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Inhibition of the Mg(II)·ATP-dependent phosphoprotein phosphatase by the regulatory subunit of cAMP-dependent protein kinase*

(enzyme regulation/phosphorylation–dephosphorylation/glycogen cascade system)

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ABSTRACT **We report potent inhibition of the Mg(II)·ATP-dependent protein phosphatase, F_C·M, by the regulatory subunit dimer of type II cAMP-dependent protein kinase, R_2^{II}. The protein kinase catalytic subunit has no effect on phosphatase activity and is unable to substitute for kinase F_A in the kinase F_A- and Mg(II)·ATP-mediated phosphatase activation reaction. Phosphatase inhibition was investigated as a function of R_2^{II} concentration. The results suggest that R_2^{II} both inhibits the active phosphatase and inhibits phosphatase activation. The inhibition is shown to be noncompetitive with respect to substrate (phosphorylase a). The potential physiological significance of this inhibition is discussed in terms of phosphorylation/dephosphorylation cascade systems involving this kinase and phosphatase.**

In recent years, increasing attention has been focused on uncovering the role of protein phosphatases in regulating the state of phosphorylation of various proteins and enzymes that control an array of cellular processes. It is known that reversible, protein phosphorylation/dephosphorylation is an important general mechanism of regulating diverse cellular processes in response to various physiological stimuli (1, 2).

The identification of protein kinases acting in protein phosphorylation cascades and the mechanisms by which they are regulated via second messengers such as cAMP and Ca^{2+} have preceded similar knowledge of the protein phosphatases involved. Recently, Ingebritsen and Cohen (3, 4) have attempted to categorize a variety of protein phosphatase forms identified in different systems as being one of four different classes of enzymes. In the nomenclature of Ingebritsen and Cohen (3, 4), type 1 protein phosphatase has been shown to be inhibited by two different heat-stable protein inhibitors termed inhibitor 1 and inhibitor 2 (5–9).

A Mg(II)·ATP-dependent protein phosphatase has been isolated from rabbit skeletal muscle and characterized (10, 11). This enzyme is a major phosphorylase phosphatase in skeletal muscle and it also readily dephosphorylates the β subunit of phosphorylase kinase as well as glycogen synthase (10, 12), suggesting an important role in the glycogen cascade system. The enzyme, isolated in its inactive form, is activated by kinase F_A (also identified as glycogen synthase kinase 3) in the presence of Mg(II)·ATP (13, 14). Inhibitor 2, also termed modulator, M, has been identified as a modulator of this phosphatase (9). It is now considered to be a subunit of the enzyme that has been designated F_C·M. Recent work on the mechanism of activation of this phosphatase by kinase F_A has implicated phosphorylation/dephosphorylation of modulator in the interconversion of active and inactive phosphatase (15). A similar enzymatic activity has been isolated from rabbit skeletal muscle by a procedure involving an acetone-precipitation step (16, 17). This enzyme consisted of a complex between a 38-kDa catalytic subunit, and the 31-kDa modulator, M. Activation of the inactive phosphatase could be accomplished either with trypsin treatment in the presence of Mn(II) or by treatment with kinase F_A and Mg(II)·ATP, resulting in phosphorylation of the modulator subunit. A Mg(II)·ATP-dependent protein phosphatase has also been reconstituted from type 1 protein phosphatase and inhibitor 2 (18, 19). The inactive complex was activated by glycogen synthase kinase 3 and Mg(II)·ATP, which resulted in phosphorylation of inhibitor 2 on threonine and caused inhibitor 2 to dissociate from the active phosphatase.

In this investigation, we report an inhibition of the Mg(II)·ATP-dependent protein phosphatase by type II cAMP-dependent protein kinase. cAMP-dependent protein kinase, when substituted for kinase F_A, failed to activate the phosphatase. However, its inclusion in the activation reaction with kinase F_A resulted in inhibition of the phosphatase. The inhibition is characterized and is shown to be due to dissociated regulatory subunit dimer R_2^{II}, dissociated from type II cAMP-dependent protein kinases holoenzyme R_2C_2.

