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Modulation of some nuclear matrix enzymatic activities by free fatty acids*

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It was shown that two of main enzymatic activities of plant nucleus and nuclear matrix, namely RNA-polymerasic and DNA-nucleolytic are susceptible to modulation with free fatty acids. The effects observed were dependent to both fatty acid length and degree of unsaturation. In nuclei a stimulation of nuclease activity was observed whereas in matrices short chain fatty acids inhibited the studied activity. The effect of fatty acids on RNA-polymerase was also different in nuclei and matrices. In nuclei all fatty acids studied inhibited polymerasic activity whereas in matrices short chain fatty acids stimulated this activity by up to 80% and the long chain fatty acids inhibited by up over 70%. The overall alteration of studied activities in nuclei and matrices by unsaturated fatty acids was similar. Nucleolytic activity was stronger inhibited and polymerasic activity was stimulated when the effects of linoleic and linolenic acids were studied. The results suggest possible importance of lipid component in nuclear matrix biological function.

The term nuclear matrix is used to denote the residual structures remaining after treatment of isolated nuclei with non-ionic detergents and high concentration of salts [1] or with lithium diiodosalicylate [2]. Besides proteinaceous components, the presence of other components like nucleic acids, carbohydrates as well as lipids was demonstrated in nuclear matrices [3]. Therefore nuclear matrices can be recognised as structures similar to biological membrane skeletons.

Our previous data show that after removal of the lipid components present in plant nuclear matrices with non denaturing solvents these structures exhibit significantly decreased apparent nucleolytic activity [4]. Furthermore, supplementation of such delipidated matrices with nuclear lipids or simple free fatty acid resulted in over 90% recovery of nucleolytic activity [4]. These observations point to possible importance of lipid components in modulation (regulation) of nuclear matrix functions.

For studying the role of individual lipid components of nuclear matrix lipids in biological activity of this structure, the experiments on the effect of various amphiphilic compounds upon plant nuclear matrix enzymatic activities were undertaken. In this report we describe for the first time (to our best knowledge) the data indicating that free fatty acids, as model lipids, affect two key enzymatic activities, namely DNA-nucleolytic and RNA-polymerasic, exerted by plant nuclear matrices.

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MATERIALS AND METHODS

Nuclear matrices were prepared from nuclei isolated according to Luthe & Quatrano [5] from 6-day *Cucurbita pepo* var. *patissoniana* seedlings (1–4 cm long) and were stored in liquid nitrogen. The matrices were isolated by the method involving washing with non-ionic detergent Triton X-100 [6]. Protein was determined according to Bradford [7]. DNA-nucleolytic activity was estimated by the modified method of Szopa *et al.* [8]. DNA-dependent RNA-polymerase activity was determined as described by Payne & Loening [9]. Fatty acids at final concentrations of 0.2 mM were injected into the incubation media in microliter volumes of methanolic solutions and incubated with matrices for 10 min at 37°C prior to determination of the enzymatic activities. Controls contained identical volumes of methanol.

RESULTS AND DISCUSSION

Isolated plant cell nuclear matrix, when incubated with free fatty acids at varying concentrations shows alterations of its enzymatic activities. Fig. 1 illustrates the effect of free fatty acid chain length dependence of nucleolytic activity of both plant nuclei and isolated matrices. In nuclei fatty acids stimulated their apparent nucleolytic activity by 25%–50% over control values (100%), depending on fatty acid chain length. Nucleolytic activity of matrices was affected by fatty acids in a different manner. Short chain fatty acids (lauric and myristic acids) induced a decrease of nucleolytic activity by 25% and 7%, respectively. Palmitic acid practically did not affect this activity, but long chain fatty acids (stearic, arachidic and behenic) stimulated the observed nucleolytic activity of the matrices (by 18% maximally).

