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COBALT-STIMULATED PROTEIN PHOSPHOKINASE ACTIVITY OF THE PORE COMPLEX-LAMINA FRACTION FROM RAT LIVER NUCLEAR ENVELOPE

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SUMMARY

The pore complex-lamina fraction obtained from nuclear envelope contains a protein phosphokinase activity capable of phosphorylating endogenous and exogenous protein substrates. Its specific activity in the presence of $MgCl_2$ is approximately twice that of intact nuclear envelope. However, when $MgCl_2$ is replaced by $CoCl_2$ in the reaction mixture, a 7 to 12-fold increase in incorporation of ^{32}P from γ-^{32}P-ATP into protein substrate occurs. This appears not to be due to an effect of the divalent cation on the substrate, or to inhibition of a phosphoprotein phosphatase activity. Substitution of $CuCl_2$, $MnCl_2$, $CaCl_2$, and $ZnCl_2$ for $MgCl_2$ results in a 20 to 30% decrease in incorporation of ^{32}P. Cyclic AMP and cyclic GMP at 1 μM were without apparent effect. Approximately 40% of the total protein phosphokinase activity of the nuclear envelope is associated with the pore complex-lamina fraction.

The post-synthetic modification of proteins by reactions involving phosphorylation and dephosphorylation occurs at numerous subcellular locations (1-3). Recent reports have documented the presence in nuclear envelope preparations of a protein phosphokinase activity capable of phosphorylating exogenous substrates as well as those endogenous to this organelle (4,5). In addition, we have described a nuclear envelope-associated phosphoprotein phosphatase activity which dephosphorylates endogenous phosphoprotein (6).

The precise location(s) of these enzyme reactions within the nuclear envelope has not been determined. However, studies of Lam and Kasper demonstrated an enrichment in the pore complex-lamina fraction of the major phosphoprotein present in their nuclear envelope preparation. Accordingly, they speculated that the kinase and its preferred substrate were components of the pore complex and that this protein kinase reaction might play a role

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in nucleocytoplasmic transport (5). In this communication, we substantiate the foregoing by presenting direct evidence for a protein phosphokinase activity associated with the pore complex-lamina fraction from liver nuclear envelope. In addition, we have observed that this protein kinase reaction is remarkably stimulated by cobalt ions. This appears to be the first demonstration of such an effect of cobalt ions on a cyclic AMP-independent protein kinase reaction.

MATERIALS AND METHODS

Preparation of Nuclei, Nuclear Envelopes, and Pore Complex-Lamina Fraction - Maintenance of animals, preparation of nuclei and nuclear envelopes from rat liver (male rats 350 gm, Sprague-Dawley, Madison, WI) were carried out as previously described (4). The pore complex-lamina fraction was obtained in a manner as described by Dwyer and Blobel (7), and contained approximately 2%, 20%, and 1% of the total nuclear envelope RNA, protein, and DNA, respectively. Transmission electron microscopy (not shown here) revealed numerous round, dense pores with no evidence of contaminating envelopes.

Chemicals and Chemical Analyses - Preparation of γ-^{32}P-ATP was as described elsewhere (8). Protein composition was determined by the method of Lowry et al. (9), using crystalline bovine serum albumin as a standard. RNA was determined by the orcinol reaction (10), and DNA by the diphenylamine reaction (11) using appropriate standards.

Enzyme Assays - Protein phosphokinase activities toward endogenous protein of intact nuclear envelope and the pore complex-lamina fraction were measured as described previously (4), except that the 0.5 ml reaction volume contained 12 mM NaCl in these experiments. Phosphoprotein phosphatase activities were measured in a manner as previously described (6).

