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Short Communication

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Modification of the lipid component modulates nuclear matrix nucleolytic activity*

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It was shown that lipid composition of plant nuclear matrix depends on procedure of its isolation. The matrix isolated with the use of lithium diodosalicylate (LiS) differs in its lipid composition from the preparation isolated with the use of nonionic detergent (Triton X-100). It was also shown that the nucleolytic activity of the matrix is related to its lipid component. Matrix depleted in lipids loses half of its nucleolytic activity which is recovered after supplementation with previously extracted lipids. The extent of recovery of the nucleolytic activity is also dependent on the presence of residual DNA in matrix preparation. The recoveries of nucleolytic activities were higher in matrices not depleted in their DNA content.

The term nuclear matrix was proposed by Berezney and Coffey [1] when they discovered a residual structure in nuclei digested with nucleases and extracted with non-ionic detergents and concentrated sodium chloride. Later it was demonstrated that nuclear matrix (Mx)[1], as a biochemically defined and highly organised nuclear structure, mainly composed of proteins, and present in both animal and plant cells [1–3], is involved in a number of nuclear activities. Soon evidence has been obtained suggesting that this structure serves some specific functions of the nucleus. It was demonstrated that nuclear matrix plays significant role in organisation of DNA domains [4], DNA replication [5] and RNA transcription and transport [6–8]. Most of nuclear matrix proteins span residual nucleolus and nuclear envelope and maintain shape and structure of the nucleus. However, other proteinaceous components, such as nucleases, polymerases or topoisomerases, exhibit enzymatic activities that are of key importance for nuclear function [6, 9, 10]. Besides proteinaceous components, the presence in nuclear matrices of other components, i.e. nucleic acids, carbohydrates as well as lipids was demonstrated [11]. Therefore nuclear matrices can be recognised as structures similar to biological membrane skeletons.

Although most of research on nuclear metabolism and nuclear matrix function is focused on the role and structure of protein-nucleic acid complexes, the literature data indicate that lipids components may also play important role in nuclear function (e.g. [12–15]). During studies on plant cell nuclear matrices the presence of 32 kDa endonuclease was demonstrated [16]. Recent data (Szopa *et al.*, unpub-

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[1]Abbreviations: HPTLC, high performance thin-layer chromatography; LiS, lithium diiodosalicylate; Mx, nuclear matrix; Mx-L, nuclear matrix isolated with the use of LiS; Mx-T, nuclear matrix isolated with the use of Triton X-100.

lished) point to the presence, in the gene encoding this protein, of a sequence highly homologous to the lipid-binding sequence in anexins [17, 18], (KGDYHRYL) [19]. Therefore it is important to recognise a possible role of the lipid component in nuclear matrix nucleolytic activity as well as nuclear function. In this report the effect of plant nuclear matrix lipid manipulation on the matrix enzymatic activities is presented.

MATERIALS AND METHODS

Nuclear matrices were prepared from nuclei isolated by the method of Luthe & Quatrano [20] from 6-day *Cucurbita pepo* var. *patissoniana* seedlings (1–4 cm long) and were stored in liquid nitrogen. The matrices were isolated with two methods: a) with the use of non-ionic detergent Triton X-100 [21], and b) by extraction with lithium diiodosalicylate [2]. Protein was determined by the method of Bradford [22]. DNA-nucleolytic activity was estimated according to Szopa *et al.* [23]. The sample was undiluted, its volume was reduced to 50 µl and centrifugation time was 5 min. Nuclear and nuclear matrix lipids were isolated by Bligh & Dyer [24] procedure and analysed chromatographically by TLC. The plates (HPTLC silica gel plates) were developed in two steps. First, at 0.7 of plate height in ethyl acetate:n-propanol:chloroform:methanol:0.25% KCl (25:25:28:10:7, by vol.), then, after drying, at full distance in n-hexane:diethyl ether:acetic acid (75:23:2, by vol.). Chromatograms were visualised with non-specific (sulphuric acid charring, iodine vapours) and with reagents specific for phosphorus, cholesterol and sugars [25]. Quantitatively, total phosphorus was determined by the method of Rouser & Siakotos [26], total cholesterol by the method of Courchaine *et al.* [25] and sugars by the method of Roughan & Batt [27]. For studies of the effect of nuclear and nuclear matrix lipid manipulation, lipids were extracted (according to Radin, [28]) and subsequently added to the nuclear matrix preparations studied as methanolic solutions and incubated for 10 min at 37°C prior nuclease activity determination.

