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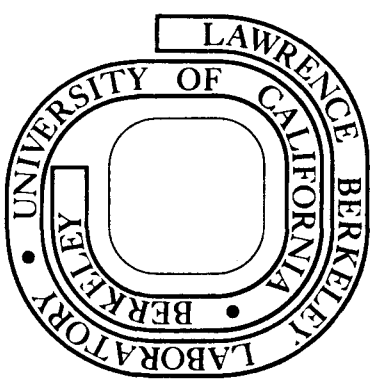

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Activation and Inhibition of Ribulose 1,5-Diphosphate Carboxylase by 6-Phosphogluconate

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ABSTRACT

Ribulose 1,5-diphosphate carboxylase, when activated by preincubation with 1 mM bicarbonate and 10 mM $MgCl_2$ in the absence of ribulose 1,5-diphosphate, remains activated for 20 min or longer after reaction is initiated by addition of ribulose diphosphate. If as little as 50 μM 6-phosphogluconate is added during this preincubation period, 5 min before the start of the reaction, a further 190% activation is observed. However, addition of 6-phosphogluconate at the same time or later than addition of ribulose diphosphate, or at any time with 50 mM bicarbonate, gives inhibition of the enzyme activity. It is proposed that enzyme activity is determined by the ratio of ribulose diphosphate bound to the enzyme to carbon dioxide bound to the enzyme (facilitated by Mg^{++}), and that 6-phosphogluconate may prevent inactivation by ribulose diphosphate, possibly by binding at certain allosteric sites in place of RuDP. It is proposed that a second effect of 6-phosphogluconate is inhibition due to binding competitively with ribulose diphosphate at active catalytic sites. Possible relevance of these effects to *in vivo* regulatory effects is discussed.

Work performed under the auspices of the U. S. Atomic Energy Commission

The carboxylation enzyme of the photosynthetic reductive pentose phosphate cycle, ribulose 1,5-diphosphate carboxylase (RuDPCase), mediates the reaction of ribulose 1,5-diphopshate (RuDP) with CO_2 and water to give two molecules of 3-phosphoglycerate (3-PGA) (25,26). This reaction appears to be subject to metabolic regulation in vivo, since it is accompanied by a high negative free energy charge under conditions of steady-state photosynthesis (1,3), and thus is a rate-limiting step. When Chlorella pyrenoidosa have been photosynthesizing with $^{14}CO_2$, and either the light is turned off (17), vitamin K_5 is added (10), or octanoate is added (16), the levels of labeled metabolite change in a way indicating a shift from operation of the reductive pentose phosphate cycle to oxidative pentose phosphate cycle. Among the manifestations of this shift are the inactivation of phosphoribulokinase (which converts ribulose 5-phosphate and ATP to RuDP and ADP) and the inactivation of RuDPCase. At the same time, labeled 6-phosphogluconate (6-PGluA) appears, indicating the onset of the operation of the oxidative pentose phosphate cycle.

Strong inhibition of isolated RuDPCase can be caused by levels of 6-PGluA (6,24) much lower than the levels of other metabolites such as fructose-1,6-diphosphate, which can also inhibit this enzyme (5,6). In our previous report of this inhibition of RuDPCase by 6-PGluA (6), the reaction was initiated by adding the enzyme to the otherwise complete reaction mixture. It had been reported that preincubation of the enzyme with bicarbonate and Mg^{++} ions increased the activity of the enzyme when assayed for 5 min (11,18). In the course of further studies of the effects of 6-PGluA on the activity of RuDPCase, we have found that preincubation of the enzyme with Mg^{++} and bicarbonate produces a long-

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lasting activation of the enzyme, and that inclusion of 6-PGluA in the preincubation mixture causes a large additional activation of the enzyme. However, when the enzyme is activated by preincubation with Mg^{++} and bicarbonate and the reaction is initiated with addition of RuDP, subsequent addition of 6-PGluA causes inhibition. These and other results in this study suggest a complex regulatory mechanism, involving inactive and active forms of the enzyme, which respond differently to 6-PGluA, with the response further modified by presence or absence of RuDP, concentration of bicarbonate, etc.

EXPERIMENTAL

Materials. In addition to the materials used in a previous report (6), glucuronic acid 1-phopshate (GlcuA 1-P), the potassium salt, was purchased from Sigma Chemical Co. Sodium salt of 6-PGluA was used in the present studies.

Enzyme Isolation. The enzyme was isolated from spinach leaves. The isolation method was the same as the one previously described (6) except in the following respects: a, the step of heating at 50 C was omitted, and b, 0.02 M phosphate buffer solution containing 0.1 mM EDTA at pH 7.4-7.5 was used instead of 0.05 M tris-HCl buffer solution with 10 mM $MgCl_2$, 2 mM glutathione, and 0.2 mM EDTA at pH 7.4. The isolated enzyme was stored in the precipitated form under 60% saturation of ammonium sulfate in 0.02 M phosphate buffer, pH 7.4-7.5. The purified enzyme had a specific activity of about 1.2 unit/mg protein.