RESULTS AND DISCUSSION

The envelope which surrounds the interphase nucleus of eukaryotic cells is composed of an inner and outer membrane which share many of the general properties of other biological membranes. A distinguishing characteristic of the nuclear envelope is the presence of pores which appear to be interconnected by an underlying proteinaceous lamina which apposes the inner membrane and surrounds the nucleus (12). Although the precise function of pore complexes is unknown, it has been suggested that they play a role in nucleocytoplasmic transport (13) and in the organization of chromatin (14). Lam and Kasper (5) have reported that a polypeptide which is preferentially phosphorylated by an endogenous nuclear envelope-associated protein kinase

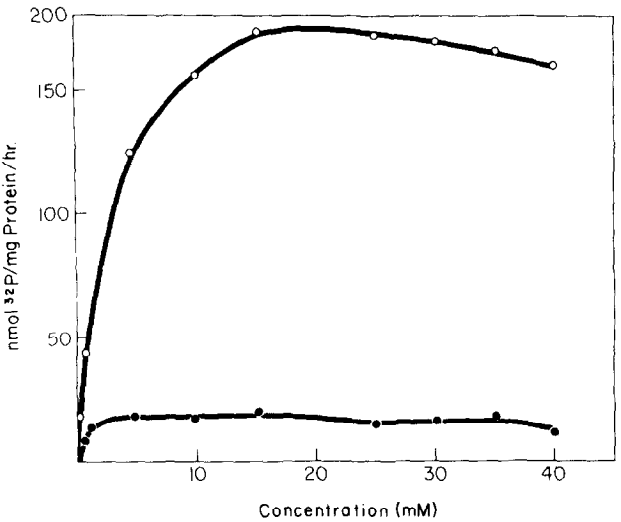

Figure 1: Effect of $CoCl_2$ on the incorporation of ^{32}P into endogenous proteins of pore complex-lamina fraction isolated from liver nuclear envelope. (o): $CoCl_2$, (·): $MgCl_2$.

appears to be associated with the pore complex-lamina fraction obtained therefrom. The data in Figure 1 demonstrate directly the presence of protein phosphokinase activity toward endogenous protein substrate in pore complex-lamina fractions reacted with γ-^{32}P-ATP. This phosphorylation shows a definite requirement for divalent cations, however, unlike other protein kinases associated with the nucleus, optimal activity is observed in the presence of $CoCl_2$ rather than $MgCl_2$. The former caused a 7 to 12-fold increase in activity when substituted for $MgCl_2$ at comparable concentrations between 5 and 40 mM. Under the same reaction conditions, $CoCl_2$ caused a 2 to 4-fold increase in kinase activity associated with intact nuclear envelope (Fig. 2).

The observed effect of $CoCl_2$ is not likely due to modification of protein substrate since it caused a similar increase of ^{32}P incorporation into the exogenous protein substrates, dephosphophosvitin and lysine-rich histones (data not reported). In addition, the $CoCl_2$ effect was not ascribed to inhibition of phosphoprotein phosphatase activity. Although the nuclear envelope does contain a phosphoprotein phosphatase activity (6), we were

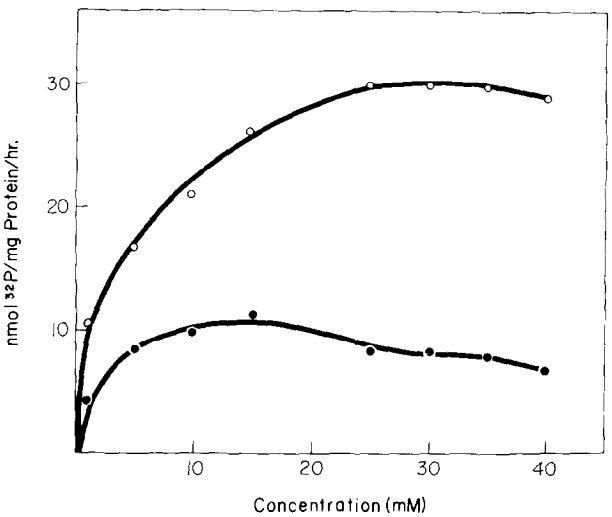

Figure 2: Effect of $CoCl_2$ on the incorporation of ^{32}P into endogenous proteins of intact liver nuclear envelope. (o): $CoCl_2$, (·): $MgCl_2$.

