Biochemical and Biophysical Research Communications **257,** 12–18 (1999)
Article ID bbrc.1999.0409, available online at http://www.idealibrary.com on IDEAL®

Role of Suc1 in the Activation of the Cyclosome by Protein Kinase Cdk1/Cyclin B

Michal Shteinberg and Avram Hershko[1]
Unit of Biochemistry, The B. Rappaport Faculty of Medicine and The Rappaport Institute for Research in the Medical Sciences, Technion-Israel Institute of Technology, Haifa 31096, Israel

Received February 16, 1999

A large complex, called the cyclosome or anaphase-promoting complex, has specific and regulated protein-ubiquitin ligase activity that targets mitotic regulators (such as cyclin B) for degradation at the end of mitosis. In early embryonic cell cycles the cyclosome is inactive in the interphase, but is subsequently converted by protein kinase Cdk1/cyclin B to an active, phosphorylated form, in a process that includes an initial lag period. This time lag may be important to prevent premature self-inactivation of Cdk1/cyclin B before the end of mitosis. We have previously observed that the phosphorylated form of the cyclosome binds to Suc1, a protein that associates with Cdk1 and with phosphate-containing compounds. We now report that low, physiological concentrations of Suc1 stimulate the activation of the interphase form of the cyclosome by the protein kinase. When Suc1 was present from the beginning of the incubation together with protein kinase Cdk1/cyclin B, activation of the cyclosome took place with the normal lag kinetics. However, when interphase cyclosome was first incubated with protein kinase Cdk1/cyclin B without Suc1, the subsequent addition of Suc1 caused a rapid burst of cyclosome activation and the lag was completely abolished. These findings are consistent with the interpretation that following initial slow phosphorylations of the cyclosome by the protein kinase, Suc1 accelerates multiple phosphorylations that culminate in the full activation of the cyclosome. In support of this interpretation, we find that Suc1 stimulates the phosphorylation of several proteins in the preparation of interphase cyclosome and that the effect of Suc1 on phosphorylation was augmented by prior incubation of interphase cyclosome with protein kinase Cdk1/cyclin B.

Exit from mitosis in eukaryotic cells requires the programmed, ubiquitin-mediated degradation of cell cycle regulators, such as mitotic cyclins, anaphase inhibitors and some proteins associated with the mitotic spindle (reviewed in Refs. 1,2). These regulatory proteins are targeted for degradation by a large complex called the cyclosome (3) or APC (4), which has specific ubiquitin-protein ligase activity. Many subunits of this complex are strongly conserved in evolution (5–8), indicating essential functions. The cyclosome was discovered in extracts of clam oocyctes as the cell cycle-regulated component of the system that ligates cyclin B to ubiquitin (3,9). In such early embryonic cells, the cyclosome is inactive in the interphase and becomes active at the end of mitosis (3,4,9). The activity of the cyclosome is regulated, at least in part, by reversible phosphorylation, as indicated by the observation that phosphatase treatment converts the active, mitotic cyclosome to an inactive form (10). It is not clear, however, whether phosphorylation is the only event involved in the modulation of cyclosome activity. In addition, the regulation of cyclosome action is much more complex in cell cycles of somatic cells or yeast, where the machinery remains active until the end of the G1 phase of the cell cycle (11,12). In early embryonic cells, the activation of the cyclosome is initiated by the major mitotic protein kinase, Cdk1/cyclin B (3,9,13). Thus, the protein kinase triggers its own inactivation by the machinery that degrades its cyclin B subunit. It is notable that a lag period precedes the activation of the cyclosome by Cdk1/cyclin B (3,9,13). This lag may represent a regulatory device that prevents premature self-inactivation of the protein kinase before the end of mitosis. The mechanisms by which Cdk1/cyclin B activates the cyclosome and those re-

[1] To whom correspondence and reprint requests should be addressed. Fax: 9724-853-5573. E-mail: hershko@tx.technion.ac.il.

Abbreviations used: APC, anaphase promoting complex; Cdk, cyclin-dependent kinase; Suc1, suppressor of cdc2; Cks, cyclin kinase subunit; rcm-BSA, reduced carboxymethylated bovine serum albumin; DTT, dithiothreitol; GST, glutathione-*S*-transferase; HEPES, 4-(2-hydroxyethyl)-1-piperazine–ethanesulfonic acid; AMP-PNP, 5′-adenylyl-β,γ-imidodiphosphate; SDS, sodium dodecyl sulfate; E1, ubiquitin-activating enzyme; E2, ubiquitin-carrier protein; MOPS, 3-(*N*-morpholino)propanesulfonic acid.

0006-291X/99 $30.00

sponsible for the lag kinetics of cyclosome activation, remain unknown.