Assay Method. The enzyme activity was determined by measurement of the radioactive acid-stable compound formed when $^{14}CO_2$ is used as a substrate in the reaction mixture. Because different assay procedures have been found to give very different results, we shall describe

detailed experimental methods used in assaying the activities. The assays were conducted in the 17x60 mm glass vials. Each vial was first flushed with N_2 gas and sealed with a rubber serum stopper. Tris-HCl buffer solution and solutions of RuDP, $NaH^{14}CO_3$, effectors, and enzyme were injected into the vials by microsyringes at different stages according to the assay method used. All the solutions, except $NaH^{14}CO_3$, had been bubbled with N_2 gas in order to remove CO_2 and O_2 dissolved in the solution from the air.

The stored enzyme was first dissolved in phosphate buffer and then dialyzed against the same buffer with at least six changes. The dialyzed enzyme solution was centrifuged. The supernatant enzyme solution usually contained 50 mg/ml of protein. Before the experiment, this enzyme solution was diluted with tris-HCl buffer, 0.1 M, pH 7.8, which contained either 10 mM or no $MgCl_2$. (Tris-HCl buffer had been bubbled with N_2 gas previously, but not the enzyme solution.) Usually a 200-fold diluted enzyme solution was used for the assay. In all assays, unless otherwise indicated, the final concentration of each component was the following: tris-HCl, 0.1 M; pH 7.8; $MgCl_2$, 10 mM; RuDP, 0.5 mM; enzyme, 10 μg; $NaH^{14}CO_3$, 50 mM (0.10 μC/μm) or 1 mM (4.1 μC/μm). The amounts of effector (inhibitor or activator), the preincubation and/or reaction time (see below) are indicated for each experiment. The final volume of the reaction mixture was 0.4 ml. /At the end of the reaction, 0.1 ml of glacial acetic acid was added to stop the reaction. Then the vials were flushed with N_2 gas at room temperature to dryness. 0.5 ml water was added to dissolve the remaining material in the vial. Six ml of Aquasol was then added. After mixing, the vials in the vial holders (IsoLab Inc., Akron, Ohio) were counted in the liquid scintillation spectrometer. With

All the preincubations and reactions were conducted at 25 C.

^{14}C-toluene as internal standard, the counting efficiency for carbon-14 with Aquasol and the small vial in the holder was about 75%.

The preincubation time is the length of time in which the enzyme was incubated with part(s) of the reaction ingredients before the start of the reaction. The reaction time is the length of time in which the reaction had taken place.

Assay Method I. The reaction was started by adding the enzyme to the reaction mixture which contained all the required ingredients.

Assay Method II. As in I, except that the enzyme had been preincubated with $MgCl_2$ for at least 10 min.

Assay Method III. The enzyme had been preincubated with $NaH^{14}CO_3$ for 10 min; then the reaction was started by adding the mixture of RuDP and $MgCl_2$.

Assay Method IV. The enzyme had been preincubated with $MgCl_2$ and $NaH^{14}CO_3$ for a specific length of time before adding RuDP to start the reaction.

For the studies of activation or inhibition, the order and procedure for adding the effector will be indicated below.

Assay Method V. Method II was used; the effector was added in the reaction mixture before the addition of the enzyme.

Assay Method VI. Method IV was used; the enzyme had been preincubated with the effector along with $MgCl_2$ and $NaH^{14}CO_3$ for a specific length of time before the addition of RuDP to start the reaction.

Assay Method VII. The enzyme had been preincubated with $MgCl_2$ and $NaH^{14}CO_3$ for a specific length of time before starting the reaction by adding the mixture of RuDP and the effector.

Assay Method VIII. The enzyme reactions were carried out in several vials according to Method II (preincubation with $MgCl_2$ but not with $NaH^{14}CO_3$); one reaction was stopped at 5 min. The effector was added to three other vials at the end of 5 min and the reactions were allowed to proceed for another 1.5, 4.5 and 7.5 min.

Assay Method IX. As in VIII, except that Method IV (preincubation with both $MgCl_2$ and $NaH^{14}CO_3$) was used instead of Method II.

For the kinetic studies, the assays were conducted in separate vials for various preincubation and/or reaction times. In the study of 6-PGluA activation, 6-PGluA was added to the enzyme which had been preincubated with $MgCl_2$ and $NaH^{14}CO_3$ so that the preincubation time with 6-PGluA in each vial was different but the total preincubation time with $MgCl_2$ and $NaH^{14}CO_3$ was kept the same.

The validity of inhibition and activation induced by 6-PGluA has been checked by the following control experiment. 6-PGluA was first generated by the reaction of 5 mM of glucose-6-phosphate (G-6-P) with 5 mM of NADP in the presence of glucose-6-phosphate dehydrogenase (10 units) in 0.1 M tris-HCl buffer, pH 7.8. In each of the other three tubes, either G-6-P, NADP, or G-6-P dehydrogenase was omitted. All the tubes were incubated for 20 min at 25 C. After the reaction, part of the reaction product in each tube was diluted 10-fold with the same buffer. An aliquot (40 µl) from each of the original and diluted reaction products was added to the vials for assay of RuDP carboxylase activity, using either Method V or Method VI, so that the concentrations of the effectors were decreased 10-fold (i.e., the final concentration in the complete system was 0.5 or 0.05 mM).

