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ALTERATION OF THE PHOSPHORYLATION STATE OF p34^{cdc2} KINASE BY THE FLAVONE L86-8275 IN BREAST CARCINOMA CELLS

CORRELATION WITH DECREASED H1 KINASE ACTIVITY

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Abstract—The flavone L86-8275 [(−)*cis*-5,7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl)-piperidinyl]-4*H*-1-benzopyran-4-one] delayed the progression of aphidicolin-synchronized MDA-468 breast carcinoma cells through S phase and prevented progression through G_2. L86-8275 prevented the G_2-related increase in histone H1 kinase activity mediated by cyclin-dependent kinase-1 (p34^{cdc2} kinase). L86-8275 inhibited [^{32}P]orthophosphate labeling of p34^{cdc2} threonine and tyrosine residues and decreased the phosphotyrosine content of p34^{cdc2}. Diminution of p34^{cdc2} phosphotyrosine appeared selective, as a general depletion of cellular phosphotyrosine was not observed. The mass of p34^{cdc2} in L86-8275-exposed cells was not decreased during the period over which these effects occurred. [^{35}S]Methionine labeling of p34^{cdc2} or other cellular proteins was not inhibited at concentrations that were effective for complete cellular growth inhibition. We hypothesize that L86-8275 interferes with the normal cell cycle-dependent phosphorylation of p34^{cdc2}, resulting in decreased kinase activity and cell cycle arrest.

We have shown recently that L86-8275, a flavone containing chlorophenyl and (3-hydroxy-1-methyl)-piperidinyl substitutions [1, 2], is a significantly more potent inhibitor of tumor cell growth (IC_{50} = 25–160 nM) than either the polyhydroxyl substituted flavone quercetin or the isoflavone genistein [3]. In these studies, L86-8275 was shown to block cell cycle progression at either G_1 or G_2, depending on the method of cell synchronization. The experiments reported herein address the mechanism of G_2 block produced by L86-8275.

Control of cell cycle progression is maintained by complex coordinated kinase and phosphatase reactions [4–8]. An enzyme central to cell cycle progression through G_2 into M phase is the cyclin-dependent kinase-1 (p34^{cdc2} kinase)‖ [9, 10]. During S phase, increased synthesis of cyclin B occurs and a complex is formed between p34^{cdc2} and cyclin B [11, 12]. p34^{cdc2} is phosphorylated at four specific sites: Ser^{277}, Thr^{14}, Tyr^{15} and Thr^{161} [13], and three of these are known to have functional significance. Thr^{14} and Tyr^{15} within the ATP binding site are phosphorylated when the complex is formed and serve as negative regulators of kinase activity until mitosis, when specific phosphatase activity dephosphorylates these residues [14–16]. Phosphorylation on Thr^{161} of p34^{cdc2} is necessary for p34^{cdc2} kinase activity [17, 18].

In the present study, we demonstrated that an early consequence of the exposure of aphidicolin-synchronized breast carcinoma cells to L86-8275 is inhibition of the normal cell cycle regulated phosphorylation of p34^{cdc2} kinase during S phase. Both threonine and tyrosine phosphorylation were affected. In addition, decreased immunoprecipitated p34^{cdc2} histone H1 kinase activity was found in the L86-8275-treated cells compared with the control cells. We hypothesize that L86-8275 prevented the development of the required kinase activity necessary to drive the MDA-468 breast carcinoma cells into mitosis.

MATERIALS AND METHODS

Cell synchronization and [^{32}P]*orthophosphate labeling.* Exponentially growing MDA-468 (obtained from R. Lupu, Georgetown University, Washington, DC) breast carcinoma cells (approximately 70% confluent) in improved minimal essential medium (IMEM) with 5% fetal bovine serum (FBS), 2 mM glutamine, and 100 U/mL each of penicillin and streptomycin were synchronized at the beginning of the S phase by a 12-hr exposure to 4 μg/mL aphidicolin. Cells were then rinsed three times with drug-free medium and placed in IMEM containing 5% FBS at 37° with or without 200 nM L86-8275 or other drugs for the period indicated in the figure legends. Flow cytometry to determine DNA content was performed as previously described [3]. Cells

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‖ Abbreviations: DTT, dithiothreitol; EGTA, ethylenebis(oxyethylene nitrilo) tetraacetic acid; FBS, fetal bovine serum; IC_x: concentration of drug which inhibits cell growth by x%; IMEM, improved minimal essential medium; p34^{cdc2} kinase, cyclin-dependent kinase 1; PAGE, polyacrylamide gel electrophoresis; and PBS, 0.015 M sodium phosphate, pH 7.4, 0.15 M NaCl.

were labeled with [^{32}P]orthophosphate by rinsing once with phosphate-free IMEM medium containing 5% dialyzed FBS, followed by the addition of the same medium with 800 μCi or carrier-free [^{32}P]-orthophosphate with or without appropriate drugs for 2 hr. Cells were rinsed three times with 0.015 M sodium phosphate, pH 7.4, 0.15 M NaCl (PBS) and harvested after the addition of 650 μL of lysis buffer [10 mM sodium phosphate buffer, pH 7.5, 1% Triton X-100, 0.5% deoxycholate, 100 mM NaCl, 0.1% sodium azide, 1 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride, 20 μg/mL aprotinin, 20 μg/mL leupeptin and 50 μg/mL *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK)]; then the cells were briefly vortexed and centrifuged (10,000 *g*, 15 min), retaining the supernatant. A 10 or 50 μg protein sample was used for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

[^{35}S]*Methionine labeling.* At set times after removal of aphidicolin and the initiation of different drug treatments, but 3 hr prior to harvesting, MDA-468 cells were washed twice with 6 mL of methionine- and cysteine-free medium, followed by 6 mL of the same medium containing dialyzed FBS and the same drugs (L86-8275, emetine or cycloheximide). Cells were then labeled with 50 μCi [^{35}S]methionine (sp. act. 1100 Ci/mmol)/mL medium.