A clue to the possible involvement of an additional component in the process of cyclosome activation was provided by the observation that the active, mitotic form of the cyclosome from clam oocytes binds to Suc1-Sepharose (14). Suc1 (also called Cks) proteins were first identified in yeasts as small (13–18 kDa) proteins that interact with Cdk1 (15–17). They are essential proteins strongly conserved in evolution (reviewed in Refs. 18,19), but their precise functions remained unclear. Suc1 is required at multiple stages of the cell cycle, including the G1/S-phase and G2/M-phase transitions in the budding yeast (20), and exit from mitosis in the fission yeast (21,22). In the latter organism, the arrest in mitosis due to the depletion of Suc1 is accompanied by the inhibition of cyclin B degradation (21,22). *In vitro* studies with immunodepleted extracts of *Xenopus* eggs further indicated that Suc1 is required both for the activation of Cdk1 and for the degradation of cyclin B (23). The crystal structures of Suc1/Cks proteins (24,25) and of their complex with Cdk2 (26) showed that in addition to the Cdk binding site, they contain a highly conserved anion binding site that can bind phosphate or related anions. It has been suggested that due to its two binding sites, Suc1 may direct Cdks to some phosphorylated proteins (23,26). Experimental evidence supporting the notion that Suc1 may indeed associate with certain phosphorylated proteins *via* its phosphate-binding site, was provided by our finding that the mitotic form of the cyclosome can be effectively eluted from Suc1-Sepharose by phosphate-containing compounds (14). These observations were used to develop an affinity procedure for the purification of the cyclosome (14).

In the present work, we show that Suc1 stimulates the activation of the interphase form of the cyclosome by protein kinase Cdk1/cyclin B. We find that the delayed addition of Suc1 abolishes the lag and causes a rapid burst of cyclosome activation. It is suggested that Suc1 may play a role in the lag kinetics of cyclosome activation by facilitation of multiple phosphorylations of partially phosphorylated proteins.

MATERIALS AND METHODS

Chemicals and reagents. Okadaic acid, hexokinase from yeast (450 units/mg of protein) and histone H1 were obtained from Boehringer. Ubiquitin aldehyde was prepared as described (27). E1 was purified from human erythrocytes (28) and recombinant E2-C (clam) was expressed and purified as described (29). Recombinant Suc1 (fission yeast) was expressed in bacteria as described (16) and was purified by gel filtration on Sephadex G-100. The monomeric form of Suc1 was collected and was further purified by chromatography on a Resource Q anion exchange column (Pharmacia) at pH 7.6. Suc1 eluted at around 150 mm NaCl and was essentially homogenous, as judged by gel electrophoresis. Recombinant GST-Δ88-cyclin B1 (human) was expressed as described (30), and was purified by affinity chromatography on glutathione-agarose (Sigma G-4510). Sea urchin cyclin B (13-91)/protein A was expressed, purified and radioiodinated as described (31).

Purification of Cdk1/cyclin B. Cdk1/cyclin B was purified by affinity chromatography on Suc1-Sepharose as described previously (9), with the following modifications. We have used a complex of Cdk1 with recombinant GST-Δ88-cyclin B for the present study, because this complex has high protein kinase activity but lacks the portion of cyclin that contains the "destruction box", and therefore does not interfere with the cyclin-ubiquitin ligation assay. The active complex was formed by the incubation of interphase clam oocycte extracts with GST-Δ88-cyclin B in the presence of ATP and okadaic acid, as described (14). Following sequential affinity purification on Suc1-Sepharose and on GSH-agarose, residual Suc1 was removed by immunodepletion with anti-Suc1 antibodies covalently linked to Protein A-Sepharose, as described (33). To facilitate description, this preparation is referred to as Cdk1/cyclin B in this paper.

Partial purification of the interphase form of the cyclosome. Since the interphase form of the cyclosome does not bind to Suc1-Sepharose (14), we had to use conventional procedures for its partial purification. Extracts of interphase clam embryos, arrested in the two-cell stage with emetine, were prepared and fractionated on DEAE-cellulose as described previously (9). The fraction not adsorbed to the resin, Fraction 1, was extracted with 0.25 M KCl, as described (3). A sample of 6 mg of this preparation was separated on a Superose-6 HR 10/30 column (Pharmacia) under conditions similar to those described earlier for the mitotic form of the cyclosome (10). The fractions were concentrated as described (10), and ^{125}I-cyclin-ubiquitin ligation activity was estimated in 1-μl samples of column fractions in the presence of 50 units/μl of Cdk1/cyclin B and 500 nM Suc1. The peak of enzyme, eluted in fractions 21–23, was collected. Attempts to further purify the interphase form of the cyclosome by a variety of chromatographic procedures (including ion exchange chromatography on MonoS and MonoQ columns and hydrophobic chromatography) resulted in a nearly complete loss of activity. It appears that the interphase form of the cyclosome is less stable than the mitotic form. Therefore, we have used the peak fractions of the Superose-6 column as the source of partially purified interphase cyclosome for the present study.