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In all the inhibition and activation studies, a control test (H_2O) and a comparison test (glucuronic acid-1-phosphate [GlcuA 1-P]) were also carried out at the same time.

Protein concentration was determined by UV absorption at 280 nm. The factor, O.D. = 1.0 for 0.61 mg enzyme/ml, was used for the calculation of protein concentration (15).

RESULTS

It was found that dissolved CO_2 in the buffer solution from air could activate the enzyme activity. Because the amount of CO_2 in the solution varies from time to time due to the storage condition, it is necessary to expel the dissolved CO_2 in the solution so that the activation effects due to preincubation with $MgCl_2$ and $NaH^{14}CO_3$ can be accurately determined.

With 1 mM $NaH^{14}CO_3$, the kinetic studies using Assay Methods I-IV showed that the enzyme gave by far the highest activity when it was preincubated with both $MgCl_2$ and $NaH^{14}CO_3$ (Method IV, Fig. 1). The enzyme when preincubated with only $MgCl_2$ (Method II), or $NaH^{14}CO_3$ (Method III), or no preincubation at all (Method I) gave only 1/10 of the activity obtained under the above preincubation conditions. While the highest rate with the enzyme preincubated with both Mg^{++} and $NaH^{14}CO_3$ was during the first 5 min, the rate remained about 6 times greater than the rate for the other assay conditions for the period from 5 to 20 min. The one which was preincubated with $MgCl_2$ had a slightly higher activity than the other two.

At a much higher concentration of $NaH^{14}CO_3$ (50 mM), the reaction rates from 9 to 20 min were almost the same (see Fig. 2), irrespective

of whether or not the enzyme was preincubated, although during the first 9 min the "preincubated" enzyme gave the higher activity.

The activities of enzymes which had been preincubated with $MgCl_2$ and $NaH^{14}CO_3$ for various lengths of time indicated that the enzyme reached the maximal activity after about 2 min preincubation at 25 C when the concentration of $NaH^{14}CO_3$ was low (1 mM) (Fig. 3). At high $NaH^{14}CO_3$ concentration (50 mM), 30 sec was enough to reach the maximum activity.

With 1 mM $NaH^{14}CO_3$, inhibition was observed when 6-PGluA was added to the reaction mixture 5 min after initiation of the reaction, whether the rate was high following preincubation with both Mg^{++} and $NaH^{14}CO_3$ (Assay Method IX, Fig. 4A) or low following preincubation with only $MgCl_2$ (Assay Method VIII, Fig. 4B). Similar inhibition was seen upon addition of 6-PGluA to reaction mixtures containing 50 mM $NaH^{14}CO_3$ 5 min after reaction initiation following preincubation according to Method IX (Fig. 5A) or Method VIII (Fig. 5B). The degree of inhibition in each case was calculated from the slope of reaction rate. Addition of GlcuA 1-P gave no appreciable inhibition in any of these cases.

It was found that 6-PGluA could either activate or inhibit the enzyme activity, and the effect was completely dependent on the assay method (Tables I, II). At low $NaH^{14}CO_3$, 6-PGluA activated the enzyme if the enzyme was preincubated with 6-PGluA as well as with $MgCl_2$ and $NaH^{14}CO_3$. A maximum of 190% activation was observed. Even after 15 min reaction time, substantial activation (about twofold) of the enzyme was observed in the presence of 50 μM 6-PGluA. In all of the other assay methods with 1 mM $NaH^{14}CO_3$, 6-PGluA gave strong inhibition. At high $NaH^{14}CO_3$, inhibition was observed in every case, although different

degrees of inhibition could be seen. In low $NaH^{14}CO_3$, every method, except Method VI ("preincubation"), gave almost the same degree of inhibition--about 60% inhibition by 0.5 mM 6-PGluA. GlcuA 1-P gave no significant effect. At high $NaH^{14}CO_3$, "no preincubation" Method V showed a stronger inhibition than the "preincubation" Method VI did (also see Fig. 9).

The enzyme reached its highest activity only after 3-4 min preincubation with 6-PGluA (0.05 mM) in the presence of $MgCl_2$ and $NaH^{14}CO_3$ (Fig. 6). This may suggest that the binding of 6-PGluA to the enzyme is rather a slow process.

When the enzymes were preincubated with various concentrations of 6-PGluA along with $MgCl_2$ and $NaH^{14}CO_3$ (1 mM), the maximum activation was obtained at 0.05-0.1 mM of 6-PGluA. As the concentration was increased beyond 0.1 mM, the activation decreased or an inhibition effect appeared. At as low concentration as 5 µM, 6-PGluA gave 70-80% activation (Fig. 7). However, with Method V ("no preincubation"), inhibition was obtained in every level of 6-PGluA (Fig. 8).