[^{32}P]*Orthophosphate uptake.* Cells released from aphidicolin block into medium with or without 200 nM L86-8275 were labeled with 800 μCi [^{32}P]-orthophosphate in phosphate-free medium in the presence or absence of L86-8275 as appropriate for the last hour of a 1-, 2-, 4-, 6- or 8-hr period. Cells were washed three times with cold PBS, pH 7.4, and lysed with 750 μL of 3.5% $HClO_4$; the lysate was centrifuged at 10,000 *g* for 15 min. An aliquot of the supernatant was removed for quantitation of total [^{32}P]phosphate.

Immunoprecipitation and immunoblotting. For immunoblotting, proteins were transferred to Immobilon-P® in 10 mM 3-[cyclohexylamino]-1-propanesulfonic acid (pH 11.0), 10% methanol at 0.5 A for 2.75 hr at 8°. Phosphotyrosine was detected by western blotting with a mouse monoclonal anti-phosphotyrosine antibody (No. 05-321, UBI, Lake Placid, NY). Co-incubation of the blot with 5 mM phosphotyrosine prevented detection of phosphotyrosine. Either a polyclonal rabbit PSTAIRE antiserum raised against the synthetic peptide EGVPSTAIREISLLKE or 3.6 μg of an affinity-purified polyclonal rabbit antibody raised against the C-terminal of p34^{cdc2} (No. 06-194, UBI, or No. 3398SA, GIBCO BRL, Grand Island, NY) was used for immunoprecipitation from 800 μg of whole cell lysate. Western blotting was done with antiserum raised against the conserved PSTAIRE motif of p34^{cdc2}-like proteins with alkaline phosphatase detection and with a monoclonal anti-phosphotyrosine antibody (UBI) or an anti-cyclin-B antibody (No. C-55, Santa Cruz Biotechnology, Inc., CA) followed by [^{125}I]protein A detection.

Phosphoamino acid analysis of immobilized proteins was done as previously described [19, 20].

Histone H1 kinase assay. C-Terminal p34^{cdc2} immunoprecipitates were resuspended in 40 μL of assay buffer [20 mM Tris–HCl, pH 7.5, 0.1 mM ethylenebis(oxyethylene nitrilo) tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 10 mM $MgCl_2$], and 5 μL of a histone H1 solution (1 mg/mL solution in 200 mM NaCl, 0.1 mM EGTA, 1 mM DTT, 20 mM Tris–HCl, pH 7.5, and 10 μg/mL of both leupeptin and aprotinin) and 5 μL of a 3-mM ATP solution containing 3.5 μCi [γ-^{32}P]ATP were added. Blanks were established by addition of buffer instead of histone H1. Kinase reactions proceeded for 15 min at 25° and were stopped by addition of 15 μL of 5X SDS–PAGE loading buffer. After SDS–

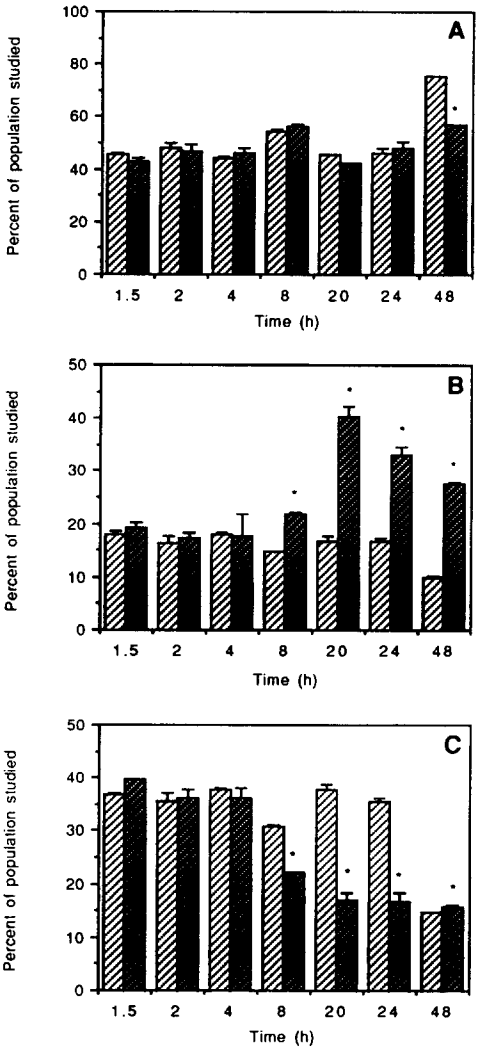
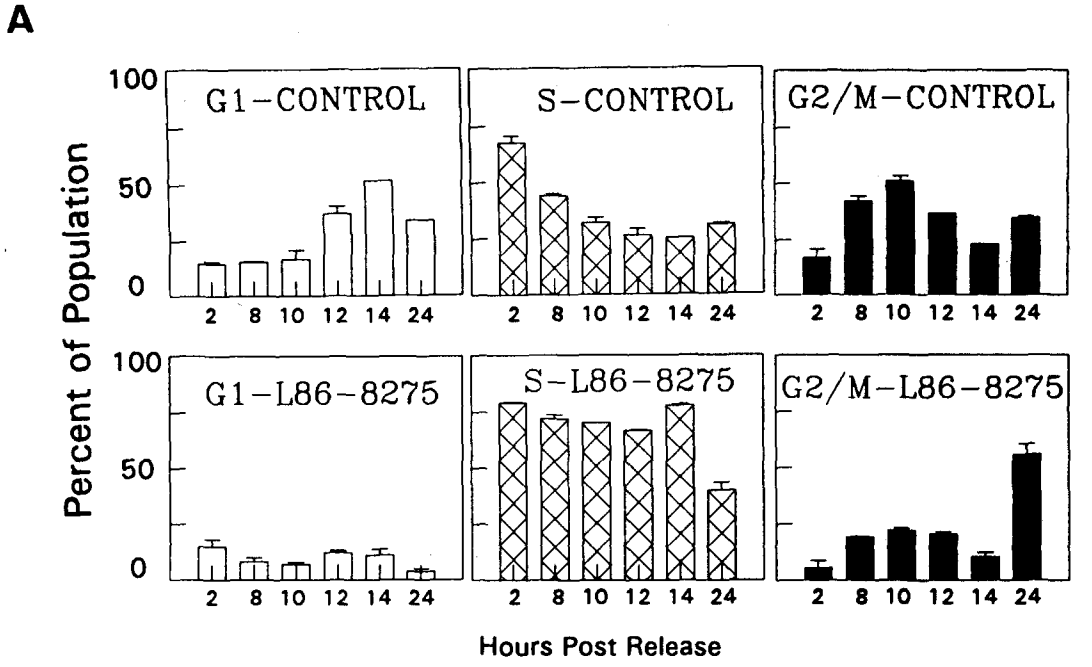
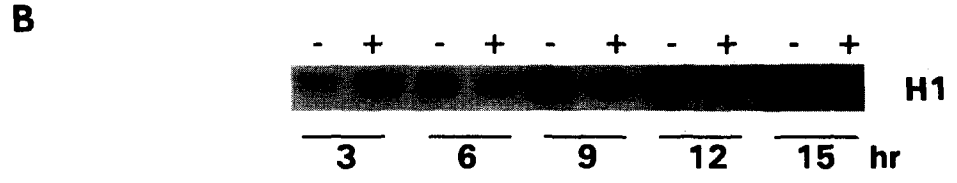