Assay of cyclin-ubiquitin ligation. The reaction mixture contained in a volume of 10 μl: 40 mM Tris-HCl (pH 7.6), 1 mg/ml rcm-BSA, 1 mM DTT, 5 mM $MgCl_2$, 10 mM phosphocreatine, 50 μg/ml creatine phosphokinase, 50 μM ubiquitin, 1 μM ubiquitin aldehyde, 1 pmol E1, 5 pmole E2-C, 1 μM okadaic acid, 1–2 pmol of ^{125}-I-labeled cyclin B (13–91)/protein A (termed "^{125}I-cyclin" subsequently; $\sim 2 \times 10^5$ cpm) and enzyme source as specified. Following incubation at 18°C for 1 hour, samples were subjected to electrophoresis on a 12.5% polyacrylamide-SDS gel. Results were quantified with a phosphorimager. The amount of radioactivity in all cyclin-ubiquitin conjugates was expressed as the percentage of the total radioactivity in each lane. One unit of activity is defined as that converting 1% of ^{125}I-cyclin to ubiquitin conjugates under the conditions described above.

Determination of protein phosphorylation by Cdk1/cyclin B. Reaction mixtures contained in a volume of 10 μl: 40 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 1 mM DTT, 0.1 mg/ml rcm-BSA, 1 μM okadaic acid, 100 μM [γ-^{32}P]ATP ($\sim$2,000 cpm/pmol), and protein kinase Cdk1/cyclin B, substrates and further additions as indicated in the Figures. Following incubation 18°C for 60 min, reaction products were separated on an 8% polyacrylamide-SDS gel. The gel was washed in 5% (w/v) trichloroacetic acid containing 1% sodium pyrophosphate, 4 times for 20–60 min each, at 4°C with shaking and then was dried and subjected to radioautography or phosphorimager analysis.

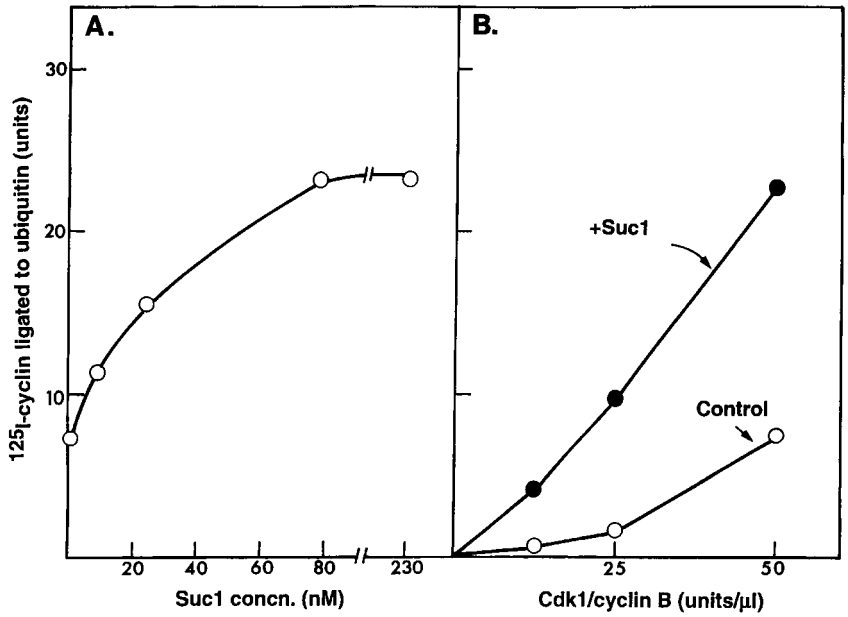

FIG. 1. Suc1 stimulates the activation of interphase cyclosome by Cdk1/cyclin B. Samples of 1 μl of interphase cyclosome preparation were incubated for 90 min at 18°C with the indicated additions in a reaction mixture similar to that described for the assay of ^{125}I-cyclin-ubiquitin ligation (see Materials and Methods). **A.** Effect of concentration of Suc1. All incubations contained 50 units/μl of Suc1-depleted Cdk1/cyclin B and the indicated concentrations of Suc1. **B.** Effect of concentration of Cdk1/cyclin B. Incubations were carried out in the absence (open circles) or presence (filled circles) of 230 nM Suc1. ^{125}I-cyclin-ubiquitin ligation was determined as described under Materials and Methods.