GlcuA 1-P was used as a comparison with 6-PGluA. Since both compounds have the same carboxyl and phosphate groups, differences in the charge effect and the ionic strength effect which might influence the enzyme activity are minimized. Preincubation of the enzyme with 2 mM GlcuA 1-P, in the presence of $MgCl_2$ and $NaH^{14}CO_3$ (Method VI), gave about 30% activation. The "activation" curve (Fig. 7) for enzyme preincubated with GlcuA 1-P was similar to curves for fructose-6-phosphate and inorganic phosphate. We were unable to confirm significant activation by 0.5 mM fructose-6-phosphate, reported by Buchanan and Schürmann (5). At that level of fructose-6-phosphate we saw less than 20% activation.

At 50 mM $NaH^{14}CO_3$, no activation could be observed by either method. Nevertheless, Method V ("no preincubation") gave stronger inhibition than Method VI ("preincubation") (see Fig. 9).

In order to check whether the above activation or inhibition effect is really induced by 6-PGluA, a control experiment was performed in which 6-PGluA was freshly generated using G-6-P, G-6-P dehydrogenase, and $NADP^+$. From the results (Table III) it is clear that 6-PGluA, not impurities in the chemicals purchased, was the cause of the activation or inhibition.

DISCUSSION

In discussing the requirement for preincubation of RuDPCase with Mg^{++} and $NaH^{14}CO_3$ to obtain maximal activity, Pon et al. (18) proposed three possible explanations: 1) The enzyme combines with both Mg^{++} and bicarbonate before the RuDP is carboxylated; 2) both Mg^{++} and bicarbonate activate the enzyme, but the activating bicarbonate is not necessarily the carboxylating species; and/or 3) since radioactive PGA is measured, the preincubation effect may reflect an exchange or displacement of unlabeled bicarbonate by labeled bicarbonate. The prolonged activation effect (20 min) which we observed, together with the fact that unlabeled bicarbonate was carefully excluded in our studies, rules out possibilities 1 (as a required reaction mechanism at the active site) and 3 as explanations of the preincubation effects. Of course, the order of binding of substrates could still be as in 1, but neither our data nor that of Pon et al. can provide an answer to the question of order of substrate binding.

It might be suggested that the even higher reaction rate of the enzyme during the first 5 min of reaction following preincubation could be at

least in part the result of the carboxylation using already bound carbon dioxide, the species which has been shown to be involved in the carboxylation mechanism (7). It should be noted that in all our experiments $H^{14}CO_3^-$ was added to the buffer at least 30 min prior to initiation of the reaction, so that complete equilibration of CO_2 and HCO_3^- species was assured. However, to account for the increased rate seen during the first 5 min (compared to the subsequent period) for the preincubated enzyme, about 100 molecules of CO_2 would have to be bound to the enzyme at the start of the reaction period. A more likely explanation of the higher initial rate, decreasing over the first 5 min, would seem to be that binding of RuDP to the enzyme decreases its activity. The enzyme would be in the most active form at the start, and binding of RuDP until some steady-state number of binding sites are occupied might take several minutes.

Since the higher rate of enzymic reaction persists for as long as 20 min, we suggest that preincubation with Mg^{++} and HCO_3^- either modifies the enzyme conformation to give a more active form, or prevents excessive binding of RuDP to the enzyme, or both. Since the enzyme presented with RuDP without preincubation with HCO_3^- and Mg^{++} is much less active and remains inactive for at least 20 min, it is suggested that RuDP binding by the non-preincubated enzyme does convert the enzyme to (or maintain it in) a less active form from which it cannot change in low levels (1 mM) HCO_3^- as long as RuDP is present. If RuDP had not had any influence on the enzyme conformation and activity, the reaction rate should increase after several minutes (in the non-preincubated case). It is proposed that the enzyme is in the active form as long as it is in an environment

of Mg^{++} and CO_2, and is never presented with RuDP in the absence of Mg^{++} and CO_2. (However, as shown in Fig. 2, the effect caused by RuDP without preincubation with CO_2 and Mg^{++} can be overcome by high levels of CO_2.)

It is known that RuDPCase contains a number of subunits and many binding sites for substrates (20,21,23,28). It is suggested that the activity of the enzyme depends in some way on the number of CO_2 molecules and RuDP molecules bound to the enzyme, and that a high number of CO_2 molecules bound to the enzyme tends to reduce the number of RuDP molecules bound to the enzyme, but not necessarily at the active catalytic sites. It is further suggested that RuDP binding at some sites tends to prevent CO_2 binding at activating sites, thus reducing the activity of the enzyme. Sufficiently high levels of bicarbonate provide enough CO_2 to overcome the effects of the RuDP binding at sites which inactivate the enzyme.

In one model, the activating molecules of CO_2 might be all bound at catalytic sites. When RuDP then became bound at one such site, and the two substrates were converted to PGA, CO_2 bound at other active sites would keep the enzyme active. Even if another RuDP were to bind immediately at that same site (before CO_2 binding), the enzyme would remain active due to CO_2 molecules bound at other sites. The enzyme could only be converted to the inactive form if CO_2 were depleted and RuDP allowed to bind enough sites without reaction to change the conformation of the enzyme to the form with a high binding constant for CO_2.

Another possible model is one in which some or all of the activating molecules of CO_2 are bound at allosteric sites. Again, the binding of CO_2 to such sites, necessary for activation of the enzyme, would be prevented by the prior binding of RuDP to a number of sites on the enzyme.