Fig. 1. Cell cycle distribtion of L86-8275 in asynchronous cultures. Asynchronous MDA-468 breast carcinoma cells received medium alone (left-hand bars) or 125 nM L86-8275 (right-hand bars) for different time periods, and the fraction of cells in the population with G_1 (panel A), G_2/M (panel B), or S (panel C) phase DNA content was calculated from DNA histograms obtained by flow cytometry as described in Ref. 3. Data are presented as means ± SEM, N = 4. The asterisks denote time points where the difference between control and L86-8275-treated cultures was significant at $P < 0.05$ (Student's *t*-test).





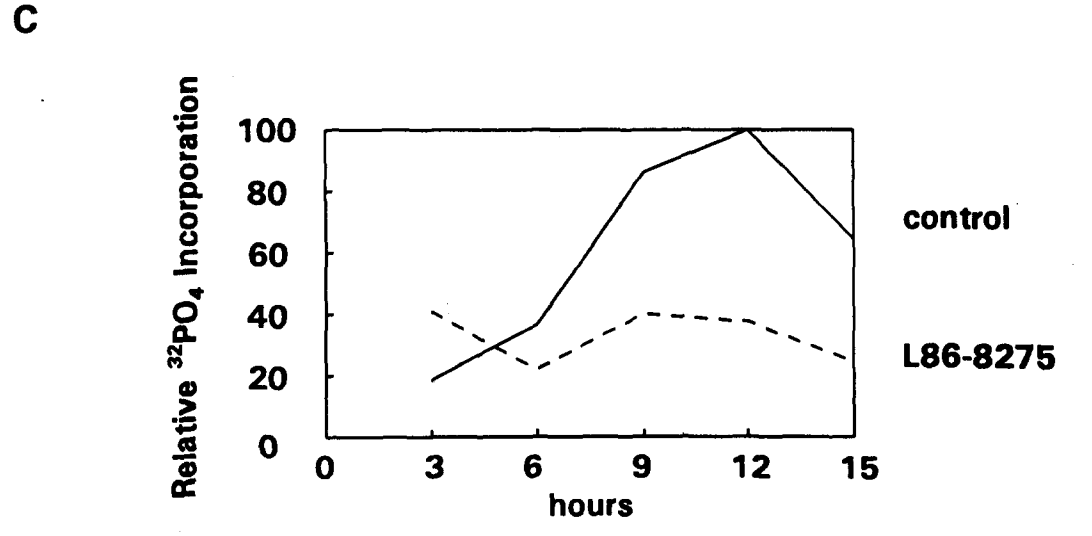


Fig. 2. Effect of L86-8275 on cell cycle progression and development of H1 kinase activity in MDA-468 breast carcinoma cells. (A) Relative number of cells with G_1 (□), S (⊠), or G_2/M (■) DNA content at 2, 8, 10, 12, 14 and 24 hr after release from aphidicolin block into medium with or without 200 nM L86-8275. All panels show the mean ± range from two replicates of two independent experiments. (B) H1 kinase activity of p34^{cdc2} immunoprecipitates derived with antiserum raised against the C-terminal of p34^{cdc2}. Aphidicolin-synchronized MDA-468 cells were released into medium with or without 200 nM L86-8275. At 3, 6, 9, 12 and 15 hr of exposure, immunoprecipitates were obtained and assayed for H1 kinase activity. (C) Quantitation of radioactivity in panel B expressed as a percentage of the maximum value. Key: untreated controls (——), and L86-8275-exposed cells (– – –).