unable to demonstrate the presence of a pore complex-lamina fraction-associated phosphatase which could dephosphorylate phosphoproteins endogenous to that fraction. For example, the presence of NaF, a potent inhibitor of phosphoprotein phosphatase (6), in the reaction did not increase the incorporation of ^{32}P into pore complex-lamina fraction proteins. In addition, EDTA, a potent inhibitor of the protein kinase reaction (6,15), when added 4 minutes after the start of incubation of the pore complex-lamina fraction sample in the presence of γ-^{32}P-ATP, did not cause a decline in incorporation of ^{32}P into endogenous protein (data not shown). Thus, these results are distinct from those obtained in a previous study (6) on intact nuclear envelope, where the addition of NaF and EDTA to the protein kinase reaction produced effects which strongly suggested the presence of a phosphoprotein phosphatase activity in that organelle.

Others have shown that 10 mM $CoCl_2$ causes a 2 to 3-fold increase in activity (as compared with activity in the presence of $MgCl_2$) of cyclic AMP-dependent protein kinase (16,17), whereas $CoCl_2$ inhibits the activity of cyclic nucleotide-independent kinases (1,18,19). The nature of the $CoCl_2$ effect observed in our study might initially suggest that the kinase reactions

associated with the intact envelope and pore complex-lamina fraction are dependent on the presence of cyclic nucleotides. However, we have previously shown (4) that cyclic AMP and cyclic GMP at 1 and 10 μM have no measurable effect on the activity of the nuclear envelope-associated kinase reactions. The activity of the pore complex-lamina-associated protein kinase toward endogenous protein substrates was also not affected by addition of these two agents (Table 1). Furthermore, addition of 100 μg of protein kinase inhibitor (20) to the reaction did not inhibit the protein kinase activity of either fraction (data not shown), suggesting that the kinase activity of nuclear envelope and pore complex-lamina fraction was not due to the presence of the catalytic subunit of a cyclic AMP-dependent kinase. The pore complex-lamina fraction-associated protein kinase activity was also observed when using exogenous substrates such as dephosphophosvitin and lysine-rich histones. These reactions were also unaffected by the presence of cyclic nucleotides (data not shown).

We have also observed that when $CoCl_2$ replaced $MgCl_2$ in the range of 0.5 to 5 mM, the protein kinase activity associated with intact nuclei was decreased by 60 to 65%, and that of chromatin 30 to 70% (data not shown). Clearly, the stimulation by $CoCl_2$ of the nuclear envelope and pore complex-lamina-associated protein kinase reactions differs from the effect of this agent on other cyclic AMP-independent kinases located at various sites within the cell. The high degree of stimulation by $CoCl_2$ might reflect a specific role for cobalt ion in the regulation of kinase reactions associated with the pore complex, dense lamina, and/or nuclear membranes.

The effects of other agents on the protein kinase activity associated with nuclear envelope and pore complex-lamina are shown in Table 1. 15 mM $CuCl_2$, $MnCl_2$, $CaCl_2$, and $ZnCl_2$ each caused a decrease in incorporation of ^{32}P into endogenous protein of each fraction. Lam and Kasper (5) found no phosphorylation of the major pore complex polypeptide in the presence of 20 mM $CaCl_2$ or 20 mM $CuCl_2$. The difference between the extent of diminished

Table 1

Effects of Various Agents on the Protein Phosphokinase Activity Toward Endogenous Protein from Intact Nuclear Envelope and Pore Complex-Lamina Fraction

Agent Added[a]	Protein Phosphokinase Activity[b]	
	Nuclear Envelope	Pore Complex-Lamina
$MgCl_2$	100	100
$CoCl_2$	250	940
$CuCl_2$	62	80
$MnCl_2$	65	70
$CaCl_2$	26	70
$ZnCl_2$	22	68
cAMP	106	100
cGMP	98	103

[a]Each cation was present at 15 mM and added in the absence of $MgCl_2$. The cyclic nucleotides (1 μM) were added in the presence of 15 mM $MgCl_2$. Values listed (arbitrary units) are relative to the activity in the presence of $MgCl_2$, which produced optimal activity at this concentration.