Just as it is not possible from our data to say whether the activation of the enzyme by Mg^{++}-mediated binding of CO_2 is due to binding at allosteric sites, catalytic sites, or both, we cannot say whether the proposed inhibitory conformational change is due to RuDP binding at allosteric sites, active sites, or both. However, the effects of 6-PGluA, discussed below, suggest that there may be both allosteric and active site binding of RuDP.

As previously reported, and as seen in these studies (Tables I and II) 6-PGluA inhibits the enzyme when added with or after RuDP, or under any condition with 50 mM HCO_3^-. Surprisingly, when the enzyme is preincubated with 1 mM $H^{14}CO_3^-$ and Mg^{++}, and when 6-PGluA is added during the preincubation period, considerable additional activation of the enzyme can be seen. The maximum such activation occurs with only 0.1 mM 6-PGluA. An activation by 75% is seen with only 5 μM 6-PGluA. Thus, the 6-PGluA is a very specific effector under these conditions. At 2 mM, a net inhibitory effect is observed.

Activation (with preincubated RuDPCase) is not seen when the 6-PGluA is added simultaneously with the RuDP. Thus, it appears that the binding of RuDP is faster than the binding of 6-PGluA at those sites involved in the conversion of active to inactive enzyme. Furthermore, binding of RuDP prior to incubation of the enzyme with Mg^{++}, CO_2 and 6-PGluA precludes not only the activation by Mg^{++} and CO_2, but also the additional activation by 6-PGluA.

As one possible model to account for these effects, we suggest that RuDP can be bound not only at active sites but also at RuDP allosteric sites (R-A sites). We further propose that such binding by RuDP decreases the binding of CO_2 in the presence of Mg^{++}, perhaps by inducing a conformational

change in the enzyme. It is also appropriate to this model to specify that the prior binding of CO_2 (with Mg^{++} present) reduces or prevents the binding of RuDP at the R-A sites. This mutual antagonism of binding seems required to account for the persistence of the active and inactive forms.

The additional activation (when enzyme is preincubated with CO_2 plus Mg^{++}) due to 6-PGluA may be due to further conformational change, or could be the result of 6-PGluA binding to the same R-A sites, but without conformational change. If 6-PGluA does bind to these R-A sites, such binding could prevent some binding by RuDP when it is subsequently added. The binding of 6-PGluA is a relatively slow process as indicated by the fact that maximum stimulation of the enzyme by preincubation with CO_2, Mg^{++} and 6-PGluA requires about 5 min (Fig. 6). The slow response of the enzyme to 6-PGluA might be an example of the hysteretic phenomenon proposed by Frieden (8). The preincubation effect of CO_2 and Mg^{++} alone required 2 min (Fig. 3), and the binding of RuDP must be assumed to be even faster, since simultaneous addition of RuDP, Mg^{++} and CO_2 gives the inactive form of the enzyme.

The inhibition by 6-PGluA reported previously, and seen under all conditions except preincubation with Mg^{++} and low CO_2, may be due to competition of 6-PGluA with RuDP at the active catalytic sites. Kinetic analysis of the inhibition of the enzyme by 6-PGluA without preincubation suggested that the inhibition might be of the linear non-competitive type, but, as we pointed out, simple kinetic treatment might not be strictly valid for RuDPCase, which has several subunits, two substrates, multiple binding sites, etc. Thus, we are now inclined to view the inhibition by 6-PGluA as due to competition with RuDP for the RuDP-catalytic binding sites, while the rather small displacement of the crossing point of the

reciprocal plots (1/V vs. 1/[RuDP]) for different 6-PGluA concentrations from the zero value of 1/[RuDP] could be due to the effect of 6-PGluA in affecting the binding of RuDP at the allosteric RuDP binding sites.

What significance can be attached to the regulation of RuDPCase by levels of RuDP, CO_2, Mg^{++} and 6-PGluA? When the light is on, and physiological conditions are favorable for photosynthesis, we may assume that the level of Mg^{++} is high, CO_2 is not too low, RuDP concentration is adequate, 6-PGluA is nearly absent, and RuDPCase is active. If CO_2 pressure falls too low, RuDP concentration should, if anything, increase. Given this condition of very low CO_2 and high RuDP concentration, the enzyme may be converted to the less active form. In vitro, RuDPCase has been found to bind O_2 competitively with CO_2 (14). This binding is reportedly followed by oxidation of RuDP to PGA and phosphoglycolate. If binding of O_2 decreases along with binding of CO_2 in the inactive form of the enzyme, this inactivation could decrease the tendency for RuDP to be oxidized. Avoidance of this oxidation of RuDP would be beneficial to the plant cell irrespective of whether oxidation of RuDP in vivo is an important route in the formation of glycolate and subsequent photorespiration, a question still subject to controversy (19,22).

Returning to normal physiological conditions, let us next consider the light to dark transition. If the level of Mg^{++} free in the soluble part of the chloroplasts decreases greatly, as indicated by the study of Lin and Nobel (12,13), this would of itself decrease the activity of the active form of RuDPCase, which has a pH dependence shift to the alkaline with reduced Mg^{++} (4). The appearance of 6-PGluA during the first minute of darkness would further inhibit the enzyme. These and perhaps other

effects (9,27) may account for the slow rate of the carboxylation reaction observed in isolated chloroplasts and in Chlorella pyrenoidosa after about 2 min darkness.