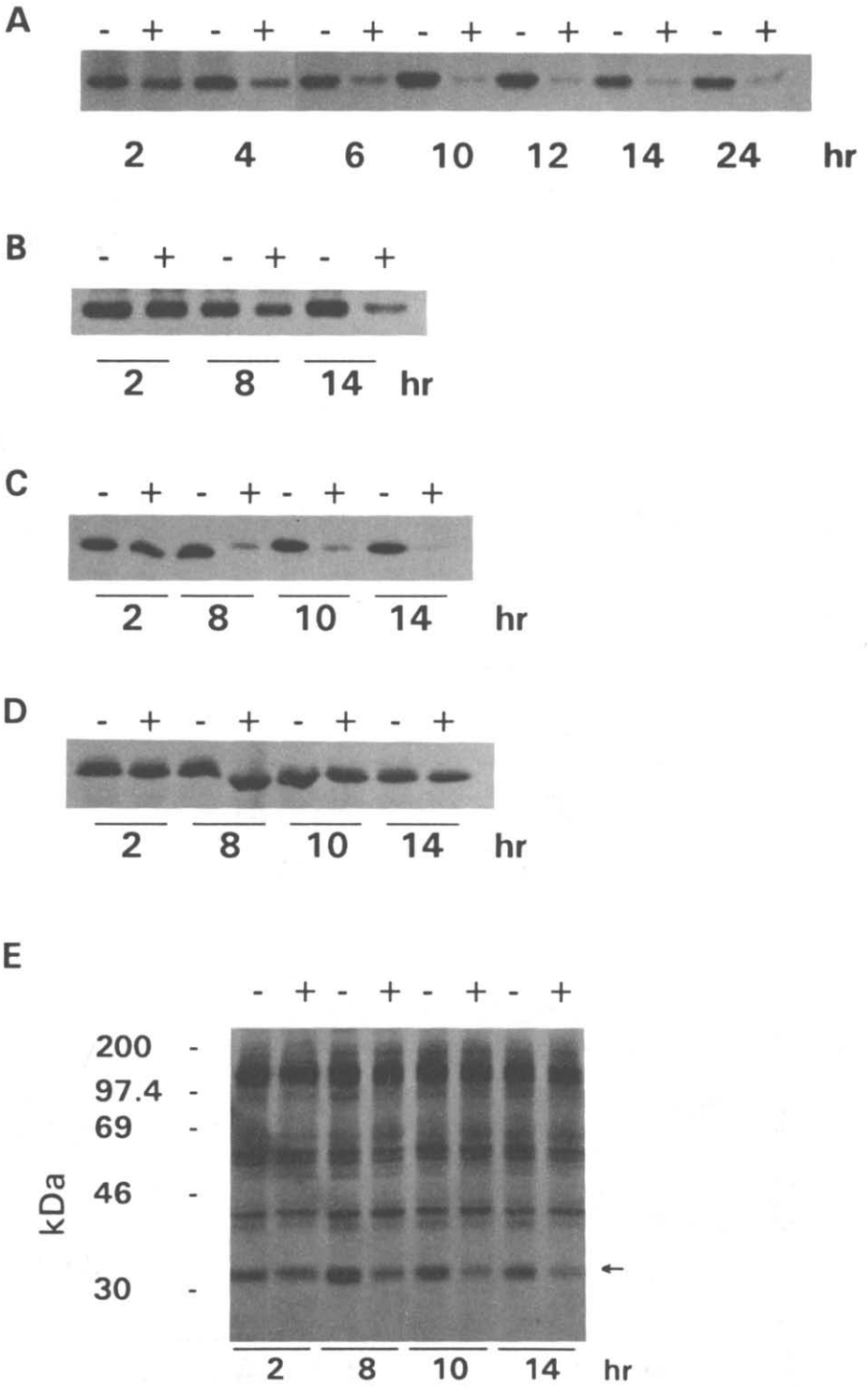


Fig. 3. Effect of L86-8275 (200 nM) on the phosphorylation of $p34^{cdc2}$ and PSTAIRE containing proteins in aphidicolin-synchronized MDA-468 cells. Samples were obtained at 2, 4, 6, 10, 12, 14 and 24 hr from MDA-468 breast carcinoma cells after release from aphidicolin block into medium with or without 200 nM L86-8275. (A) [^{32}P]Orthophosphate-labeled $p34^{cdc2}$ immunoprecipitates, derived with C-terminal specific antiserum. (B) [^{32}P]Orthophosphate-labeled PSTAIRE immunoprecipitates, derived with antiserum raised against the conserved PSTAIRE motif of CDK proteins. (C) Phosphotyrosine western blot showing phosphotyrosine content of PSTAIRE immunoprecipitates. (D) Western blot using PSTAIRE specific antiserum of PSTAIRE immunoprecipitates. (E) Phosphotyrosine western blot of total cellular proteins from cells with or without 200 nM L86-8275 for 2, 8, 10 and 14 hr after release from aphidicolin synchronization (the 34 kDa band is indicated by an arrow).