EXPERIMENTAL PROCEDURES

Materials. [γ-^{32}P]ATP was purchased from New England Nuclear. Phosphorylase *b* was isolated from rabbit skeletal muscle and converted to ^{32}P-labeled phosphorylase *a* as described (20, 21). Phosphatase F_C·M and kinase F_A were isolated as described (10, 13). Two different preparations of phosphatase F_C·M were tested for inhibition by R_2^{II} with similar findings. However, most experiments were performed by using a preparation with lower specific activity (≈1,500 units/mg) compared to that described previously (10, 15). This preparation was devoid of other known phosphorylase phosphatases, since the expression of phosphatase activity required activation by kinase F_A and Mg(II)·ATP. The phosphatase activity unit is defined as the amount of enzyme that releases 1 nmol of [^{32}P]phosphate per min at 30°C from ^{32}P-labeled phosphorylase *a* (2 mg/ml). Bovine heart type II cAMP-dependent protein kinase, purified as described (22), was kindly donated by Emily Shacter (National Heart, Lung, and Blood Institute). R_2^{II} and the catalytic subunit C were isolated from porcine heart and purified to near homogeneity as described (23).

Enzyme Assays. Assays of phosphorylase phosphatase activity were performed by using an organic extraction procedure as described (15) or with a modified extraction procedure (24). Dephosphorylation of phosphorylase *a* in the assay was usually 10% or less.

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The concentration of phosphorylase was determined by using the absorbance index of $A_{280}^{1\%} = 13.1$ (25). The concentration of other proteins was determined by the method of Bradford (26).

RESULTS

Identification of R_2^{II} as a Phosphatase Inhibitor. The Mg(II)·ATP-dependent phosphatase F_C·M is activated by kinase F_A in the presence of Mg(II)·ATP (13, 14). We found that bovine heart (type II) cAMP-dependent protein kinase, when substituted for kinase F_A, and in the presence of cAMP and Mg(II)·ATP, was unable to activate the phosphatase. However, including cAMP-dependent protein kinase in the activation-preincubation with kinase F_A resulted in a cAMP-dependent inhibition of phosphatase activity. The isolated subunits of the type II cAMP-dependent protein kinase from porcine heart were used to test whether phosphatase inhibition derived from C or R_2^{II}. C did not inhibit phosphatase activity when it was included with kinase F_A and Mg(II)·ATP during F_C·M activation (Fig. 1). However, R_2^{II} did inhibit the phosphatase. Inhibition by R_2^{II} was slightly enhanced by the addition of cAMP, which by itself had no effect on phosphatase activity. The R_2^{II} preparation contained some bound nucleotide derived from the affinity purification step in which cGMP was used to elute the protein. The addition of cAMP (4 μM in assay) should assure near saturation of the cAMP binding sites of R_2^{II} and also cause exchange of any bound cGMP for cAMP. Control experiments showed that cGMP alone at 4 μM had no effect on phosphatase activity. The inhibition of phosphatase by added R_2^{II} was reversed by the addition of an excess of C, which caused the reformation of the protein kinase holoenzyme $R_2^{II}C_2$. Dissociation of the reformed holoenzyme by addition of cAMP restored the F_C·M inhibitory activity. The inhibitory activity of R_2^{II} was heat labile and was destroyed by heating at 90°C for 5 min.

Inhibition of Active Phosphatase and of Phosphatase Activation. Inhibition of phosphatase F_C·M by R_2^{II}, added either before initiating activation of phosphatase or after phosphatase activation, was compared (Fig. 2). In assays 1–3 in Fig. 2*A*, R_2^{II} and cAMP were added immediately before initiating the activation of F_C·M. In assays 4–6, addition of R_2^{II} was made 4.5 min after initiating activation. There was significantly less phosphatase inhibition when R_2^{II} was added after activating the phosphatase (assay 6) compared to when it was added before initiating phosphatase activation (assay 3). This suggests that R_2^{II} inhibits both the activation of F_C·M and the catalytic activity of the active phosphatase.

Previous results have shown that different steady state levels of phosphatase activity occur when different concentrations of kinase F_A are used during F_C·M activation (10). The inhibitory effect of R_2^{II} was investigated with activation

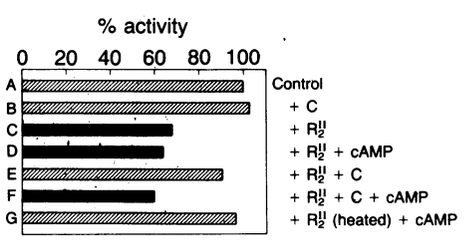

FIG. 1. Inhibition of phosphatase F_C·M by R_2^{II}. F_C·M was activated with kinase F_A and Mg(II)·ATP for 5 min in the presence or absence of R_2^{II}, C, and cAMP as indicated and then was assayed for activity in a subsequent 5-min assay. The final concentrations of assay components were: F_C·M, 0.3 μg/ml; F_A, 0.4 μg/ml; R_2^{II}, 31 nM (3.3 μg/ml); C, 94 nM (3.9 μg/ml); cAMP, 4 μM; and phosphorylase *a*, 1 mg/ml (10.5 μM).