After a long period of darkness, the level of RuDP would have gone to zero. The level of CO_2 inside chloroplasts would be much higher in the dark, due to absence of photosynthetic uptake, and generation of CO_2 by the oxidative pentose phosphate cycle. When the light is turned on again, particularly if the intensity is low, low levels of 6-PGluA will still be present even in bright light for the few minutes after a period of darkness (2). When the light comes on, the Mg^{++} level will also increase, so that the enzyme in vivo would be "preincubated" with CO_2, Mg^{++} and a low concentration of 6-PGluA, the conditions required for activation. Thus, RuDP can be actively carboxylated as soon as it is formed from ribulose-5-phosphate.

1. BASSHAM, J. A., AND M. CALVIN. 1957. The path of carbon in photosynthesis. Prentice-Hall, Inc., Englewood Cliffs, N. J. pp. 1-107.

2. BASSHAM, J. A., AND M. KIRK. 1968. Dynamic metabolic regulation of the photosynthetic carbon reduction cycle. In: K. Shibata, A. Takamiya, A. T. Jagendorf, and R. C. Fuller, eds., Comparative Biochemistry and Biophysics of Photosynthesis. University of Tokyo Press. pp. 365-378.

3. BASSHAM, J. A., AND G. H. KRAUSE. 1969. Free energy changes and metabolic regulation in steady-state photosynthetic carbon reduction. Biochim. Biophys. Acta 189: 207-221.

4. BASSHAM, J. A., P. SHARP, AND I. MORRIS. 1968. The effect of Mg^{++} concentration on the pH optimum Michaelis constants of the spinach chloroplast ribulosediphosphate carboxylase. Biochim. Biophys. Acta 153: 898-900.

5. BUCHANAN, B. B., AND P. SCHURMANN. 1972. A regulatory mechanism for CO_2 assimilation in plant photosynthesis: Activation of ribulose-1,5-diphosphate carboxylase by fructose-6-phosphate and deactivation by fructose-1,6-diphosphate. FEBS Letters 23: 157-159.

6. CHU, D. K., AND J. A. BASSHAM. 1972. Inhibition of ribulose-1,5-diphosphate carboxylase by 6-phosphogluconate. Plant Physiol. 50: 224-227.

7. COOPER, T. G., D. FILMER, M. WISHNICK, AND M. D. LANE. 1969. The active species of "CO_2" utilized by ribulose diphosphate carboxylase. J. Biol. Chem. 244: 1081-1083.

8. FRIEDEN, C. 1970. Kinetic aspects of regulation of metabolic processes. The hysteretic enzyme concept. J. Biol. Chem. 245: 5788-5799.

9. JENSEN, R. G., AND J. A. BASSHAM. 1968. Photosynthesis by isolated chloroplasts. III. Light activation of the carboxylase reaction. Biochim. Biophys. Acta 153: 227-234.

10. KRAUSE, G. H., AND J. A. BASSHAM. 1969. Induction of respiratory metabolism in illuminated Chlorella pyrenoidosa and isolated spinach chloroplasts by the addition of vitamin K_5. Biochim. Biophys. Acta 172: 553-565.

11. KUEHN, G. D., AND B. A. McFADDEN. 1969. Ribulose 1,5-diphosphate carboxylase from Hydrogenomonas eutroph and Hydrogenomonas facilis. I. Purification, metallic ion requirements, inhibition and kinetic constants. Biochemistry 8: 2394-2402.

12. LIN, D. C., AND P. S. NOBEL. 1971. Control of photosynthesis by Mg^{++}. Arch. Biochem. Biophys. 145: 622-632.

13. NOBEL, P. S. 1969. Light-induced changes in the ionic content of chloroplasts in Pisum sativum. Biochim. Biophys. Acta 172: 134-143.

14. OGREN, W. L., AND G. BOWES. 1971. Ribulose diphosphate carboxylase regulates soybean photorespiration. Nature New Biology 230: 159-160.

15. PAULSEN, J. M., AND M. D. LANE. 1966. Spinach ribulose diphosphate carboxylase. I. Purification and properties of the enzyme. Biochemistry 5: 2350-2357.

16. PEDERSEN, T. A., M. KIRK, AND J. A. BASSHAM. 1966. Inhibition of photophosphorylation and photosynthetic carbon cycle reactions by fatty acids and esters. Biochim. Biophys. Acta 112: 189-203.

17. PEDERSEN, T. A., M. KIRK, AND J. A. BASSHAM. 1966. Light-dark transients in levels of intermediate compounds during photosynthesis in air-adapted Chlorella. Physiol. Plant. 19: 219-231.

0 0 3 3 3 9 0 0 4 9 7

18. PON, N. G., B. R. RABIN, AND M. CALVIN. 1963. Mechanism of the carboxydismutase reaction. I. The effect of preliminary incubation of substrates, metal ion and enzyme on activity. Biochem. Z. 338: 7-19.