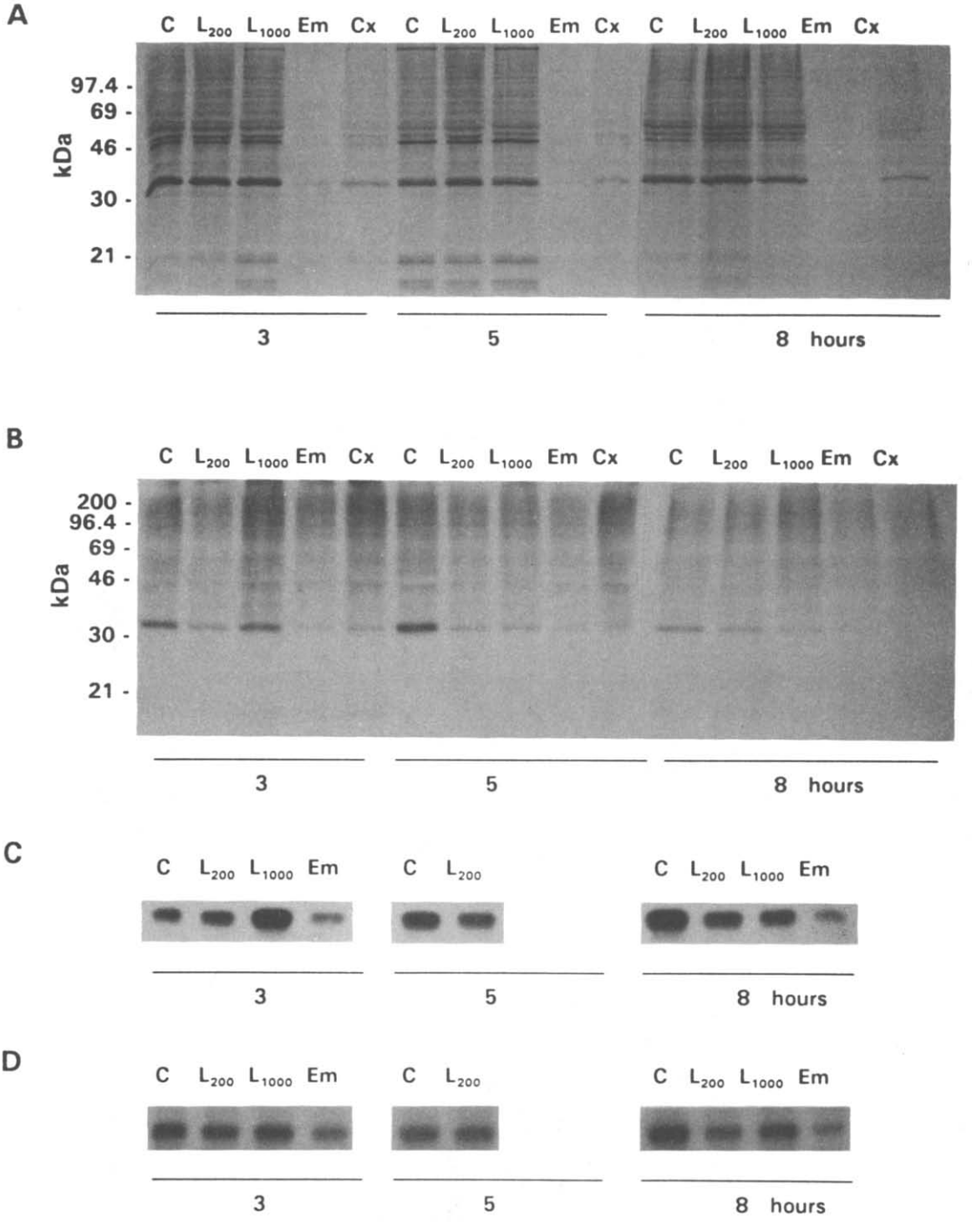


Fig. 4. Synthesis, phosphorylation, and kinase activity of p34^{cdc2} immunoprecipitates extracted from aphidicolin-synchronized MDA-468 cells. (A) [^{35}S]Methionine-labeled p34^{cdc2} immunoprecipitates. (B) [^{32}P]Orthophosphate-labeled p34^{cdc2} immunoprecipitates. (C) H1 kinase activity of p34^{cdc2} immunoprecipitates. (D) H1 kinase activity of cyclin-B immunoprecipitates. Immunoprecipitates were obtained from cells released from aphidicolin block with C, no drug; L_{200}, 200 nM L86-8275; L_{1000} 1000 nM L86-8275; Em, 2 μM emetine; and Cx, 10 μM cycloheximide for 3, 5, and 8 hr.

PAGE radioactive bands were visualized by autoradiography, they were quantified by densitometry with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

RESULTS AND DISCUSSION

The effect of L86-8275 on the cell cycle distribution of asynchronously growing MDA-468 breast car-

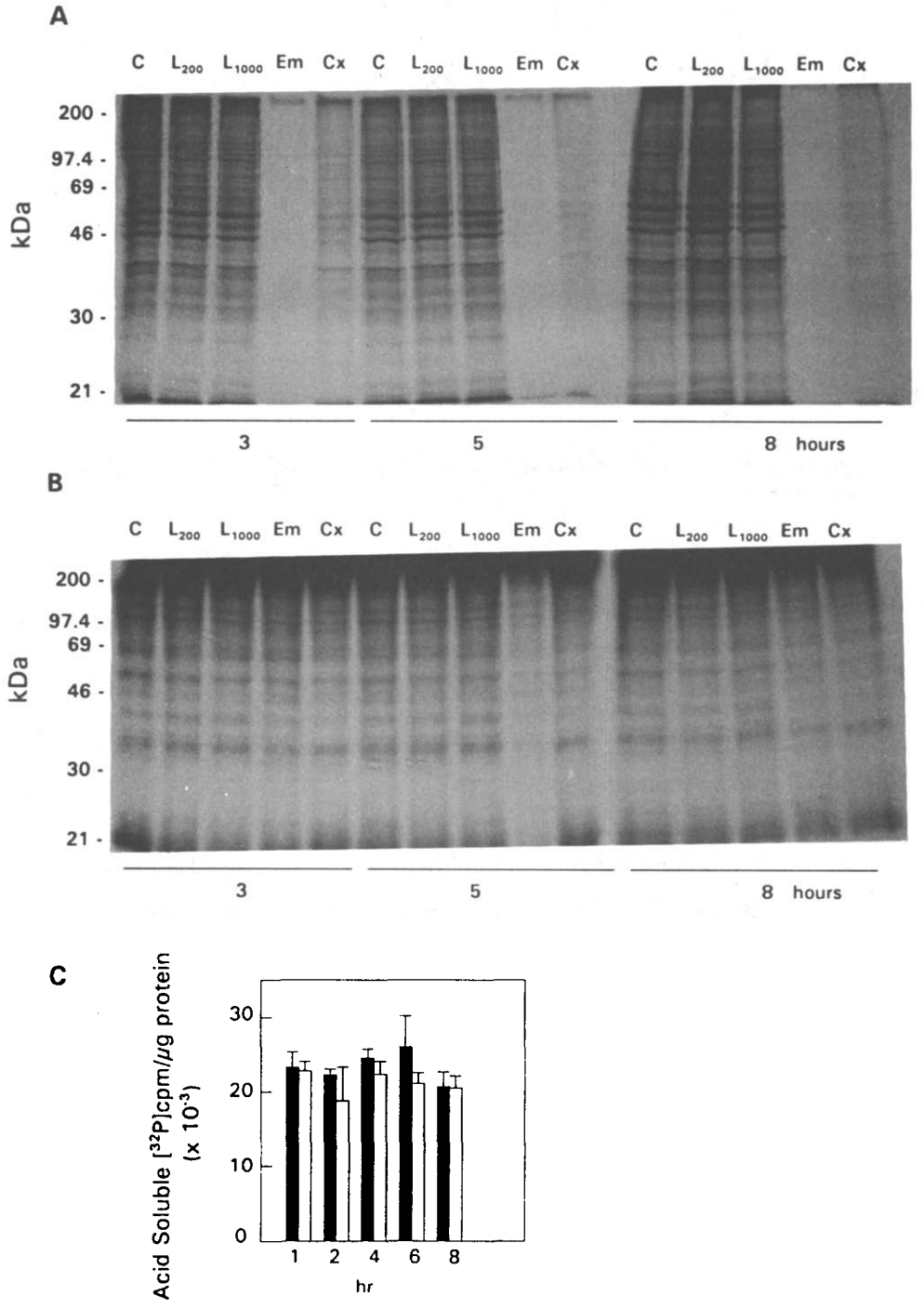


Fig. 5. Protein synthesis, phosphorylation, and orthophosphate uptake into aphidicolin-synchronized MDA-468 cells. (A) [^{35}S]Methionine-labeled cellular proteins. (B) [^{35}P]Orthophosphate-labeled cellular proteins. Proteins were obtained from cells released from aphidicolin block with C, no drug; L_{200}, 200 nM L86-8275; L_{1000} 1000 nM L86-8275; Em, 2 μM emetine; and Cx, 10 μM cycloheximide for 3, 5, and 8 hr. (C) Acid-soluble [^{32}P] extracts from MDA-468 cells. Key: (■) control untreated cells; and (□) exposed to cells 200 nM L86-8275 for 1–8 hr after release from aphidicolin block. Values are means ± SEM, N = 3.