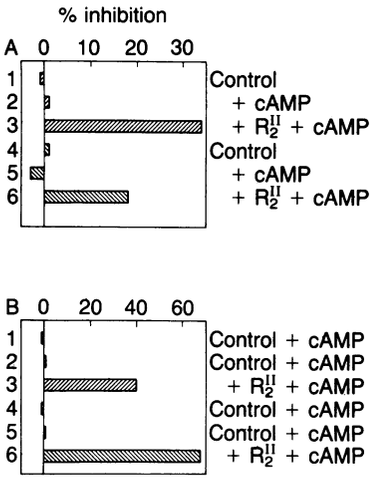

FIG. 2. (*A*) Comparison of phosphatase F_C·M inhibition by R_2^{II} added either before or after activation of phosphatase. In assays 1–3, phosphatase F_C·M was activated for 5 min with kinase F_A and Mg(II)·ATP. R_2^{II} and cAMP were added immediately before initiating the activation of F_C·M. At 5 min, ^{32}P-labeled phosphorylase *a* was added, and phosphatase activity was assayed. In assays 4–6, F_C·M was also activated for 5 min; however, the addition of R_2^{II} (or buffer control) was made 4.5 min after initiating phosphatase activation. The average of control assays (with or without cAMP) was used as the baseline activity. The values shown for assays in the presence of R_2^{II} are the average of duplicate assays. Typically, duplicates vary by 2% or less from their average. The final concentrations of R_2^{II} and cAMP were 50 nM (5.3 μg/ml), and 4 μM, respectively. The concentrations of F_C·M, F_A, and phosphorylase *a* in the assay were 0.3 μg/ml, 0.4 μg/ml, and 1 mg/ml ($\approx$10 μM), respectively. (*B*) Comparison of phosphatase inhibition by R_2^{II} at two different levels of activation. Phosphatase F_C·M was activated for 5 min with kinase F_A and Mg(II)·ATP in the presence or absence of cAMP and R_2^{II}. ^{32}P-labeled phosphorylase *a* was then added, and phosphatase activity was assayed. In assays 1–3, 400 ng/ml of kinase F_A (final assay concentration) was used to activate F_C·M completely. In assays 4–6, the kinase F_A was reduced to 80 ng/ml, which results in 72% of the maximal activation of the phosphatase. The average of duplicate control assays in the presence of cAMP was used as the baseline activity. The values shown for assays in the presence of R_2^{II} are the average of duplicates. The final assay concentrations of components other than F_A were the same as in *A* except for R_2^{II}, which was at 100 nM (10.6 μg/ml).

of F_C·M at two different concentrations of kinase F_A. In assays 1–3 in Fig. 2*B*, 400 ng of kinase F_A per ml was used; this concentration completely activated the phosphatase. In assays 4–6, kinase F_A was reduced to 80 ng/ml, which resulted in 72% of the maximal activation of the phosphatase. At the lower level of F_C·M activation, there was a greater degree of inhibition by R_2^{II}. In light of the decreased phosphatase inhibition by R_2^{II} at the higher level of kinase F_A, we considered the possibility that kinase F_A was converting R_2^{II} to a less (or non) inhibitory form via a phosphorylation reaction. This idea was tested in the following experiment. R_2^{II} was preincubated with kinase F_A and Mg(II)·ATP for 10 min at 30°C and then F_C·M was added, thereby initiating phosphatase activation. This pretreatment of R_2^{II} did not cause any decrease of its phosphatase inhibitory activity. Similar preincubation of R_2^{II} with Mg(II)·ATP alone also had no effect on phosphatase inhibition. These results indicate that under the conditions used to activate F_C·M, kinase F_A is not converting R_2^{II} to a less inhibitory form.

