Menadione is Not Selective for Inactivation of Different cdc25 Phosphatases

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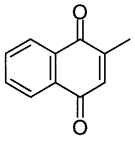
Received October 21, 1999

Menadione formerly known as vitamin K_3 is a synthetic derivative of naphthoquinone that has been widely used to study superoxide stress in mammalian system.[1] Menadione has also attracted attention because of its significant anticancer activity *in vitro*[2] and *in vivo*.[3] Since the last decade, the cell growth inhibitory effect of this compound has been attributed to two principle mechanisms: oxidative stress[4] and arylation of cellular thiol.[5]



Menadione (Vitamin K_3)

Recent study has shown differences at the level of cell cycle arrest between the response of cells to hydrogen peroxide and superoxide stress.[1f] Thus, hydrogen peroxide and menadione treatment cause cells to arrest in G_2 and G_1, respectively. However, in the experiment reported by others, superoxide dismutase did not antagonize the growth inhibitory effects by menadione, suggesting that cell cycle arrest is not mainly due to generation of superoxide anion radical.[5c] Another possible mechanism of toxicity of menadione is inhibition of sulfhydryl-dependent protein. Indeed, menadione has been proposed to inhibit cdc25 phosphatase.[6] Work from our laboratory has also shown that cdc25A phosphatase was inactivated by menadione and that the loss of enzyme activity was due to the modification of the active site.[7] Cdc25 exists as three human homologues termed cdc25A, -B, and -C.[8] Since cdc25A is predominantly expressed in G_1,[9] in contrast to cdc25B and cdc25C, which are mostly expressed in G_2-M,[10] it is of interest to know whether the inactivation by menadione on cdc25A phosphatase is specific. Therefore, we decided to determine the effect of menadione on *p*-nitrophenyl phosphate (*p*-NPP) hydrolysis catalyzed by cdc25B and -C phosphatases.

To produce the cdc25B and -C phosphatases, standard molecular cloning procedures were followed. Reverse transcriptase PCR on total RNA from human HeLa cells with primers[11] produced the expected 1719-bp and 1440-bp product for cdc25B and -C, respectively. These fragments were inserted into *Bam*HI and *Hind*III sites of pGEX-KG, an expression vector encoding GST. The resultant plasmids were introduced into the *Escherichia coli* BL21 strain and the GST-cdc25B and -C proteins were produced and purified to apparent homogeneity as described previously.[12]

Table 1. Kinetic constants for the inactivation of phosphatases by menadione

Enzyme	K_I (μM)	k_{inact} (sec^{-1})
cdc25A	38 ± 4	1.1 ± 0.1×10 $^{-2}$
cdc25B	95 ± 3	4.0 ± 0.1×10 $^{-2}$
cdc25C	20 ± 4	1.1 ± 0.1×10 $^{-2}$

Next purified phosphatases were incubated with the chromogenic substrate, 40 mM *p*-nitrophenyl phosphate in 20 mM Tris (pH 8.0), 1mM EDTA, and 0.2 mM DTT at 37.0 ± 0.1 °C. However, the result indicates that menadione also inactivates the cdc25B and -C phosphatases as well as cdc25A phosphatase (Table 1). Since evaluation of the rate of enzyme inactivation by menadione using the method of Kitz and Wilson[13] shows the similar range of K_I and k_{inact}, we conclude that there is no significant selectivity associated with the inactivation of different cdc25 phosphatases.

Cdc25 phosphatases are dual-specificity phosphatases which dephosphorylate the phosphotyrosine residue as well as phosphoserine and phosphothreonine residues. Therefore, we subsequently tested whether or not menadione affects other phosphatases and found out to be that protein tyrosine phosphatase such as LAR, PTP1B, and *Yersinia* PTP, and protein-serine/threonine phosphatase such as PP1 and PP2A (up to 50 μM) were not inactivated by menadione (data not shown). Protein tyrosine phosphatases have an 11-residue sequence motif that contains the catalytically essential Cys and Arg residue and that is also shared by cdc25 phosphatases. Aside from the sequence His-Cys-$(X)_5$-Arg, cdc25 phosphatases have no sequence homology with the catalytic domains of other protein tyrosine phosphatases or serine/threonine phosphatases. Crystallographic investigation has also revealed that the active site of cdc25 phosphatases is extremely shallow in contrast to other non dual-specificity phosphatases.[14] We believe that these structural features may control the inactivation specificity between the dual- and non dual-specificity phosphatases by menadione, although menadione did not selectively inactivate the different cdc25 homologs.

Acknowledgment. The authors wish to acknowledge the financial support of the Korea Research Foundation made in the program year of 1998 (Grant No. 98-105-D00178).

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