cinoma cells is shown in Fig. 1. After 8 hr of exposure to 125 nM L86-8275 (approximately the 50% growth inhibitory concentration, IC_{50}; Ref. 3), there was a significant decrease in the S phase fraction (Fig. 1C) with a corresponding increase in the fraction of cells with G_2/M DNA content first observed at 8 hr, but persisting for at least 48 hr of exposure (Fig. 1B). By 48 hr, non-drug-treated control cells were becoming confluent, with the expected increase in G_1 fraction and decrease in control S phase fraction. The L86-8275-treated cultures remained fixed with G_2/M DNA content (Fig. 1B). Inspection of the cells confirmed that cells had excluded trypan blue and had not become rounded, indicative of not having entered the M phase.

To address the mechanism for the apparent block to cell cycle progression through G_2, cells were synchronized at the G_1/S boundary by use of aphidicolin. After release from aphidicolin synchronization, control cells progressed through S phase by 10 hr and at 14 hr had largely completed mitosis (Fig. 2A). In contrast, cells treated with 200 nM L86-8275 (approximately at the IC_{90}, [3]) were greatly retarded in progression through S phase, but by 24 hr were accumulating in G_2 (Fig. 2A, right panel). Figure 2B shows an initial increase of immunoprecipitated p34^{cdc2} histone H1 kinase activity after a 3-hr exposure to L86-8275 following release from aphidicolin synchronization, but then inhibition of histone H1 kinase activity relative to control activity at every time observed in the S, G_2 or M phase. We concluded that the L86-8275-mediated block to cell cycle progression, first manifested as a delay in progression through S phase, with ultimate block in G_2, was accompanied by decreased p34^{cdc2}-associated histone H1 kinase activity after release from aphidicolin block.

Further investigation of the mechanism by which L86-8275 delays progression through the cell cycle was directed at potential effects on either the post-translational modification (phosphorylation) or the synthesis of p34^{cdc2}. Previous studies have shown that when mitosis is blocked, either with pharmacologic agents or with damaged and unreplicated DNA, there is an accumulation of hyperphosphorylated p34^{cdc2} [21–24]. However, data presented in Fig. 3 show that L68-8275 altered the normal phosphorylation of p34^{cdc2}. Using specific immunoprecipitates obtained with antiserum directed against the C-terminal of p34^{cdc2} from [^{32}P]-orthophosphate-labeled MDA-468 cells, differences were found in the phosphorylation status (Fig. 3A). After a 2-hr exposure of aphidicolon-synchronized cells to L86-8275, only a small decrease in phosphate labeling of the p34^{cdc2} immunoprecipitate was observed. At 6–10 hr after release from aphidicolin synchronization, when control cells were completing S phase, but drug-treated cells were retarded in S (Fig. 2A), there was clearly a decrease in [^{32}P]-orthophosphate labeling of the p34^{cdc2} immunoprecipitate in L86-8275-exposed cells. When antiserum raised against the conserved PSTAIRE motif present in p34^{cdc2} and related proteins [25] was used for immunoprecipitation, an analogous inhibition of [^{32}P]orthophosphate labeling was found by 8 hr (compare panels A and B of Fig. 3). The phosphotyrosine content of anti-PSTAIRE immunoprecipitates (Fig. 3C) was also greatly decreased by 8 hr in cells exposed to L86-8275. This result is in marked contrast to other growth inhibitory agents such as nitrogen mustard [21], camptothecin or etoposide (inhibitors or topoisomerase I and II, respectively) [22, 23], where preserved or increased tyrosine phosphorylation of p34^{cdc2} has been observed. Methotrexate, a known antifolate, S-phase inhibitor, does not cause decreased phosphorylation of p34^{cdc2} [24]. A western blot using polyclonal PSTAIRE antiserum on immunoprecipitates derived with the same PSTAIRE antiserum (Fig. 3D) or with the p34^{cdc2} C-terminal directed antiserum (not shown) showed no alteration in the amount of p34^{cdc2}-related proteins expressed in cells exposed to L86-8275. Cellular phosphotyrosine in whole cell lysates was preserved in aphidicolin-synchronized cells exposed to L86-8275 for up to 14 hr with the exception of a protein species at approximately 34 kDa, where a clear decrease in phosphotyrosine was apparent by 8 hr (see arrow, Fig. 3E).

Since the p34^{cdc2} histone H1 kinase activity depends on both its phosphorylation status and the availability of the appropriate newly synthesized cyclin [11], the results shown in Figs. 2 and 3 could derive from inhibition of protein synthesis. We have shown (Fig. 3D) that the relative mass of p34^{cdc2} was not altered within the time during which phosphorylation and H1 kinase activity were decreased. Furthermore, Fig. 4A demonstrates that neither 200 nor 1000 nM L86-8275 inhibited [^{35}S]methionine incorporation into immunoprecipitated p34^{cdc2} or into proteins in whole cell lysates (Fig. 5A). In contrast, the protein synthesis inhibitors emetine (2 μM) and cycloheximide (10 μM), at the approximate IC_{90} for growth inhibition of MDA-468 cells, inhibited [^{35}S]-methionine labeling of p34^{cdc2} immunoprecipitates and of proteins obtained in whole cell lysates (Fig. 5A).