It should be pointed out that glycogen synthase kinase 3 (kinase F_A) and glycogen synthase kinase 5 have been shown to phosphorylate R_2^{II} *in vitro* (27). However, in those experiments, in which the concentration of R_2^{II} was about 1.5 mg/ml, phosphorylation by glycogen synthase kinase 3

occurred slowly with an incorporation of about 0.3 mol of P_i per mol of subunit after 2 hr of incubation. The rate of phosphorylation was even further reduced to <0.1 mol of P_i per mol of subunit in 2 hr, if R_2^{II} was dephosphorylated first at all sites containing endogenous covalently bound phosphate. In the present investigation, the R_2^{II} concentrations used to inhibit phosphatase F_C·M were considerably lower. In the experiment presented in Fig. 1, for example, the concentration of R_2^{II} was 3.3 μg/ml. The concentration of kinase F_A used to activate phosphatase F_C·M was also somewhat less than the concentration used in the R_2^{II} phosphorylation experiments mentioned above. This makes it unlikely that significant phosphorylation of R_2^{II} can occur during the activation-preincubation of F_C·M.

Effect of R_2^{II} on Kinetics of Phosphatase Activation. The time course of the activation of phosphatase by kinase F_A and Mg(II)·ATP was determined in the presence and absence of R_2^{II} and cAMP (Fig. 3*A*). The degree of phosphatase inhibition by R_2^{II} changed during the time course of activation. The percent inhibition decreased as a function of activation time, asymptotically approaching a final level of inhibition (Fig. 3*B*). This observation may be explained by R_2^{II} inhibiting the activation of the phosphatase as well as causing partial inhibition of the activated enzyme.

R_2^{II} Concentration-Dependence of Phosphatase Inhibition. Phosphatase inhibition curves determined under several conditions are plotted as a function of R_2^{II} concentration in Fig. 4*A*. At a given concentration of R_2^{II}, the inhibition curve shifts towards greater inhibition, when a lower concentration of kinase F_A was used (curve with open circles) compared to when a higher concentration was used (curve with open squares). In these assays, R_2^{II} was added immediately before initiating the activation of F_C·M. The degree of inhibition by R_2^{II} was significantly reduced when F_C·M was activated first for 10 min and then R_2^{II} was added (curve with open triangles). The phosphatase inhibition data describing each of these

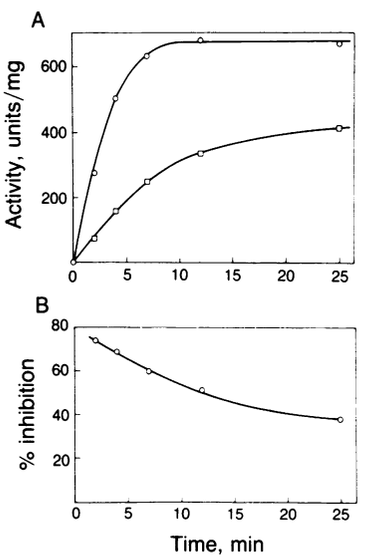

FIG. 3. (*A*) Time course of phosphatase activation in the presence and absence of R_2^{II}. F_C·M was activated with kinase F_A and Mg(II)·ATP in the presence of cAMP and in the presence (□) or absence (○) of R_2^{II}. Aliquots of the activation mixture taken at time intervals during the activation were assayed in a 1-min assay. Concentrations of components in the assay were: F_C·M, 0.25 μg/ml; R_2^{II}, 100 nM (10.6 μg/ml); cAMP, 4 μM; and phosphorylase *a*, 2 mg/ml (21 μM). The assay concentration of kinase F_A was 80 ng/ml, which results in about 70% of the maximal activation of phosphatase F_C·M. (*B*) The percentage of phosphatase F_C·M inhibition by R_2^{II} (in *A*) during the time course of activation. The percentage of F_C·M inhibition is plotted versus the time of activation.