No specific effects of fatty acids on various classes of nuclear polymerases were detected, therefore total DNA-dependent RNA-polymerase activity was chosen for the experiments. This RNA-polymerase activity (Fig. 2) was also found to be affected by fatty acids. With the increasing chain length all the fatty acids studied inhibited nuclear RNA-polymerase activity with effect increasing with fatty acid chain

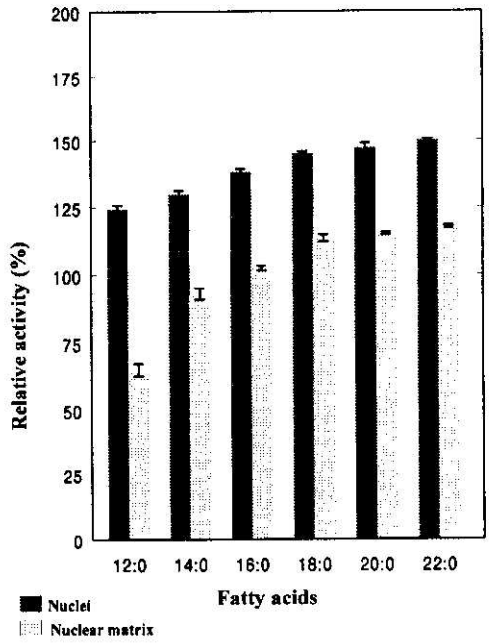

Fig. 1. Chain length dependence of the effect of free fatty acids upon nucleolytic activity of plant cell nuclei and nuclear matrix.
Fatty acids were added at 1:5 lipid/protein ratio. Determinations were performed at 37°C. Preincubation time was 10 min.

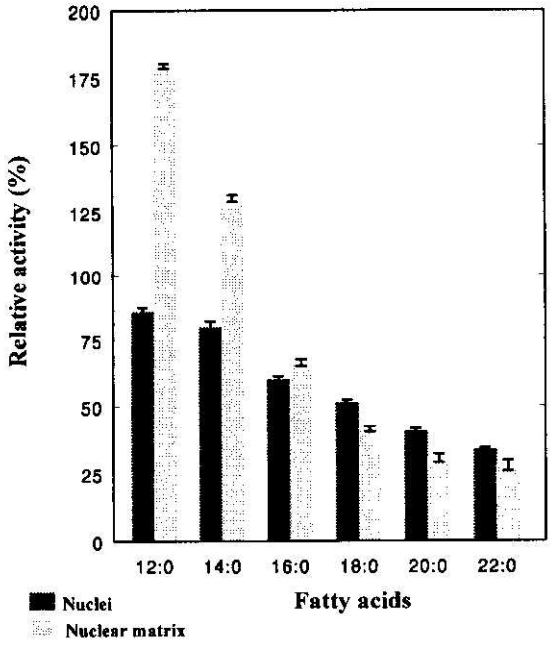

Fig. 2. Chain length dependence of the effect of free fatty acids upon DNA-dependent RNA-polymerase activity of plant cell nuclei and nuclear matrix.

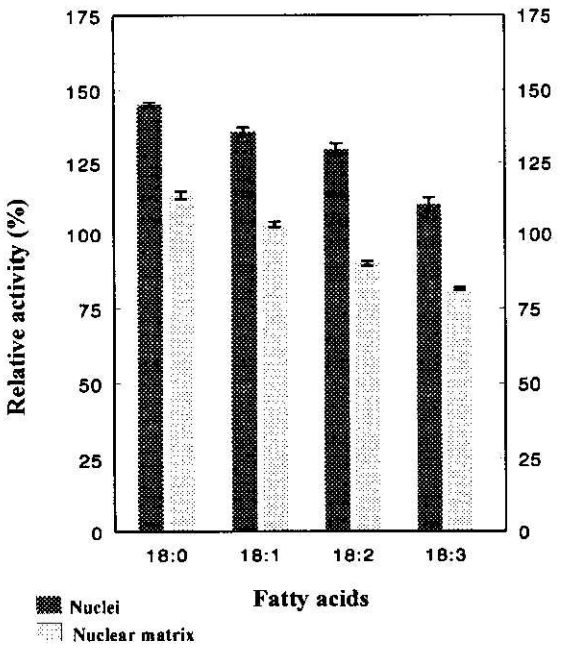

Fig. 3. Dependence of the effect of free fatty acids upon nucleolytic activity of plant cell nuclei and nuclear matrix on the degree of fatty acyl chain unsaturation.