Figure 4B demonstrates that phosphorylation of the p34^{cdc2} was decreased by treatment of cells with L86-8275, emetine or cycloheximide. Western blot analysis of the relative amount of p34^{cdc2} present with antiserum directed at the conserved PSTAIRE region showed essentially no difference in the mass of the fastest migrating form on SDS–PAGE for either L86-8275 or the protein synthesis inhibitors emetine and cycloheximide (not shown). Therefore, alteration of the normal regulatory phosphorylation of p34^{cdc2} can occur after exposure of the cells either to protein synthesis inhibitors or to L86-8275; in the latter case alteration occurs without a decrease of [^{35}S]methionine label (Figs. 4A and 5A). These results imply that protein synthesis inhibitors may decrease the synthesis of proteins (e.g. cyclins) necessary for phosphorylation of p34^{cdc2} and p34^{cdc2} histone H1 kinase activity, while L86-8275 may inhibit phosphorylation of p34^{cdc2} and H1 kinase activity by other mechanisms.

Figure 4C revealed initial increased immunoprecipitated p34^{cdc2} histone H1 kinase activity at 3 hr after release from aphidicolin synchronization into medium containing either 200 or 1000 nM L86-8275. In contrast, at this time emetine had decreased p34^{cdc2} histone H1 kinase activity and caused virtually

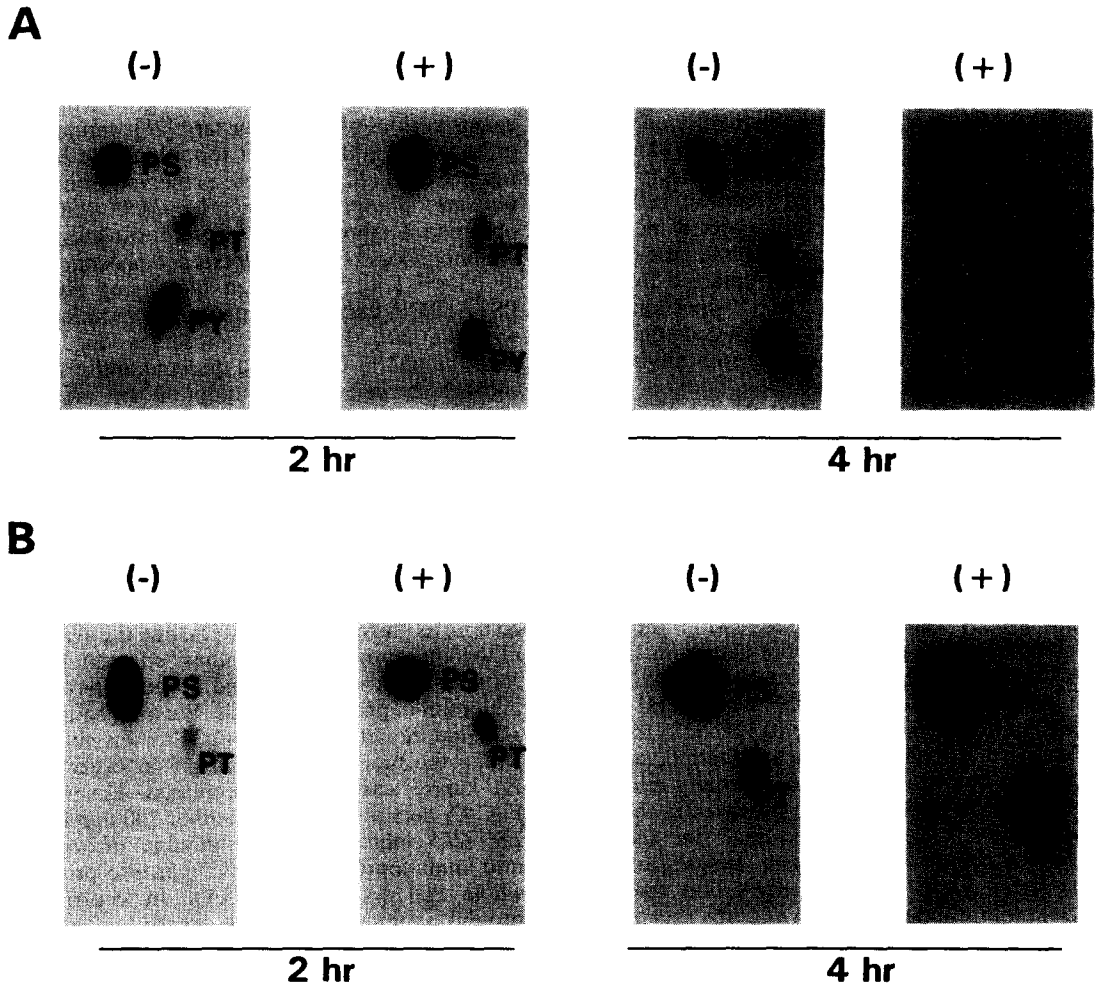


Fig. 6. Phosphoamino acid analysis. (A) Phosphoamino acids of $p34^{cdc2}$ immunoprecipitates derived with C-terminal specific antiserum from aphidicolin-synchronized MDA-468 breast carcinoma cells released into medium with (+) or without (−) 200 nM L86-8275 for 2 or 4 hr. (B) Unknown 80-kDa protein from whole cell lysate with (+) or without (−) 200 nM L86-8275 for either 2 or 4 hr. Cells were labeled with [^{32}P]orthophosphate for the last 2 hr prior to cell harvesting. Abbreviations: PS, phosphoserine; PT, phosphothreonine; and PY, phosphotyrosine.

complete inhibition of protein synthesis (Figs. 4 and 5). Significantly, immunoprecipitates using an anti-cyclin B antiserum showed increased histone H1 kinase activity from L86-8275-treated cells at 3 hr after release from aphidicolin synchronization into medium with 1000 nM L86-8275, but decreased histone H1 kinase activity from the emetine-treated cells (Fig. 4D). At 8 hr, when the L86-8275-exposed cells still had intact protein synthesis, immunoprecipitates derived with either anti-C-terminal $p34^{cdc2}$ or anti-cyclin B antisera showed decreased histone H1 kinase activity (Figs. 4A and 5A).