three curves can be accounted for by a single binding interaction between R_2^{II} and phosphatase F_C·M. Fig. 4*B* presents a computer fit of the data based on such an assumption. The computer-generated binding isotherm is in reasonable agreement with the data. The amplitude of the inhibition was reduced when R_2^{II} was added after activation of the phosphatase because the activated phosphatase was only partially inhibited by R_2^{II} binding. Addition of R_2^{II} before initiating activation of the phosphatase (curve with open squares) resulted in an increased amplitude of inhibition because activation of the phosphatase was inhibited as well as the activated enzyme activity. The apparent K_ds for the binding interaction calculated from these two curves (curves with open triangles and squares) are in reasonable agreement, 89 and 112 nM, respectively. When the kinase F_A concentration was reduced 80%, there was a shift in the inhibition curve (curve with open squares to curve with open circles) and a decrease in the apparent K_d to 42 nM. This suggests an antagonism between the binding of R_2^{II} and of kinase F_A to the phosphatase. These data are insufficient to distinguish between mutual exclusion and partial antagonism. Nevertheless, by assuming a mutual exclusion mechanism, one can estimate the value of K_d for the R_2^{II}·F_C·M complex to be ≈24 nM. This value is in reasonable agreement with the estimated K_i (15 nM) for R_2^{II} inhibition of the phosphatase catalytic subunit isolated by the ethanol treatment procedure (see below). This mechanism also gives an estimate of the K_d (≈3 nM) for kinase F_A binding to phosphatase F_C·M.

The spontaneously active type 1 phosphatase was partially purified following a procedure using ethanol treatment (28), except that the final gel filtration step was omitted. This type 1 phosphatase could not be further activated by kinase F_A and Mg(II)·ATP. As shown in Fig. 4*A*, the addition of R_2^{II} also resulted in inhibition of this phosphatase (curve with solid circles). Inhibition occurred at a low concentration of added R_2^{II}, but the maximum degree of inhibition achieved was only about 30–35%. The inhibitory activity of R_2^{II} was shown again to be heat labile; it was destroyed by heating for 5 min at 90°C. The inhibition of type 1 phosphatase caused by 50 nM R_2^{II} was not affected by the addition of a molar excess of kinase F_A (data not shown). This indicates that even at high concentrations of F_A, there is no direct interaction between kinase F_A and R_2^{II} that has an effect on the ability of R_2^{II} to inhibit type 1 phosphatase. This is of interest in light of the observations (*i*) of decreased inhibition of F_C·M when higher concentrations of kinase F_A were used and (*ii*) of decreased inhibition of F_C·M when R_2^{II} was added after, as compared to before, F_C·M activation. In another experiment (not shown) R_2^{II} (50 nM) was preincubated for different times from 0–10 min at 30°C with the spontaneously active type 1 phosphatase before adding substrate to start the assay. This preincubation did not affect the ability of R_2^{II} to inhibit the phosphatase. Similar preincubation of the phosphatase alone did not alter its activity. This result indicates that there is not a slow time dependence for the formation of an inhibited phosphatase after addition of R_2^{II}.

It is known that R_2^{II} may contain covalently bound phosphate at several different sites (27, 29). Thus, it might be possible for R_2^{II} to inhibit phosphatase by binding at the phosphatase substrate binding site, in which case the inhibition by R_2^{II} would be expected to be competitive with respect to phosphorylase *a*. However, the inhibition of phosphatase by R_2^{II} was observed to be noncompetitive. The data from an initial rate kinetic experiment performed at different concentrations of R_2^{II} is shown in Fig. 5. In the presence of R_2^{II} a decrease in the V_{max} was observed with no effect on the K_m for phosphorylase *a*. In addition, inhibition of phosphatase by R_2^{II} occurred with the concentration of phosphorylase *a* in large excess over the concentration of R_2^{II}. Together, these data indicate that the inhibition is neither due to R_2^{II} binding