The data were analysed statistically using paired *t*-test.

RESULTS AND DISCUSSION

Table 1 shows the level and composition of lipid component isolated from plant cell nuclear matrices which were isolated by two different methods. Nuclear matrices, irrespective of the isolation method, show the presence of a residual lipid component that varies from 0.9 to 1.5 mg/mg of protein. This amount, however, is dependent on the method of matrix isolation. The nuclear matrix isolated by the method involving non-ionic detergent (Mx-T) is more depleted in lipids whereas the diiodosalicylate procedure (Mx-L) results in almost 70% higher amount of lipids remaining in the preparation. The level of neutral lipids that is doubled in Mx-L is the most striking difference between the results obtained by these two matrix isolation methods.

To study the role of the lipid component in biological activity of nuclear matrix, first the effect of alteration in lipid component in nuclei was examined. It was demonstrated that nuclei extracted with n-hexane:2-propanol, (3:2, v/v), a solvent mixture, which extracts lipids without causing protein denaturation exhibited 50% decrease of their nucleolytic activity. This decrease, however, could not be attributed to denaturation of nuclear proteins with the solvents as after supplementation of

Table 1
Lipid components of plant cell nuclear matrices isolated by two different methods

Component	Nuclear matrix isolated with the use of non-ionic detergent Triton X-100 (mg/mg of protein)	Nuclear matrix isolated with the use of lithium diiodosalicylate (mg/mg of protein)
Total lipids	0.90 ± 0.12	1.50 ± 0.21
Phospholipids	0.14 ± 0.05	0.17 ± 0.04
Sterols	0.15 ± 0.04	0.18 ± 0.03
Glycolipids	0.10 ± 0.02	0.13 ± 0.04
Other neutral lipids	0.51 ± 0.06	1.02 ± 0.09

Data are mean values from 4 different determinations ±S.D.

delipidated nuclei with the lipids previously extracted from the nuclei or nuclear matrices (lipid-protein ratio 1:1, by weight), resulted in recovery of nuclease activity. When matrix lipids were used, nuclei recovered up to 87% of nuclease activity (Table 2). A similar recovery was observed when delipidated nuclei were supplemented with lipids previously extracted from nuclei (91% of native nuclear activity). Slight differences observed between the effect of nuclear and nuclear matrix lipids, although statistically not significant, may indicate the presence of some specific interactions between various lipid and proteinaceous components in nuclei. Further studies on the effect of removal and subsequent supplementation with lipids of nuclear matrices isolated by the two methods showed that only Mx-L do respond to lipid depletion-reconstitution. The nucleolytic activity of Mx-T, although several times lower than that of Mx-L matrix was practically unaffected by either delipidation or lipid supplementation (Table 3). This indicates that stronger delipidation occurring during Mx-T preparation results in matrix in which enzymatic activities are partially inhibited either directly by the action of detergent or indirectly by excessive removal of lipids. The lack of recovery of activity by Mx-T after lipid supplementation may be explained by very tight binding to matrix proteins of the non-ionic detergent used for matrix preparation. In this case the replacement of artificial amphiphilic molecules of the detergent by more physiological ones of matrix lipids would be drastically restricted. To check whether supplementation of delipidated matrix by only a simple amphiphilic lipid molecule would re-

Table 2
The effect of alterations in lipid component on nuclear nucleolytic activity

Nucleus	Activity, units	Relative activity (%)
Native	21.1 ± 2.3	100
Delipidated*	10.6 ± 1.5	50
Delipidated supplemented with nuclear matrix lipids (1:1 lipid/protein, by weight)	18.3 ± 0.9**	87
Delipidated supplemented with nuclear lipids (1:1 lipid/protein, by weight)	19.2 ± 1.3**	91

An activity unit was equal to an increase of absorbance by 0.01/min at 37°C. *Delipidation with n-hexane:2-propanol (3:2, v/v) mixture. Data are mean values from 4 different determinations ±S.D. **Statistically different with respect to delipidated, P-value < 0.05.

