Chemistry of Natural Compounds, Vol. 31, No. 1, 1995

PHOSPHORYLATION OF PROTEINS OF ISOLATED COTTON PLANT CHLOROPLASTS UNDER THE ACTION OF VARIOUS PHYTOHORMONES AND VARIOUS CONCENTRATIONS OF MAGNESIUM IONS

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UDC 577.152.2

cAMP and Ca-phospholipid-dependent protein kinases have been detected in isolated cottonplant chloroplasts. A change in the activity of these enzymes in the presence of phytohormones and of various concentrations of magnesium ions has been shown.

The necessity of endogenous phytohormones, especially cytokinins, for the normal formation and functioning of chloroplasts and their photosynthetic activity is known [1].

It may be assumed that there is definitely something in common in the action of the hormones of animals and plants. In particular, cytokinin, abscisic acid (ABA), and auxin cause the direct activation of nuclear protein kinases in barley leaves, of the phosphorylating proteins of chromatin, and of RNA polymerase [2]. ABA stimulates the activity of the protein kinase from the cytosol of barley leaves, while BAP does not affect the activity of protein kinases over a wide range of concentrations (10^{-8}-10^{-4} M) but completely eliminates the stimulating action of ABA [3].

There are reports of a change in the matrix activity of the chromatin of isolated makhorka chloroplasts under the action of a cytokinin [4]. Confirmatory results have been obtained on cottonplant chloroplasts [5].

Nothing was known of the presence of protein kinases in cotton plant chloroplasts and their possible role in the realization of the action of phytohormones, although protein kinases of chloroplasts have been described for a number of cultivated plants [2, 6, 7, 8]. Our aim was to identify the protein kinases of isolated cottonplant chloroplasts and to determine their role in the realization of the hormonal effect.

Isolated cottonplant chloroplasts obtained from three-week leaves were used.

The addition of cytokinin directly to the reaction medium for determining protein kinase activity led a considerable increase in the activity of the enzyme (Fig. 1). This was shown both in the presence of minimal amounts of magnesium ions and, to an even greater degree, at a magnesium ion concentration of 40 mM.

In the presence of IAA, the activity of protein kinase C almost doubled, and it increased slightly with a change in the concentration of magnesium ions to 10 mM. At a concentration of 40 mM its activity had increased fourfold.

We obtained almost the same response to the presence of gibberellin in the reaction medium. When ABA was added the pattern changed greatly. While in the absence of magnesium ions activity increased fourfold, in the presence of 1 mM $MgCl_2$ it rose eightfold and reached a maximum at a concentration of 20 mM.

It must be mentioned that protein kinase C reacted clearly on the addition of each of the phytohormones, although we observed a marked increase in activity in the presence of magnesium ions. All the phytohormones showed a maximum effect on the activity of protein kinase C at a magnesium ion concentration of 20 mM, and this was particularly appreciable for BAP and ABA.

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 0009-3130/95/3101-0114$12.50

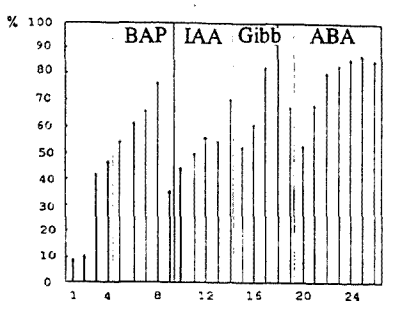

Fig. 1

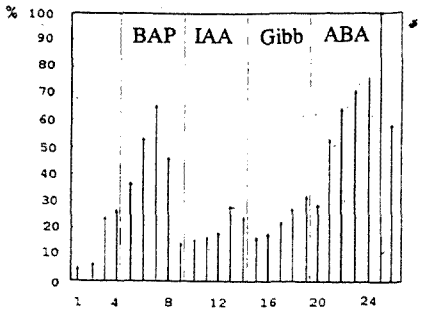

Fig. 2

Fig. 1. Protein kinase activity; *1*) without phytohormones, activators, and magnesium ions; *2*) in the presence of Ca^{2+} and a phospholipid, without phytohormones and magnesium ions; *3, 9, 15, 21*) at a concentration of magnesium ions of 0; *4, 10, 16, 22)* 1 mM; *5, 11, 17, 23)* 5 mM; *6, 12, 18, 24)* 10 mM; *7, 13, 19, 25)* 20 mM; *8, 14, 20, 26*) 40 mM; from point 3 to 26 Ca^{2+} and phospholipids were present in the reaction mixture.