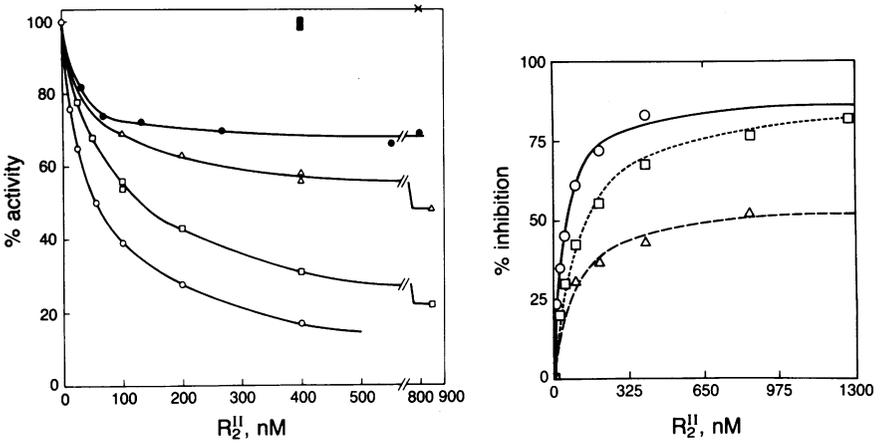

FIG. 4. (*A*) Phosphatase inhibition as a function of R_2^{II} concentration. F_C·M was activated for 5 min in the presence of R_2^{II}, cAMP, kinase F_A (final concentration, 80 ng/ml), and Mg(II)·ATP, and activity was determined in a subsequent 5-min assay (○). The inhibition curve obtained when F_C·M was activated as described above, except that the concentration of kinase F_A was increased 5-fold to 400 ng/ml, is shown (□). There was no inhibition observed when the R_2^{II} was first heated for 5 min at 90°C (■). The inhibition curve when F_C·M was activated with kinase F_A (final concentration, 400 ng/ml) and Mg(II)·ATP for 10 min prior to addition of R_2^{II} which was followed by further incubation for 5 min and then assayed is indicated by -△-. The assay concentrations of other components were: F_C·M, 0.25 μg/ml; cAMP, 4 μM; and phosphorylase *a*, 10 μM. The inhibition curve for ethanol-treated type 1 phosphatase activity is shown (●). The inhibitory activity of R_2^{II} was destroyed by heating for 5 min at 90°C (see "X"). The cAMP and phosphorylase *a* concentrations in these assays were the same as above. (*B*) Computer fit of phosphatase inhibition by R_2^{II} based on the assumption of a single binding site. Symbols show data from *A*. Curves were generated by a computer fit based on a single-binding-site assumption. The apparent K_d for the binding interaction and the amplitude of inhibition were variables in the data fitting.

at the substrate binding site nor due to R_2^{II} forming a complex with the substrate and thus reducing its effective concentration.

DISCUSSION

In this investigation, we show that the regulatory subunit of type II cAMP-dependent protein kinase, R_2^{II}, is a potent inhibitor of the phosphatase F_C·M. This inhibition is distinct from the inhibition of this phosphatase by the heat-stable protein inhibitors, inhibitor 1 and inhibitor 2. The inhibitory activity of R_2^{II} is both heat labile and requires the dissociated regulatory subunit. Inhibition by R_2^{II} is reversed by reforming the protein kinase holoenzyme $R_2^{II}C_2$.

Gergely and Bot (30) have also reported inhibition of phosphorylase phosphatase, isolated by using ethanol treatment as in ref. 28, by the regulatory subunit of cAMP-dependent protein kinase isolated from rabbit skeletal muscle. In that report, however, higher concentrations of regulatory subunit were required for inhibition of phosphatase activity. These workers suggested that phosphatase inhibition by the protein kinase regulatory subunit resulted from its interaction with substrate, phosphorylase *a*. However, in the present investigation, inhibition occurred with phosphorylase *a* concentrations in large excess over the concentration of R_2^{II}. In addition, the kinetic experiment presented in Fig. 5 demonstrates that inhibition by R_2^{II} follows a simple noncompetitive inhibition pattern. If the inhibition were derived from an interaction between R_2^{II} and phosphorylase *a*, one would not obtain the observed linear plot (Fig. 5) when R_2^{II} was present in the reaction mixture. These results indicate that the phosphatase inhibition by R_2^{II} is not a substrate-directed effect.

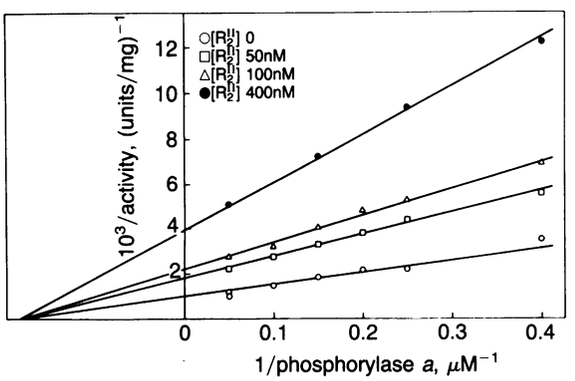

FIG. 5. Double reciprocal plot of F_C·M inhibition by R_2^{II}. F_C·M was activated for 5 min in the presence of different concentrations of R_2^{II} and then assayed with different concentrations of substrate (phosphorylase *a*). Curves: ○, double reciprocal plot determined in the absence of R_2^{II}; □, in the presence of 50 nM R_2^{II}; △, in the presence of 100 nM R_2^{II}; and ●, in the presence of 400 nM R_2^{II}. The concentration of F_C·M was 0.25 μg/ml and of kinase F_A was 0.4 μg/ml. The K_m for phosphorylase *a* calculated from the intercept with the *x* axis is 5.5 μM (subunit concentration).

