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COMMUNICATION

Studies on Menadione as an Inhibitor of the cdc25 Phosphatase

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It has been recently reported that some of the CDC25 genes have been implicated in the progression of cell cycle and might display oncogenic properties in mammalian cells. Therefore, the inhibition of this phosphatase may hold promise in the treatment of human cancer. In this study, we demonstrated that cdc25 phosphatase can be inactivated by menadione and that the loss of enzymatic activity was due to the modification of the active site.

Menadione (vitamin K_3) has increasingly been of interest because it was shown to exhibit a broad range of antitumor activity in human cell and imposes a lower level of toxicity than other cancer chemotherapeutic drugs of quinone structure (*1–4*). In this study, we investigated the effects of menadione on the activity of cdc25A phosphatase, because (i) it has been reported that menadione induced alterations in the phosphorylation status and the activity of protein tyrosine phosphatase (*5*); (ii) it has been suggested that cdc25A phosphatase is a novel potential oncogene (*6*); (iii) menadione shares a critical enone structure with some mechanism-based inhibitors of phosphatases that has been previously reported (*7*).

The cdc25A phosphatase used in this study was purified to apparent homogeneity using the method of Beach *et al.* (*8*). The assay for cdc25 phosphatase activity was performed as described, using *p*-nitrophenyl phosphate as substrate (*9*). The initial experiments with cdc25 phosphatase were conducted in the usual incubation buffer containing 20 mM Tris (pH 8.0), 1 mM EDTA, 0.1% β-mercaptoethanol, and 10 mM DTT. The yield of *p*-nitrophenolate from *p*-nitrophenyl phosphate in this system was found to be linearly dependent on time for periods up to 45 min or even longer if low levels of enzyme are employed. Attention was then turned to the determination of the ability of menadione to inhibit the phosphatase. Increasing amounts of menadione, however, did not result in increasing inhibition of cdc25 phosphatase. At this point, it was not unreasonable that menadione might be reduced to the corresponding hydroquinone during the incubation, since it is well known that DTT is a reducing agent. The resulting hydroquinone does not have an electrophilic center leading to the irreversible inhibition. To verify this assumption we studied the chemical behavior of menadione in the presence of DTT. Indeed, treatment of menadione with DTT rapidly yielded naphthohydroquinone,

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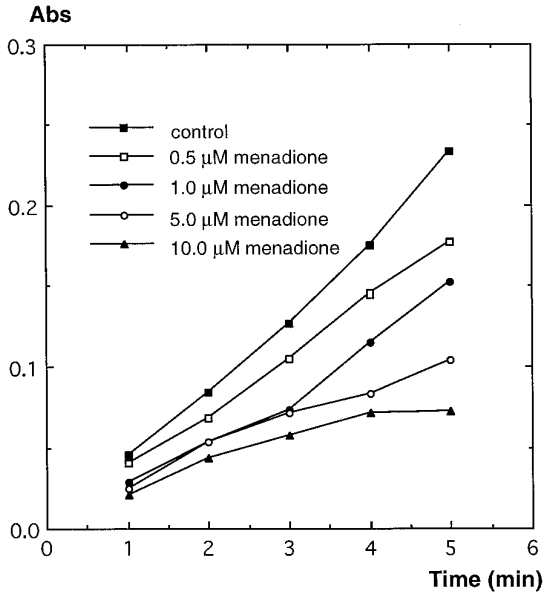

FIG. 1. Time and concentration dependencies of inactivation of cdc25 phosphatase with menadione.

as judged by its characteristic absorption spectrum. Moreover, when we tested the inhibitory effect with the synthesized hydroquinone obtained by treatment of sodium hydrosulfite, no inhibition from the incubation mixture was detectable.

In view of these experiments we decided to examine the inhibitory effect in the reaction buffer without DTT (*10*). Although the enzymatic activity was slightly decreased in the buffer without DTT, this system could be still employed for the inhibition assay. The concentration dependence of inhibition at various times is shown in Fig. 1. Thus, there is an observable decrease in the enzyme activity as the menadione concentration is increased. For menadione concentrations of >10 μM, the absorbance stays fairly constant, which indicated the almost complete inhibition within 5 min. Moreover, when the cdc25 phosphatase treated by menadione was dialyzed for 2 days at 4°C against the buffer containing 20 mM Tris (pH 8.0), 1 mM EDTA, 0.1% β-mercaptoethanol, and 10 mM DTT, the enzyme was not reactivated, and of course, the uninhibited enzyme was found to be stable with little or no loss in activity at the same dialysis. Therefore our results suggest that the formation of a covalent bond between the enzyme and inhibitor results in the inhibition.

It was also important to know whether the active site of the enzyme is involved. To investigate this question, menadione was incubated with cdc25 phosphatase in the presence of the competitive reversible inhibitor, arsenate (*11*). As shown in Fig. 2, 100 μM arsenate provides moderate protection against the inactivation, suggesting that menadione interacts with the active site of the enzyme.