These findings suggest that L86-8275 and emetine inhibit phosphorylation of the $p34^{cdc2}$ protein and reduce markedly the activity of $p34^{cdc2}$ histone H1 kinase by different pharmacologic mechanisms. Indeed, when cells were treated with the higher increase of both anti-$p34^{cdc2}$ and anti-cyclin B immunoprecipitated histone H1 kinase activity could be seen compared with control cells (Figs. 2B and 4C). The effect of emetine was to reduce the H1 kinase activity at all times, concordant with the expected result after inhibition of protein synthesis (see Fig. 5).

A possible global effect of L86-8275 on protein synthesis was also discounted by the data shown in Fig. 5. L86-8275 at 200 or 1000 nM did not affect the incorporation of [^{35}S]methionine into total cellular proteins over the period of the experiment shown, in contrast to emetine or cycloheximide. There was also no effect from L86-8275 on the global [^{32}P]orthophosphate labeling within this period in the same experiment (Fig. 5B). There was no difference in the uptake of [^{32}P]orthophosphate into total phosphate pools

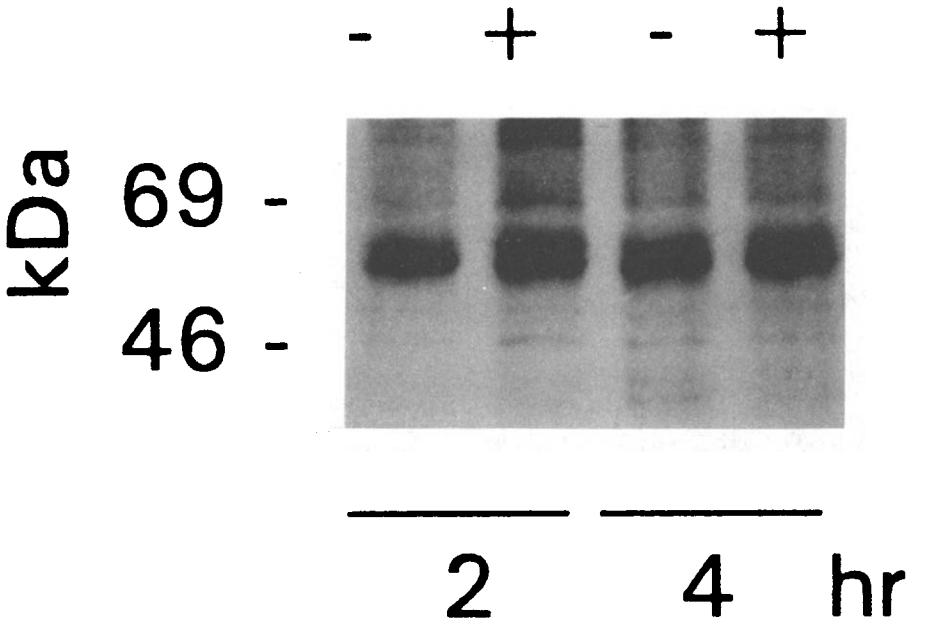


Fig. 7. Cyclin-B content of C-terminal p34^{cdc2} kinase (CDK1) immunoprecipitates. Cells were treated as described in the legend of Fig. 6, but without $^{32}PO_4$ labeling. Cyclin B, present in western blots of CDK1 immunoprecipitates, was detected using [^{125}I]protein A detection.

extracts, between control cells and cells exposed to L86-8275 (200 nM) (Fig. 5C).

Phosphoamino acid analysis from p34^{cdc2} immunoprecipitates derived with the C-terminal antiserum after release from aphidicolin into either drug-free medium or medium containing L86-8275, showed little change in either phosphothreonine or phosphotyrosine after a 2-hr exposure (Fig. 6A). However, at 4 hr, both phosphotyrosine and phosphothreonine of p34^{cdc2} were reduced markedly in cells exposed to L86-8275 compared with the control cells. Phosphoserine remained unchanged by exposure of the cells to L86-8275 within the 4-hr period. For comparison, phosphoamino acid analysis of a different, unknown protein (approx. 80 kDa) obtained from the same cell lysate used to prepare the p34^{cdc2} immunoprecipitates is shown in Fig. 6B, with no effect on the phosphothreonine over the same period of exposure to L86-8275.

To relate these changes in p34^{cdc2} immunoprecipitate $^{32}PO_4$-labeling after treatment with L86-8275 to the presence of cyclin in the immunoprecipitates, we probed western blots of p34^{cdc2} kinase immunoprecipitates with an anti-cyclin-B antibody. Figure 7 shows that up to 4 hr after release from aphidicolin block, at a time when there was clearly decreased labeling by $^{32}PO_4$ of p34^{cdc2} kinase Tyr and Thr (see Fig. 6), there was no decrease in an approximately 60 kDa species detected by the anti-cyclin-B antibody in those immunoprecipitates. These data, consistent with Figs. 4 and 5, strongly suggest that L86-8275 does not affect cyclin-B synthesis within 4 hr of addition to cells released from aphidicolin block. This result is also consistent with the ability of anti-cyclin-B antibodies to immunoprecipitate histone H1 kinase activity at least up to 5 hr after release from aphidicolin block in the presence of L86-8275 (Fig. 4D).

In these experiments, we have shown that one potential mechanism of the growth inhibitory activity of a novel flavonoid structure, L86-8275, relates to interference with the normal phosphorylation state of p34^{cdc2}, which, in turn, relates to the molecular mechanism responsible for successful transit through the cell cycle. Using synchronized populations of MDA-468 breast carcinoma cells, we observed a selective decrease in both the threonine and tyrosine phosphorylation of a 34-kDa protein species immunoprecipitated by both PSTAIRE and C-terminal directed anti-p34^{cdc2} antisera. The decreased p34^{cdc2} phosphorylation is contemporaneous with decreased histone H1 kinase activity. We hypothesize that L86-8275 represents a novel structure that is potently directed against the activation of p34^{cdc2} and related proteins.

Future experiments will clarify whether L86-8275 interacts directly with the p34^{cdc2} to block post-translational regulatory phosphorylations, or if the drug interacts directly with an upstream regulator of p34^{cdc2}. Also, it is possible that the drug can block the proper assembly or function of the p34^{cdc2} kinase–cyclin-B complex. These observations suggest that L86-8275 represents an important lead structure for the design of inhibitors of the activation or function of cyclin-dependent kinases.

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