The results indicate that R_2^{II} inhibits both the active phosphatase and the activation of F_C·M by kinase F_A and Mg(II)·ATP. This conclusion is supported by the fact that (*i*) a higher degree of inhibition was observed when R_2^{II} was added before initiating F_C·M activation compared to that when R_2^{II} was added after F_C·M has been activated (Figs. 2*A* and 4*A*); (*ii*) when R_2^{II} was added prior to F_C·M activation, the percent inhibition decreases with time until reaching a final level (Fig. 3*B*); and (*iii*) preincubation of R_2^{II} with kinase F_A and Mg(II)·ATP causes no decrease in the R_2^{II} inhibitory capacity. Binding of R_2^{II} to both active and inactive forms of phosphatase F_C·M could affect both the activity of the activated phosphatase and the activation of the phosphatase by kinase F_A and Mg(II)·ATP.

The direct inhibition of phosphatase F_C·M by the regulatory subunit, R_2^{II}, of type II cAMP-dependent protein kinase may be an important regulatory feature of phosphorylation/dephosphorylation cascade systems involving this kinase and phosphatase. Significant phosphatase inhibition is observed with concentrations of isolated R_2^{II} on the order of 100 nM. Cellular concentrations of cAMP-dependent protein kinase in a variety of tissues have been estimated to range from 0.2 to 0.7 μM (20, 31). However, compartmentalization within the cell may raise local concentrations of protein kinase to even higher levels. Recent reports have indicated that compartmentalization of cAMP and cAMP-dependent protein kinase

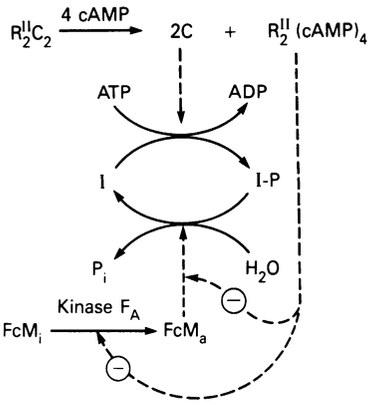

FIG. 6. Scheme for the synchronous regulation of cAMP-dependent protein kinase and the Mg(II)·ATP-dependent phosphatase. F_CM_i and F_CM_a, inactive and active forms of F_C·M; I and I-P, dephosphorylated and phosphorylated forms of the interconvertible substrate I; $R_2^{II}C_2$, cAMP-dependent protein kinase holoenzyme; C, protein kinase catalytic subunit; $R_2^{II}(cAMP)_4$, protein kinase regulatory subunit dimer with bound cAMP.

may be involved in segregating responses to different stimuli, which are mediated through cAMP-dependent protein kinase (32). We are currently investigating the possible inhibition of the Mg(II)·ATP-dependent phosphatase by the regulatory subunit of other types of cAMP-dependent protein kinase.

The role envisaged for the coordinate regulation of type II cAMP-dependent protein kinase and this phosphatase is summarized in Fig. 6. This represents the situation for a monocyclic cascade utilizing these enzymes as converter enzymes that respectively phosphorylate and dephosphorylate the interconvertible substrate I. Increased cAMP concentration dissociates the protein kinase into its regulatory R_2^{II} and catalytic C subunits. C catalyzes phosphorylation of I, and simultaneously R_2^{II} inhibits the activated phosphatase and the activation of the phosphatase. This inhibition contributes to the increased fractional phosphorylation of I. Such a synchronous regulatory mechanism provides both signal enhancement and an enhanced sensitivity to the effector, cAMP. That is, a lower concentration of cAMP will be required to achieve an intermediate level of fractional phosphorylation, and changes in fractional phosphorylation will be more sensitive to changes in cAMP.

This effect may be multiplied further due to the fact that phosphatase F_C·M has a broad substrate specificity and dephosphorylates many of the phosphorylation sites of the phosphoproteins involved in the glycogen cascade system. Furthermore, the coincident activation of protein kinase activity and inhibition of protein phosphatase activity reduces the ATP consumption of this system, thus making it more energetically efficient (33).

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