RESULTS

Suc1 stimulates the activation of the of the interphase form of the cyclosome by protein kinase Cdk1/cyclin B. We have first asked whether Suc1 has an influence for the activation of the interphase form of the cyclosome by protein kinase Cdk1/cyclin B. When interphase cyclosome preparation was incubated with Cdk1/cyclin B, some activation of the cyclosome took place in the absence of Suc1. However, cyclosome activation was significantly stimulated by the addition of Suc1 at low, physiological concentrations (Fig. 1A). Half-maximal stimulation was observed with about 30 nM Suc1, which is much lower than the levels observed in Xenopus eggs (~500 nM, ref. 23). The stimulatory effect of Suc1 was more marked at low concentrations of Cdk1/cyclin B (Fig. 1B). The effect of Suc1 was not due to the stabilization of Cdk1/cyclin B during incubation, as indicated by the observation that preincubation of the protein kinase in the presence or absence of Suc1, followed by the subsequent addition of interphase cyclosome, had no further effect on cyclosome activation (data not shown). In different experiments using different enzyme preparations, some variations were observed in the degree of partial activation of the cyclosome that took place in the absence of Suc1. Accordingly, the extent of the additional stimulatory effect of Suc1 varied between 1.5- to 5-fold. However, significant stimulation of cyclosome activation by Suc1 was observed in all experiments.

Our next question was whether Suc1 is indeed involved in the activation of the interphase form of the cyclosome by protein kinase Cdk1/cyclin B, or whether it converts the cyclosome to a catalytically active form following the action of the protein kinase. For example, it is possible that following the phosphorylation of the cyclosome by the protein kinase, the binding of Suc1 to the phosphorylated cyclosome triggers a conformational change that converts the cyclosome to a form of higher enzymatic activity. This problem was examined in the experiments described in Table 1. The experimental design included two stages: in the first incubation, a preparation of interphase cyclosome was incubated with Cdk1/cyclin B, MgATP and the additions indicated. In the second incubation, the indicated additions were supplemented and the reaction of ^{125}I-cyclin-ubiquitin ligation was carried out. This two-stage experimental design allowed to distinguish between the activation of the cyclosome in the first incubation and the assay of cyclin-ubiquitin ligation activity in the second incubation. To terminate protein kinase action, we have used two different agents. One is staurosporine, a potent inhibitor of many protein kinases, including Cdk1/cyclin B (34). The other was a mixture of hexokinase, 2-deoxyglucose and AMP-PNP, a β-γ non-hydrolyzable analogue of ATP ("hexokinase mix"). Hexokinase efficiently traps ATP, but it cannot act on AMP-PNP. This non-hydrolyzable analogue cannot replace ATP in the protein kinase reaction, but it

TABLE 1

Suc1 Does Not Stimulate the Catalytic Activity of the Cyclosome following Activation by Cdk1/cyclin B

Additions		^{125}I-cyclin ligated to ubiquitin, units
Incubation 1	Incubation 2	
A.		
None	None	36.3
Suc1	None	47.7
Staurosporine	None	0
Hexokinase mix	None	0
B.		
None	Staurosporine	33.1
None	Hexokinase mix	28.2
None	Staurosporine + Suc1	32.2
None	Hexokinase mix + Suc1	36.8
C.		
Suc1	Staurosporine	46.5
Suc1	Hexokinase mix	49.0

Note. In incubation 1, samples of interphase cyclosome (1 μl) were incubated with Cdk1/cyclin B (25 units/μl) for 2 hours at 18°C, in a reaction mixture similar to that described for the assay of cyclin-ubiquitin ligation (see Materials and Methods), except that ^{125}I-cyclin and E1 were omitted. In incubation 2, ^{125}I-cyclin and E1 were added, and incubation was continued for a further 2 hours at 18°C. Where indicated, 380 nM Suc1 or 5 μM staurosporine was added. The "Hexokinase mix" consisted of 10 μg/ml hexokinase, 20 mM 2-deoxyglucose, and 2 mM AMP-PNP.

can do so in the E1 reaction (required for cyclin-ubiquitin ligation), because the latter reaction involves the scission of the α-β bond of ATP (35). As shown in Table 1, Part A, the addition of staurosporine or hexokinase mix in the first incubation effectively prevented the activation of the interphase cyclosome by protein kinase Cdk1/cyclin B. On the other hand, when the same agents were added in the second incubation, considerable activity of cyclin-ubiquitin ligation was observed (Table 1, Part B). These findings suggest that staurosporine and hexokinase mix prevent the activation of the interphase cyclosome, but do not interfere with the cyclin-ubiquitin ligation reaction following the activation process. When Suc1 was added in the second incubation together with staurosporine or hexokinase mix, there was no significant stimulation of cyclin-ubiquitin ligation activity (Table 1, Part B). By contrast, when Suc1 was added in the first incubation and staurosporine or hexokinase mix were added in the second incubation, there was a 1.5-fold increase in activity as compared to a similar incubation without Suc1 (Table 1, compare Parts B and C). It should be noted that in this experiment, there was considerable activation of cyclosome in the absence of Suc1, resulting in relatively low (1.5-fold) further stimulation when Suc1 was added in the first incubation. Still, the lack of any significant effect of Suc1 when added in the second incubation in the presence of protein kinase inhibitors, clearly indicates that it has no influence on the enzymatic activity of the cyclosome, following the activation process. It rather appears that Suc1 stimulates the activation of the interphase form of the cyclosome by protein kinase Cdk1/cyclin B.