[b]Protein phosphokinase activity was measured as referred to in Materials and Methods section. Values represent average of those obtained in 2 to 7 experiments involving 2 separate preparations of each fraction.

kinase activity in the presence of these agents in that and the present study may be due to differences in experimental details, such as composition of standard assay mixtures or methods of envelope preparation.

The foregoing is a first demonstration of a protein phosphokinase activity associated with the pore complex-lamina fraction of nuclear envelope. Approximately 40% of the total endogenous protein kinase activity of intact nuclear envelope is associated with the pore complex-lamina, however, this fraction does not appear to contain an endogenous phosphoprotein phosphatase activity, despite the presence of such an activity in intact nuclear envelope (6). Although such a distribution of the protein kinase and phosphatase reactions in nuclear envelope (i.e., enrichment of kinase in the pore complex-lamina, and phosphatase elsewhere in the nuclear envelope) may be the result of

fractionation procedures, it is also likely that these findings might indeed reflect a specific topographic distribution of these reactions in the nuclear envelope. Further studies are needed to elaborate on this matter and on the possible physiologic significance of the observed cobalt effect.

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REFERENCES

1. Rubin, C.S., and Rosen, O.M. (1975) Ann. Rev. Biochem. 44, 831-887.
2. Ahmed, K., and Wilson, M.J. (1978) Chromatin Controls in the Prostate, Chapter 12, in The Cell Nucleus, Chromatin, Part C, Vol. VI, pp. 409-459 (H. Busch, ed.) Academic Press, New York.
3. Jungmann, R.A., and Kranias, E.G. (1977) Int. J. Biochem. 8, 819-830.
4. Steer, R.C., Wilson, M.J., and Ahmed, K. (1979) Exptl. Cell Res. 119, 403-406.
5. Lam, K.S., and Kasper, C.B. (1979) Biochemistry 18(2), 307-311.
6. Steer, R.C., Wilson, M.J., and Ahmed, K. (1979) Biochem. Biophys. Res. Commun. 89(4), 1082-1087.
7. Dwyer, N., and Blobel, G. (1976) J. Cell Biol. 70, 581-591.
8. Ahmed, K., Wilson, M.J., and Davis, A.T. (1975) Biochim. Biophys. Acta 377, 80-83.
9. Lowry, O.H., Rosebrough, N.J., Farr, A.R., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
10. Hutchinson, W.C., and Munro, H.N. (1961) Analyst 86, 768-813.
11. Burton, K. (1956) Biochem. J. 62, 315-323.
12. Aaronson, R.P., and Blobel, G. (1975) Proc. Nat. Acad. Sci. USA 72(3), 1007-1011
13. Stevens, B.J., and Swift, H. (1966) J. Cell Biol. 31, 55-77.
14. Comings, D.E., and Okada, T.A. (1970) Exptl. Cell Res. 62, 293-302.
15. Ahmed, K., and Wilson, M.J. (1975) J. Biol. Chem. 250, 2370-2375.
16. Kuo, J.F., Krueger, B.K., Sanes, J.R., and Greengard, P. (1970) Biochim. Biophys. Acta 212, 79-91.
17. Miyamoto, E., Kuo, J.F., and Greengard, P. (1969) J. Biol. Chem. 244(23), 6395-6402).
18. Kleinsmith, L.J., and Allfrey, V.G. (1969) Biochim. Biophys. Acta 175, 123-135.
19. Thornburg, W., Gamo, S., O'Malley, A.F., and Lindell, T.J. (1979) Biochim. Biophys. Acta 571, 35-44.
20. Walsh, D.A., Ashby, C.D., Gonzalez, C., Calkins, D., Fischer, E.H., and Krebs, E.G. (1971) J. Biol. Chem. 246 (7), 1977-1985.