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BRIEF COMMUNICATION

Effect of ATP concentration and temperature on firefly luciferase activity

I. GÁLIS and J. JIRÁSKOVÁ

Institute of Plant Molecular Biology. Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic

Abstract

The dependence of luciferase activity in the homogenate of leaves of transgenic tobacco plants with chimeric firefly luciferase gene on ATP concentration and temperature was studied. The optimum ATP concentration was between 0.625 mM and 2.5 mM. The activity rapidly decreased if the homogenate was kept in 25 °C and is completely lost during 30 min.

Important parts of plant gene expression vectors are signal genes like chloramphenicol acetyl transferase, β-glucuronidase, neomycin phosphotransferase and firefly luciferase (for review see Weising *et al.* 1988), the expression of which can be quantitatively studied. The luciferase gene constructs have been expressed successfully in a variety of cell types (Ow *et al.* 1986, De Wet *et al.* 1987, Wood and DeLuca 1987, Fromm *et al.* 1990 and Jarvis and Lewis 1990). Some aspects of activity of luciferase, coded by firefly luciferase chimeric transgene introduced into the plant genome were studied. The purpose of this study was to find the range of optimum conditions for luciferase activity. The chimeric gene which consists of 35S promotor, firefly luciferase coding sequence and nos terminator sequence, constructed by Komari (1989) was used.

Tobacco (*Nicotiana tabacum* cv. Samsun) was transformed by *A. tumefaciens* AL4404 strain, which carried the helper plasmid pAL4404 (Hoekema *et al.* 1983) and the vector plasmid pTOK. The last plasmid was obtained by courtesy of Dr. Komari (Japan Tobacco Inc., Plant Breeding and Genetics Research Laboratory, Japan) and introduced into *Agrobacterium* strain by three-parental conjugation (Comai *et al.* 1983). Leaf disc transformation was used and regenerated plants were selected on kanamycin.

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Plant leaves was homogenized (4 °C) in extraction buffer (Riggs *et al.* 1989): 50 mM Tris pH 8.5, 0.5 mM EDTA, 1 % (v/v) β-mercaptoethanol and 0.1 % (v/v) Triton X-100, centrifugated (10 000 *g*, 4 °C) and stored in 4 °C for the following work. Luciferase activity was measured as emitted light on the scintilation counter (*Beckmann LS7000 Liquid Scintillation System*): 200 µl 0.3 mM D-luciferin (*Sigma*), 100 µl final concentration 25 mM glycylglycine (*Sigma*) and 5 mM $MgCl_2$ pH 7.8 and 100 µl ATP (*Sigma*) in corresponding concentration (0.04 - 10 mM) was mixed in scintillation vial and temperature was established to 26 °C. Enzymatic reaction was initiated by injection of 100 µl plant extract (at temperature 4 °C) immediately before sample adjustment into counting chamber. The values continuously measured and displayed by counter in 10 s intervals were registred. Measurement was finished after 90 s, when equilibration was established (Fig. 1).

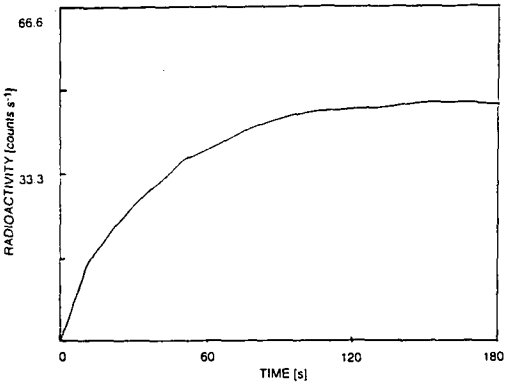

Fig. 1. Reaction course of luciferase activity.

Dependence of the luciferase reaction on ATP concentration was studied in the first series of experiments. It is well known that luciferase reaction depends on ATP concentration (Subramani and DeLuca 1988) which is employed for measurement of the ATP amount. We focused our work on finding of the optimal ATP level for luciferase activity measurement in plant extracts from transformed tissues (Fig. 2.).

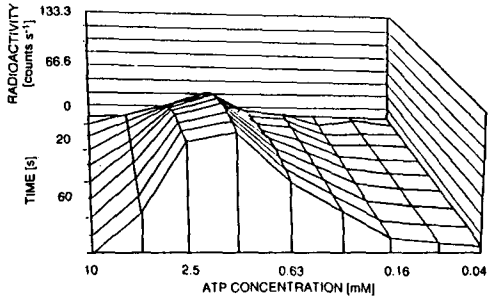

Fig. 2. Time dependence of luciferase activity on ATP concentration (enzyme dilution 1:20).

In the enzyme dilutions 1:20 and 1:50 (data not shown) optimal ATP concentration lies between 0.625 mM to 2.5 mM and in higher or lower ATP concentrations the enzyme activity sharply decreased (in 10 mM or 0.04 mM ATP concentrations the reaction course is almost undetectable). Not only low but also high ATP concentrations inhibit luciferase reaction. The aim of the second series of experiments was to test the degree of thermostability of luciferase. According to our experience GUS in homogenates is resistant to higher temperature (25 °C) and the homogenate can be handled in the laboratory temperature for several hours without loss of enzyme activity. This is not the case of luciferase, which, as our results show, is very unstable at 25 °C (Fig. 3). Light emission was completely inhibited after 30 min.

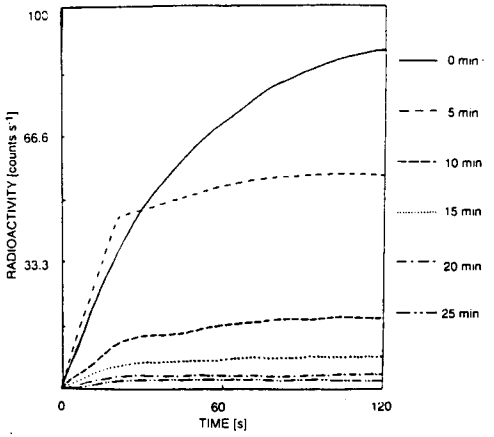

Fig. 3. Time dependence of activity of homogenate exposed at 25 °C.

It follows from our findings that it is very important to establish optimum ATP concentration before working with plant luciferase extracts and to work at low temperature (4 °C).

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