Table 3
The effect of lipid component alteration on nucleolytic activity of nuclear matrices isolated with two different methods

	Nuclear matrix isolated with the use of non-ionic detergent Triton X-100		Nuclear matrix isolated with the use of lithium diiodosalicylate	
Nuclear matrix	Activity, units	Relative activity, %	Activity, units	Relative activity, %
Native	7.6 ± 0.60	100	19.1 ± 0.90	100
Delipidated*	7.6 ± 0.90	100	10.0 ± 1.10	52
Delipidated supplemented with palmitic acid (1:5 lipid/protein, by weight)	7.8 ± 0.95	103	18.6 ± 1.20**	98
Delipidated supplemented with nuclear matrix lipids (1:1 lipid/protein, by weight)	7.7 ± 0.92	101	20.0 ± 1.35	107

Activity units as in Table 2. *Delipidation with n-hexane:2-propanol (3:2, v/v) mixture. Data are mean values from 4 different determinations ± S.D. **Statistically different with respect to delipidated, P-value < 0.05.

sult in recovery of the Mx-L activity, similarly to that observed for total matrix lipids, free fatty acid was used. Supplementation of delipidated matrix (Mx-L) with palmitic acid, even at its ratio to matrix proteins of 1:5, resulted in recovery of the matrix nucleolytic activity. When fatty acid was replaced by another simple amphiphilic molecule (resorcinolic lipid) the recovery of matrix nucleolytic activity was not observed (Łubocka & Kozubek, unpublished results). Some difference in recovery of matrix activity when fatty acid or nuclear lipids were used (significant only in paired *t*-test) together with the opposite effect of resorcinolic lipid suggests possible significance of the nature of the lipids in modulating of nuclear matrix physiological activities. The studies on the role of lipid type and composition on the activity observed are being confirmed.

In the studies on nuclear matrix structure and function, one of the crucial problems is the isolation of the structures such a way that only the necessary elements remains. Recent data (Rzepecki, unpublished) show that the level of residual DNA in plant nuclear matrix is preparation procedure-dependent. It was found that depletion of DNA from nuclear matrix achieved by the modified procedure (removal of copper ions, extended incubation with magnesium ions and application of higher, 45 mM, LiS concentration) gave as a result preparations exhibiting by almost 50% lower nuclease activity in comparison to those determined in preparation not depleted of DNA (Table 4). Despite lower nucleolytic activity, the modified matrix also exhibited susceptibility to modulation of its nuclease activity by alteration of the lipid component. Delipidation of DNA-poor matrix resulted in a further decrease (by 40%) of the apparent nuclease activity (Table 4) whereas subsequent supplementation with nuclear matrix lipids resulted in recovery of up to 77% of the activity exhibited prior delipidation. Interestingly, although supplementation of DNA-poor matrix with matrix lipids resulted in a statistically significant effect, supplementation with fatty acid gave a statistically nonsignificant result. Moreover, differences between the effects of matrix lipids and palmitic acid were statistically not significant, either. These results indicate, that nuclear matrix lipids, from statistical point of view, are slightly more active in recovery of the activity. The less pronounced effect of lipids on DNA-poor matrices, in comparison to that on native matrices, i.e. not depleted of DNA (compare data in Table 3 and 4) suggests that a possible interaction between DNA and lipids (already reported in literature, e.g. [29]) might be involved in retaining of the enzymatic activities by native nuclear matrix. Removal of lipids subsequent to DNA depletion affects probably overall structural integrity of the matrix which is difficult to compensate by supplementation with lipids only. On the other hand, these experiments show that a portion of matrix-bound DNA, remaining during

Table 4

Effect of lipid component alteration in nuclear matrices depleted of DNA

Nuclear matrix	Activity, units	Relative to native matrix activity (%)	Relative to DNA-depleted matrix activity (%)
Native	18.7 ± 1.4	100	–
Native, DNA depleted	8.7 ± 1.10	46.5	100
DNA depleted delipidated*	5.2 ± 0.85	27.8	59.7
DNA depleted delipidated* supplemented with palmitic acid (1:5 lipid/protein, by weight)	6.0 ± 1.31**	32.1	68.9
DNA depleted delipidated* supplemented with nuclear matrix lipids(1:1 lipid/protein, by weight)	6.7 ± 1.02**	35.8	77.0

Activity units as in Table 2. *Delipidation with n-hexane:2-propanol (3:2, v/v) mixture. Data are mean values from 4 different determinations ± S.D. **Statistically different with respect to delipidated, *P*-value < 0.05.

nonmodified preparation procedure is necessary for maintaining the biological activity of the matrix, at least regarding its nucleolytic activity.

The data presented in this work indicate that interactions between all components of such a complex, membrane skeleton-like structure, as nuclear matrix seems to play a key role in expression and regulation of its biological function.

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