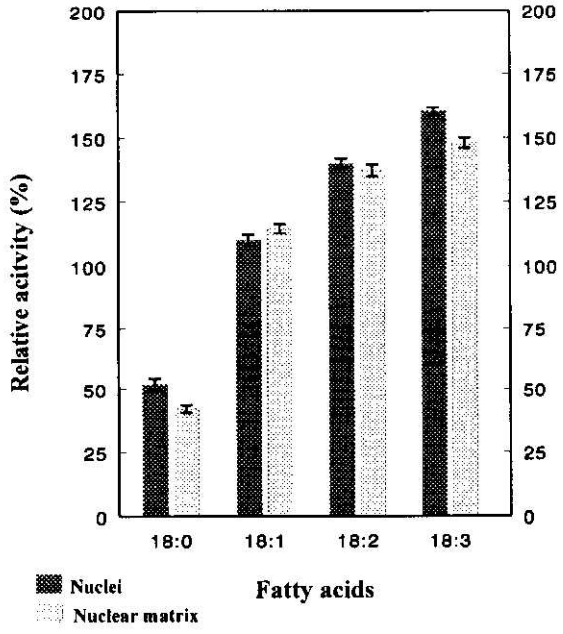

Fig. 4. Dependence of the effect of free fatty acids upon RNA-polymerase activity of plant cell nuclei and nuclear matrix on the degree of fatty acyl chain unsaturation.

length. The inhibition was the strongest with behenic acid which caused a decrease of activity by 66%. Although nuclear matrix isolated from plant nuclei exhibited only about 25% of the DNA-dependent RNA-polymerase activity of intact nuclei, the matrix activity was also susceptible to modulation by fatty acids. The activity was stimulated (up to 80%) by short chain fatty acids (lauric and myristic), whereas other fatty acids studied inhibited RNA-polymerase activity by over 70% (Fig. 2).

The effect of unsaturation of the fatty acid chain upon the nucleolytic activity of nuclei and matrices (Fig. 3) was similar, its extent was however different. In nuclei all the fatty acids studied stimulated the nucleolytic activity. The effect was however diminishing with the increase of fatty acid chain unsaturation. In matrices, saturated and monoenoic fatty acids stimulated RNA-polymerase activity whereas dienoic and trienoic fatty acids induced a decrease of the activity observed. The extent of the decrease was slightly larger than that observed for the stimulation (19% *versus* 13%).

The effect of unsaturation of fatty acid chain upon the pool of DNA-dependent RNA-polymerase was stronger than on the nuclease in both nuclei and matrices and dependent to the number of double bonds (Fig. 4). Oleic acid stimulated the activity by 10% and 14% in nuclei and matrices, respectively. Linolenic acid induced the maximal activity increase of 60% in nuclei and of 48% in matrices.

The results presented show that enzymatic activities of nuclear matrices are susceptible to modulation by lipid components. Observation of dependence of the effect on amphiphile structure suggests the presence of specific lipid components in matrices that could be directly responsible for modulation of the physiological activity of structure studied. The presence in some of matrix proteins of specific regions, directly undergoing modulation by a lipid component can therefore be suggested. Recent findings of Szopa (Szopa *et al.*, in preparation) pointing to the presence, in plant nuclear matrix 32 kDa nuclease, of a region of high homology to the lipid binding region of anexin strongly support this suggestion. Further studies on the lipid specificity of 32 kDa nuclease are in progress.

REFERENCES

1. Berezney, R. & Coffey, D.S. (1977) *J. Cell Biol.* **73**, 616–637.
2. Slatter, R.E., Dupree, P. & Gray, J. (1991) *Plant Cell* **3**, 1239–1250.
3. Cocco, L., Maraldi, N.M. & Manzoli, A. (1980) *Biochim. Biophys. Res. Commun.* **96**, 890–897.
4. Łubocka, J., Szopa, J. & Kozubek, A. (1995) *Acta Biochim. Polon.* **42**, 211–215.
5. Luthe, D.S. & Quatrano, R.S. (1980) *Plant. Physiol.* **65**, 305–308.
6. Pieck, A.C., Rijken, A.M. & Wanka, F. (1987) *FEBS Lett.* **212**, 267–280.
7. Bradford, M. (1976) *Anal. Biochem.* **272**, 248–254.
8. Szopa, J., Bocher, M. & Wagner, K.G. (1983) *J. Plant. Physiol.* **109**, 291–300.
9. Payne, P.I. & Loening, U.E. (1970) *Biochim. Biophys. Acta* **224**, 128–135.