19. RICHARDSON, K. E., AND TOLBERT, N. E. 1961. Phosphoglycolic acid phosphatase. J. Biol. Chem. 236: 1285-1290.

20. RUTNER, A. C. 1970. Estimation of the molecular weight of ribulose diphosphate carboxylase sub-units. Biochem. Biophys. Res. Commun. 39: 923-929.

21. RUTNER, A. C., AND M. D. LANE. 1967. Nonidentical subunits of ribulose diphosphate carboxylase. Biochem. Biophys. Res. Commun. 28: 531-537.

22. SHAIN, Y., AND GIBBS, M. 1971. Formation of glycolate by a reconstituted spinach chloroplast preparation. Plant Physiol. 48: 325-330.

23. SUGIYAMA, T., AND T. AKAZAWA. 1970. Subunit structure of spinach leaf ribulose 1,5-diphosphate carboxylase. Biochemistry 9: 4499-4504.

24. TABITA, F. R., AND B. A. McFADDEN. 1972. Regulation of ribulose 1,5-diphosphate carboxylase by 6-phospho-D-gluconate. Biochem. Biophys. Res. Commun. 48: 1153-1159.

25. WEISSBACH, A., AND B. L. HORECKER. 1955. Enzymatic formation of phosphoglyceric acid from ribulose diphosphate and CO_2. Fed. Proc. 14: 302-303.

26. WEISSBACH, A., B. L. HORECKER, AND J. HURWITZ. 1956. The enzymatic formation of phosphoglyceric acid from ribulose diphosphate and carbon dioxide. J. Biol. Chem. 218: 795-810.

27. WILDNER, G. F., AND R. S. CRIDDLE. 1969. Ribulose diphosphate carboxylase. I. A factor involved in light activation of the enzyme. Biochem. Biophys. Res. Commun. 37: 952-960.

28. WISHNICK, M., AND M. D. LANE. 1970. The interaction of metal ion with ribulose 1,5-diphosphate carboxylase from spinach. J. Biol. Chem. 245: 4939-4947.

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Figure Captions

Fig. 1. Fixation of $^{14}CO_2$ via the carboxylation reaction with or without preincubation *vs.* time of reaction. Preincubation time: 10 min. $NaH^{14}CO_3$, 1 mM.

Fig. 2. Fixation of $^{14}CO_2$ via the carboxylation reaction with or without preincubation *vs.* time of reaction. Preincubation time: 10 min. $NaH^{14}CO_3$, 50 mM.

Fig. 3. Dependence of carboxylation reaction on time of preincubation of carboxylase with $MgCl_2$ and $NaH^{14}CO_3$ (1 mM). Reaction time: 5 min.

Fig. 4. The effects induced by adding effectors into the reacting carboxylation reaction mixture. Arrows indicate the time of additions of effectors. $NaH^{14}CO_3$, 1 mM. A. Enzyme had been preincubated with $MgCl_2$ and $NaH^{14}CO_3$ for 5 min (see Assay Method IX). B. Enzyme had been preincubated with $MgCl_2$ only (see Assay Method VIII).

Fig. 5. Same as in Fig. 4 except $NaH^{14}CO_3$ was 50 mM.

Fig. 6. Dependence of the carboxylation reaction on the time of preincubation of carboxylase with 6-PGluA (0.05 mM) in the presence of $MgCl_2$ and $NaH^{14}CO_3$ (1 mM). Total preincubation time with $MgCl_2$ and $NaH^{14}CO_3$: 5 min; reaction time: 5 min.

Fig. 7. The effects on carboxylase activity caused by various concentrations of effectors with two different assay methods. $NaH^{14}CO_3$: 1 mM. Reaction time: 5 min (between 0 and 5 min). A. Enzyme had been preincubated with effector in the presence of $MgCl_2$ and $NaH^{14}CO_3$ for 5 min (see Assay Method VI). B. Enzyme had been preincubated with $MgCl_2$ only (see Assay Method V).

Fig. 8. Expanded curve of B from Fig. 7.

Fig. 9. Same as in Fig. 7 except $NaH^{14}CO_3$ was 50 mM. Reaction time: 5 min (between 5 and 10 min).

Table I. Inhibition and Activation by 6-PGluA with 1 mM $NaH^{14}CO_3$

	% Activity of control (H_2O)				
	Assay Method				
Effector	V*	VI*	VII*	VIII**	IX**
H_2O	100	100	100	100	100
6-PGluA, 0.5 mM	41	177	43	39	45
GlcuA 1-P, 0.5 mM	93	128	94	95	105
6-PGluA, 0.05 mM	89	288	87	85	84
GlcuA 1-P, 0.05 mM	103	105	104	105	102

*Activities were the reaction rates between 0 and 5 min.

**Activities were measured from the slopes in Fig. 4.

Table II. Inhibition and activation by 6-PGluA with 50 mM $NaH^{14}CO_3$

	% Activity of control (H_2O)				
	Assay Method				
Effector	V*	VI*	VII*	VIII**	IX**
H_2O	100	100	100	100	100
6-PGluA, 0.5 mM	50	55	58	40	41
GlcuA 1-P, 0.5 mM	99	104	98	104	103
6-PGluA, 0.05 mM	87	89	85	84	85
GlcuA 1-P, 0.05 mM	104	99	103	98	102

*Activities were the reaction rates between 2.5 and 7.5 min; during this period, the rates were linear with time (see Fig. 2).