Influence of Suc1 on the different phases of cyclosome activation. In the experiments described above, the effects of Suc1 were examined at a single time point of incubation. Thus, the results represented the cumulative formation of cyclin-ubiquitin conjugates in the various phases of the activation of the cyclosome, that include the initial lag, followed by a period of rapid activation. To gain some insight into the question of whether Suc1 affects the initial lag, the subsequent phase of activation, or both, we have examined the effects of Suc1, added at different times of incubation of interphase cyclosome with Cdk1/cyclin B, on the kinetics of the activation process. For accurate estimation of the kinetics of activation, we have used the above-described two-stage assay. First, cyclosome activation was carried out in different experimental conditions, and at various times, cyclosome activation was terminated by the addition of staurosporine. Subsequently, ^{125}I-cyclin-ubiquitin ligation assay was carried out for an identical time in all samples. As shown in Fig. 2, in the absence of Suc1 ("Control"), activation of the cyclosome took place slowly. The addition of Suc1 from the start of the incubation considerably accelerated the

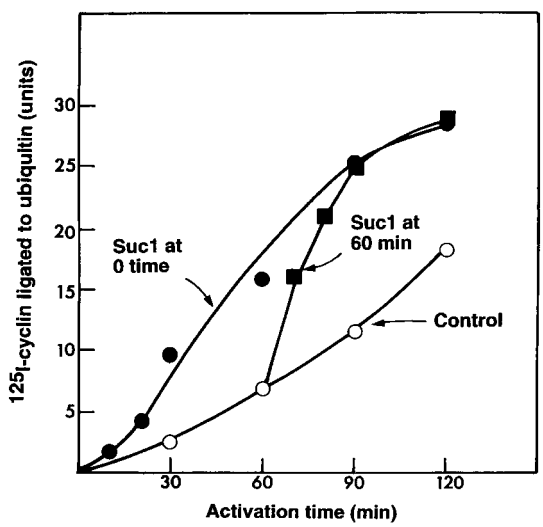

FIG. 2. Effects of Suc1 on the kinetics of cyclosome activation. Samples of 1 μl of interphase cyclosome preparation were incubated with Suc1-depleted Cdk1/cyclin B (25 units/μl) in a reaction mixture similar to that described for the assay of ^{125}I-cyclin-ubiquitin ligation (see "Experimental Procedures") except that ^{125}I-cyclin was omitted. Suc1 (380 nM) was added either at time zero (filled circles) or after 60 min (filled squares). Control samples (open circles) were incubated without Suc1. At the times indicated in the Figure, activation of the cyclosome was stopped by the addition of staurosporine (5 μM). Subsequently, ^{125}I-cyclin was added and all samples were incubated for 2 hours at 18°C for the determination of cyclin-ubiquitin ligation, as described under Materials and Methods.

rate of cyclosome activation. However, an initial lag of about 10 min remained, even though Suc1 was added at a large excess. This lag kinetics of cyclosome activation confirmed our previous observations with less precise assays (3,9). To examine the possible effect of Suc1 on the second phase of the activation, interphase cyclosome was first incubated with protein kinase Cdk1/cyclin B for 60 min, and subsequently Suc1 was added. Following the delayed addition of Suc1, a rapid burst of cyclosome activation took place, without any measurable lag (Fig. 2). We conclude that Suc1 accelerates the second phase of cyclosome activation that follows the initial lag. These results are compatible with the interpretation that following initial partial phosphorylation, Suc1 accelerates further phosphorylations by protein kinase Cdk1/cyclin B (see "Discussion").

Effects of Suc1 on phosphorylation of proteins by Cdk1/cyclin B. Our results on the influence of Suc1 to accelerate cyclosome activation by protein kinase Cdk1/cyclin B raised the question of whether Suc1 stimulates some phosphorylation event required for this process. It has been reported previously that Suc1 has no influence on the protein kinase activity of Cdk1/cyclin B (21,36). In these studies, histone H1 has been used as the substrate for the protein kinase. Using our presently employed experimental conditions, we have confirmed that Suc1 has no detectable effect on the phosphorylation of histone H1 by Cdk1/cyclin B (Fig. 3A). By contrast, when the protein kinase was incubated with a partially purified preparation of interphase cyclosome, the phosphorylation of several proteins was noticeably stimulated by Suc1 (Fig. 3B). Without added Suc1, many proteins in the partially purified preparation were phosphorylated by Cdk1/cyclin B (Fig. 3B, lane 4). The addition of Suc1 stimulated the phosphorylation of several proteins (indicated by asterisks next to lane 5 in Fig. 4B), most markedly three of apparent molecular mass of approximately 80, 125 and 165 kDa. It is notable that the increased phosphorylation of these proteins by Suc1 was accompanied by retardation of their electrophoretic migration, as compared to the corresponding proteins phosphorylated in the absence of Suc1. The phosphorylation of several proteins in the molecular mass region of approx. 200-300 kDa was also stimulated by Suc1. On the other hand, Suc1 had no influence on the phosphorylation of some other proteins in the interphase cyclosome preparation. It thus seems that that Suc1 affects specifically the phosphorylation of some proteins and not of some others. Because of the necessary use of partially purified interphase cyclosome preparations (see "Materials and Methods") and the lack of antibodies directed against subunits of the clam cyclosome, it was not possible to determine with certainty which phosphorylated subunits are actually subunits of the clam cyclosome. However, while this work was in progress, Patra and Dunphy (38) have reported that Suc1 stimulates the phosphorylation of the Cdc27 and BIME subunits of the *Xenopus* cyclosome/APC by Cdk1/cyclin B. It thus appears reasonable to assume that at least some of the phosphorylated proteins seen in our preparation are subunits of the clam cyclosome.