Fig. 2. Protein kinase activity: *1*) without phytohormones, activators, and magnesium ions; *2*) in the presence of cAMP without phytohormones and magnesium ions; *3, 9, 15, 21*) concentration of magnesium ions — 0; *4, 10, 16, 22*) 1 mM; *5, 11, 17, 23*) 5 mM; *6, 12, 18, 24*) 10 mM; *7, 17, 19, 25*) 20 mM; *8, 14, 20, 26*) 40 mM; from point 3 to 26 cAMP was present in the reaction mixture.

To examine the reaction of cAMP-dependent protein kinase to the presence of phytohormones in the reaction medium we added cAMP instead of Ca^{2+} and phospholipid. In this case, a change in the activity of cAMP-dependent protein kinase was observed at the same concentrations as for the Ca-phospholipid-dependent protein kinase: 0, 1, 5, 10, 20, and 40 mM (Fig. 2).

As in the case of protein kinase C (PKC), the cAMP-dependent protein kinase reacted to the addition of each of the phytohormones used, and while the gibberellins and IAA did not increase the activity of the PKC in the presence of magnesium ions so strongly as BAP and ABA, here we saw an increase in activity to almost the same extent for all the phytohormones. A fairly pronounced dependence of the increase in the activity of the enzyme on the concentration of magnesium ions was observed, and while for PKC the maximum activity appeared at a $MgCl_2$ concentration of 20 mM in the presence of each of the phytohormones, here the maximum activity of cAMP-dependent protein kinase in the presence of BAP and IAA appeared at a concentration of 40 mM. In the presence of a gibberellin, the maximum was observed at 10 mM $MgCl_2$, and for ABA this concentration was 20 mM. While ABA increased the activity of protein kinase C to the maximum extent in the presence of 20 mM $MgCl_2$, the activity of cAMP-dependent protein kinase was increased to the maximum extent in the presence of a gibberellin when the concentration of $MgCl_2$ was 10 mM.

Thus, it may be concluded that phytohormones realize their action through protein kinases. The pathways of realization may depend on a multiplicity of factors and, in particular, on the concentration of bivalent metals present in the medium. The action on the cell of phytohormones and other factors is obviously realized through a phosphorylation-dephosphorylation process [9, 10]. The question of to what extent the pathways of the realization of the action of hormones and of other factors coincide for animal and plant cells remains open, although the fact that they have something in common, even if partial, is undoubted.

EXPERIMENTAL

Protein kinase activity was determined by a known method [11]. The phytohormones were added in a concentration of 10^{-4} M.

REFERENCES

1. E. G. Romanko, Kh. Ya. Khein, and O. N. Kulaeva, Biokhimiya, **33**, 547 (1968).
2. S. Yu. Selivankina, E. G. Romanko, G. V. Novikova, and O. N. Kulaeva, Fiziol. Rast., **292**, 766 (1987).
3. S. Yu. Selivankina, E. G. Romanko, N. I. Karavaiko, I. S. Moshkov, G. V. Novikova, and O. N. Kulaeva, Dokl. Akad. Nauk SSSR, **299**, 254 (1988).
4. O. N. Kulaeva and E. G. Romanko, Dokl. Akad. Nauk SSSR, **77**, 464 (1967).
5. N. A. Evgrafova, Kh. K. Avlyanov, O. N. Veshkurova, A. A. Takanaev, and Sh. I. Salikhov, Lectures at the First International Symposium on the Chemistry of Plant Substances, [in Russian], Tashkent (1994).
6. J. Soll, I. Fischer, and K. Keegstra, Planta, **176**, 488 (1988).
7. A. Hector, A. Zucero, Zhe-Fu Lin, and E. Rascher, J. Biol. Chem., **257**, 12157 (1982).
8. R. K. Ralph, P. J. A. McCombe, G. Tener, and S. L. Wojcik, Biochem. J., **130**, 901 (1972).
9. Zh. A. Abdurakhmanova and D. Yu. Shcherbakov, Abstracts of the Fifth Conference of Biochemists of the Republics of Central Asia and Kazakhstan [in Russian], Tashkent (1991), p. 129.
10. F. G. Karimova and S. N. Zhukov, Dokl. Akad. Nauk SSSR, **316**, No. 5, 1277 (1991).
11. Zh. A. Abdurakhmanova, L. N. Abdusalyamova, V. V. Kim, and Sh. I. Salikhov, Khim. Prir. Soedin., 100 (1993).