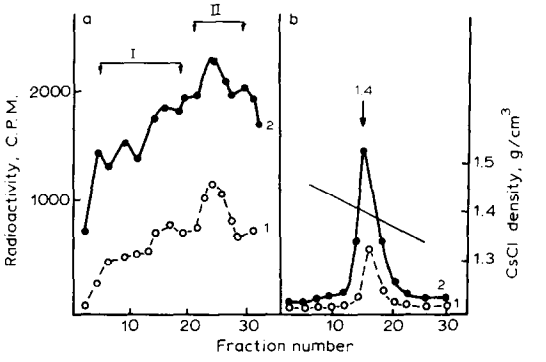

Fig.5. Sedimentation patterns (a) and CsCl density distribution (b) of material extracted from nuclei of wheat embryos in the presence (1) and absence of 10^{-4} M cytisine (2). Samples from zones I and II were collected as indicated in a and analyzed in a CsCl gradient. Since the density profiles of material from both zones were identical, data only for one zone are shown.

3.3. *Inhibition of informosome release from nuclei in the process of their extraction with cytisine*

For further elucidation of the mechanisms of inhibition of protein synthesis, it was necessary to study the process of mRNA transport from nuclei to the cytoplasm, one of the stages of protein synthesis. The results summarized in table 1 show that the alkaloid at concentrations of 10^{-4} M strongly inhibits the release of mRNP particles from nuclei.

The sedimentation and density distribution in a CsCl gradient of extracted material (fig.5a,b) demonstrate that in both cases RNA is released from nuclei in the form of RNP particles identical to the informosomes described for plants [10].

4. DISCUSSION

In this work the effect of the alkaloid cytisine on protein synthesis has been studied. It is shown for the first time that alkaloid does not affect the synthesis of DNA and RNA, and intranuclear processing of RNA in plant cells. Inhibition of protein synthesis in wheat embryos is not due to the action of cytisine on the stability of polysomes and mRNA translation. Inhibition of protein synthesis is demonstrated to be a consequence of a sharp decrease in mRNA transport from the nucleus to the cytoplasm. It is likely that the site of action of cytisine appears to be the interaction with nuclear membranes or components responsible for the

transfer of mRNA from the nucleus. This viewpoint is supported by data on colchicine inhibition of messenger RNA release in a reconstituted cell-free system from rat liver nuclei which provides the processing and transport of functional mRNA [8]. These investigators have shown that such a phenomenon is not mediated by the disruption of microtubular elements. Autoradiographic and histological studies carried out earlier indicated that colchicine localizes at the nuclear membranes and interacts with nuclear pores. The observed effect of cytisine on the transport of mRNP particles from the nucleus to cytoplasm for plants and the previously described action of the alkaloid colchicine on a similar process in the case of animal cells may have a common mechanism.

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