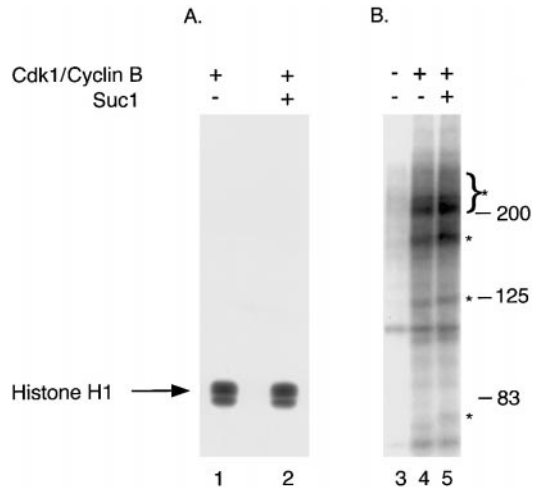

FIG. 3. Stimulatory effect of Suc1 on the phosphorylation of some proteins in **interphase** extracts by Cdk1/cyclin B. Reaction conditions of protein phosphorylation were as described under Materials and Methods, except that incubation was carried out for 60 min. Where indicated, 25 units/μl of Cdk1/cyclin B or 380 nM Suc1 was added. **A.** Phosphorylation of histone H1. The reaction was carried out in the presence of 0.1 μg/μl histone H1, and the products were separated on a 12.5% polyacrylamide–SDS gel. **B.** Phosphorylation of proteins in the preparation of interphase cyclosome. 1 μl of interphase cyclosome preparation was added to all incubations. Reaction products were separated on an 8% polyacrylamide–SDS gel. Numbers on the right indicate the position of molecular mass markers in the latter gel. Asterisks indicate proteins whose phosphorylation is augmented by Suc1.

We have next asked whether the effect of Suc1 to stimulate protein phosphorylation is enhanced by prior incubation with protein kinase Cdk1/cyclin B, as observed for cyclosome activation in the above-described experiments. In the experiment shown in Fig. 4, interphase cyclosome preparation was preincubated (or not) with protein kinase Cdk1/cyclin B and unlabeled ATP, to partially phosphorylate proteins. Subsequently, [γ-^{32}P]ATP was added in the presence or absence of Suc1, and the time-course of the incorporation of [^{32}P]-phosphate into the ~165-kDa protein was followed. This protein was selected for quantitation, because it was best resolved from other phosphorylated proteins (cf. Fig. 3B). The results were expressed as the relative incorporation of [^{32}P]-phosphate, with the 60-min control value taken as unity. It may be seen that without preincubation, Suc1 stimulated only slightly protein phosphorylation at early times of incubation, but stimulation increased at later times of incubation. Following preincubation (that causes partial phosphoryla-

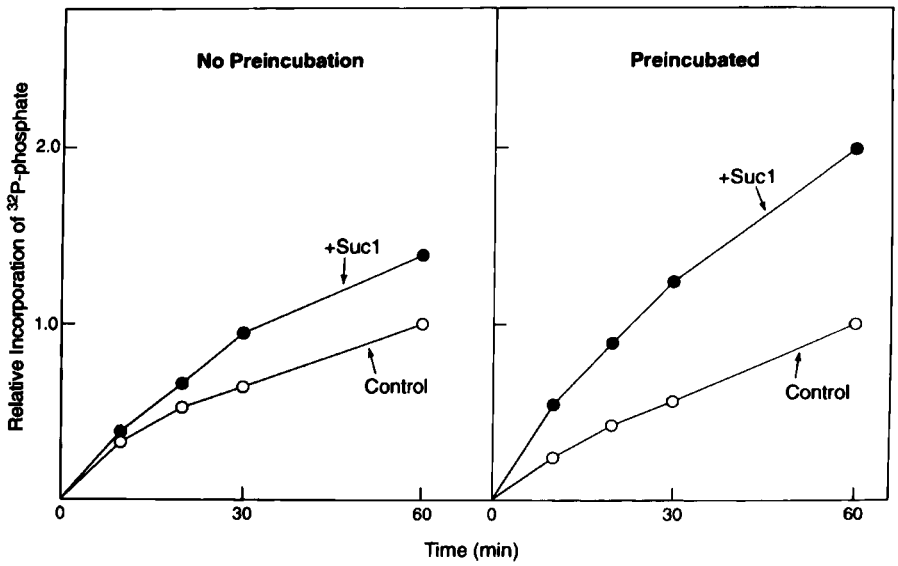

FIG. 4. Effect of Suc1 on the kinetics of protein phosphorylation. Reaction mixtures contained interphase cyclosome and Cdk1/cyclin B as described under Fig. 3B, except that samples were either not preincubated (left) or preincubated (right) with a similar mixture that contained 50 μM unlabeled ATP. Subsequently, [γ-^{32}P]ATP and Suc1 (where indicated, 380 nM) were added. The final concentration of ATP was 100 μM and the specific radioactivity was 2000 cpm/pmol in all incubations. Incubation was continued at 18°C for the time periods indicated and the incorporation of ^{32}P-phosphate into the ~165-kDa protein was quantitated by phosphorimager analysis. Results were expressed relative to the values of the control incubations at 60 min. These were 9542 and 3724 PSL for the "No preincubation" and "Preincubated" treatments, respectively. Open circles ("Control"), without Suc1; closed circles, with Suc1.

tion), the stimulatory effect of Suc1 on [^{32}P]-phosphate incorporation was greatly increased, and was prominent already at early times of incubation (Fig. 4). These quantitative kinetic data are consistent with the notion that Suc1 facilitates the multiple phosphorylation of partially phosphorylated proteins (see "Discussion").

DISCUSSION

This study was initiated by the observation that the active, phosphorylated form of the cyclosome binds to Suc1-Sepharose and that this interaction is mediated by the phosphate-binding site of Suc1 (14). It appeared possible that due to the binding of Suc1 to both the cyclosome and Cdk1, it may be involved in the activation of the cyclosome by Cdk1/cyclin B. This notion is in line with the observed requirement of cyclin B degradation for Suc1 both in yeast *in vivo* (21,22) and in *Xenopus* extracts *in vitro* (23). However, it could not be concluded from these studies whether Suc1 affects the cyclin degradation machinery indirectly or directly. In the present study, we have found that low, physiological concentrations of Suc1 stimulated the activation of the interphase form of the cyclosome by protein kinase Cdk1/cyclin B (Fig. 1) and that following activation, Suc1 has no influence on the catalytic efficiency of the mitotic form of the cyclosome (Table 1). Most significantly, the delayed addition of Suc1 after a prior incubation of interphase cyclosome with Cdk1/cyclin B, caused a rapid activation of the cyclosome and abolished the lag kinetics of this process (Fig. 2). One possible interpretation of these results is that the binding of Suc1 to both the cyclosome and Cdk1 may increase the affinity of Cdk1 to the partially phosphorylated cyclosome and thus accelerate multiple phosphorylations required for full activation of the cyclosome. Such sequence of events may explain, at least in part, the lag kinetics of cyclosome activation. In line with this interpretation, we find that the phosphorylation of some proteins in the preparation of interphase cyclosome is accelerated by Suc1, accompanied by retardation of their electrophoretic migration (Fig. 3), and that the effect of Suc1 on protein phosphorylation is markedly augmented by preincubation with Cdk1/cyclin B (Fig. 4).

While this work was in progress, Dunphy and Patra (38) have reported that in extracts of *Xenopus* eggs, a Suc1/Cks protein is required for the activation of the degradation of cyclin B and for the hyperphosphorylation of the several subunits of the cyclosome/APC by protein kinase Cdk1/cyclin B. These findings are in agreement with our results, though the influence of Suc1 on the lag kinetics of cyclosome activation was not addressed in their study. The combined information from both studies strongly suggest that Suc1-assisted multiple phosphorylations may have a role in the delayed activation of the cyclosome at the end of mitosis.

ACKNOWLEDGMENTS

We thank Valery Sudakin for participating in the early stages of this work, Clara Segal for skillful technical assistance, and the

collecting staff of the Marine Resources Facility at the Marine Biological Laboratory, Woods Hole, MA, for their expert help in the collection and maintenance of clams. This work was supported by grants from the United States-Israel Science Foundation, the Israel Science Foundation, and the Ministry of Science and Arts, Israel.

REFERENCES

1. Hershko, A. (1997) *Curr. Opin. Cell Biol* **9,** 788–799.
2. King, R. W., Deshaies, R. J., Peters, J.-M., and Kirschner, M. W. (1996) *Science* **274,** 1652–1659.
3. Sudakin, V., Ganoth, D., Dahan, A., Heller, H., Hershko, J., Luca, F. C., Ruderman, J. V., and Hershko, A. (1995) *Mol. Biol. Cell* **6,** 185–198.
4. King, R. W., Peters, J.-M., Tugendreich, S., Rolfe, M., Hieter, P., and Kirschner, M. W. (1995) *Cell* **81,** 279–288.
5. Zachariae, W., Shin, T. H., Galova, M., Obermeier, B., and Nasmyth, K. (1996) *Science* **274,** 1201–1204.
6. Peters, J.-M., King, R. W., Höög, C., and Kirschner, M. W. (1996) *Science* **274,** 1199–1201.
7. Zachariae, W., Shevchenko, A., Andrews, P. D., Ciosk, R., Galova, M., Stark, M. J. R., Mann, M., and Nasmyth, K. (1998) *Science* **279,** 1216–1219.
8. Yu, H., Peters, J.-M., King, R. W., Page, A. M., Hieter, P., and Kirschner, M. W. (1998) *Science* **279,** 1219–1222.
9. Hershko, A., Ganoth, D., Sudakin, V., Dahan, A., Cohen, L. H., Luca, F. C., Ruderman, J. V., and Eytan, E. (1994) *J. Biol. Chem.* **269,** 4940–4946.
10. Lahav-Baratz, S., Sudakin, V., Ruderman, J. V., and Hershko, A. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 9303–9307.
11. Amon, A., Irniger, S., and Nasmyth, K. (1994) *Cell* **77,** 1037–1050.
12. Brandeis, M., and Hunt, T. (1996) *EMBO J.* **15,** 5280–5289.
13. Félix, M.-A., Labbé , J.-D., Dorée, M., Hunt, T., and Karsenti, E. (1990) *Nature* **346,** 379–382.
14. Sudakin, V., Shteinberg, M., Ganoth, D., Hershko, J., and Hershko, A. (1997) *J. Biol. Chem.* **272,** 18051–18059.
15. Hayles, J., Beach, D., Durkatz, B., and Nurse, P. (1986) *Mol. Gen. Genet.* **202,** 291–293.
16. Brizuela, L., Draetta, G., and Beach, D. (1987) *EMBO J.* **6,** 3507–3514.
17. Hadwiger, J. A., Wittenberg, C., Mendenhall, M. D., and Reed, S. I. (1989) *Mol. Cell. Biol.* **9,** 2034–2041.
18. Endicott, J. A., and Nurse, P. (1995) *Structure* **3,** 321–325.
19. Pines, J. (1996) *Curr. Biol.* **6,** 1399–1402.
20. Tang, Y. and Reed, S. I. (1993) *Genes Dev.* **7,** 822–832.
21. Moreno, S., Hayles, J., and Nurse, P. (1989) *Cell* **58,** 361–372.
22. Basi, G., and Draetta, G. (1995) *Mol. Cell. Biol.* **15,** 2028–2036.
23. Patra, D., and Dunphy, W. G. (1996) *Genes Dev.* **10,** 1503–1515.
24. Parge, H. E., Arvai, A. S., Murtari, D. J., Reed, S. I., and Tainer, J. D. (1993) *Science* **262,** 387–395.
25. Endicott, J. A., Noble, M. E., Garman, E. F., Brown, N., Rasmussen, B., Nurse, P., and Johnson, L. N. (1995) *EMBO J.* **14,** 1004–1014.
26. Bourne, Y., Watson, H. M., Hickey, M. J., Holmes, W., Rocque, W., Reed, S. I., and Tainer, J. A. (1996) *Cell* **84,** 863–874.
27. Mayer, A. N., and Wilkinson, K. D. (1989) *Biochemistry* **28,** 166–172.
28. Hershko, A., Heller, H., Elias, S., and Ciechanover, A. (1983) *J. Biol. Chem.* **258,** 8206–8214.
29. Townsley, F. M., Aristarkhov,A., Beck, S., Hershko, A., and Ruderman, J. (1997). *Proc. Natl. Acad. Sci. USA.* **94,** 2362–2367.
30. Zheng, X.-F., and Ruderman, J. V. (1983) *Cell* **75,** 155–164.
31. Glotzer, M., Murray, A. W., and Kirschner, M. W. (1991) *Nature* **349,** 132–138.
32. Labbé, J.-D., Cavadore, J.-C., and Dorée, M. (1991) *Methods Enzymol.* **200,** 291–301.
33. Harlow, E., and Lane, D. (1988) *Antibodies: A Laboratory Manual,* pp. 521–523, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
34. Gadbois, D. M., Hamaguchi, J. R., Swank, R. A., and Bradbury, E. M. (1992) *Biochem. Biophys. Res. Commun.* **184,** 80–85.
35. Hershko, A., and Ciechanover, A. (1992). *Annu. Rev. Biochem.* **61,** 761–807.
36. Brizuela, L., Draetta, G., and Beach, D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4362–4366.
37. Langan, T. A. (1978) *Methods Cell Biol.* **19,** 127–142.
38. Patra, D., and Dunphy, W. G. (1998). *Genes Dev.* **12,** 2549–2559.