**Activities were measured from the slopes in Fig. 5.

Table III. Control Experiment

6-PGluA was generated from G-6-P, NADP, and G-6-P dehydrogenase. This 6-PGluA as well as other reaction products from the reaction systems in which either G-6-P, NADP, or G-6P dehydrogenase was omitted were used for the studies of inhibition and activation.

Incubation system	Assay Method V*		Assay Method VI†,*	
	activity	%	activity	%
All ingredients	871	38	50974	183
Minus NADP	2267	99	29296	105
Minus G-6-P	2173	95	34003	122
Minus G-6-P dehydrogenase	2239	98	30487	109
Minus all ingredients (control)	2291	100	27861	100
10-fold dilution of above				
All ingredients	1886	82	79978	287
Minus NADP	2234	98	28602	103
Minus G-6-P	2245	98	29090	104
Minus G-6-P dehydrogenase	2225	97	27490	99

*Reaction time: 5 min; $NaH^{14}CO_3$: 1 mM

†Preincubation time: 5 min

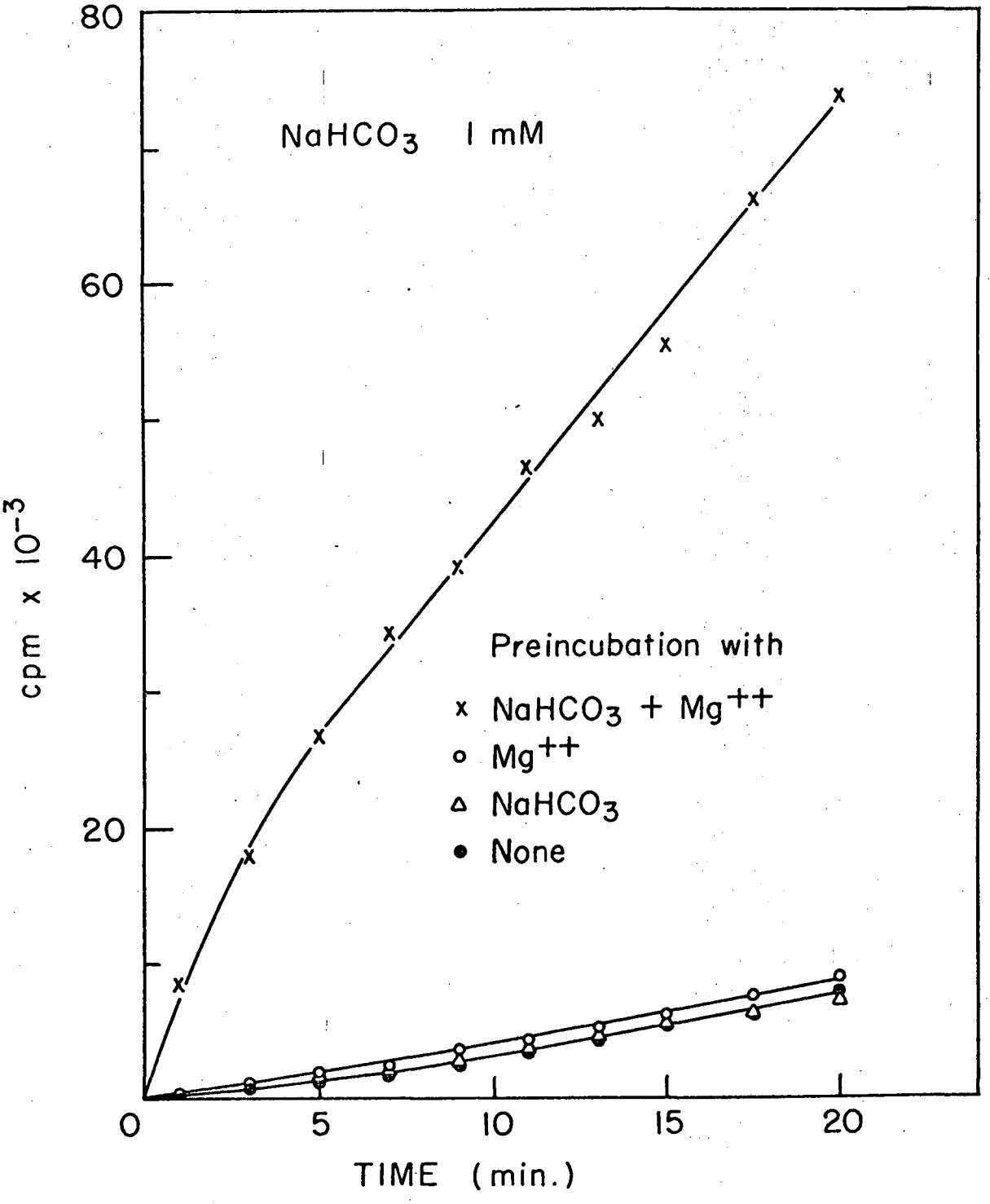

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Fig. 1

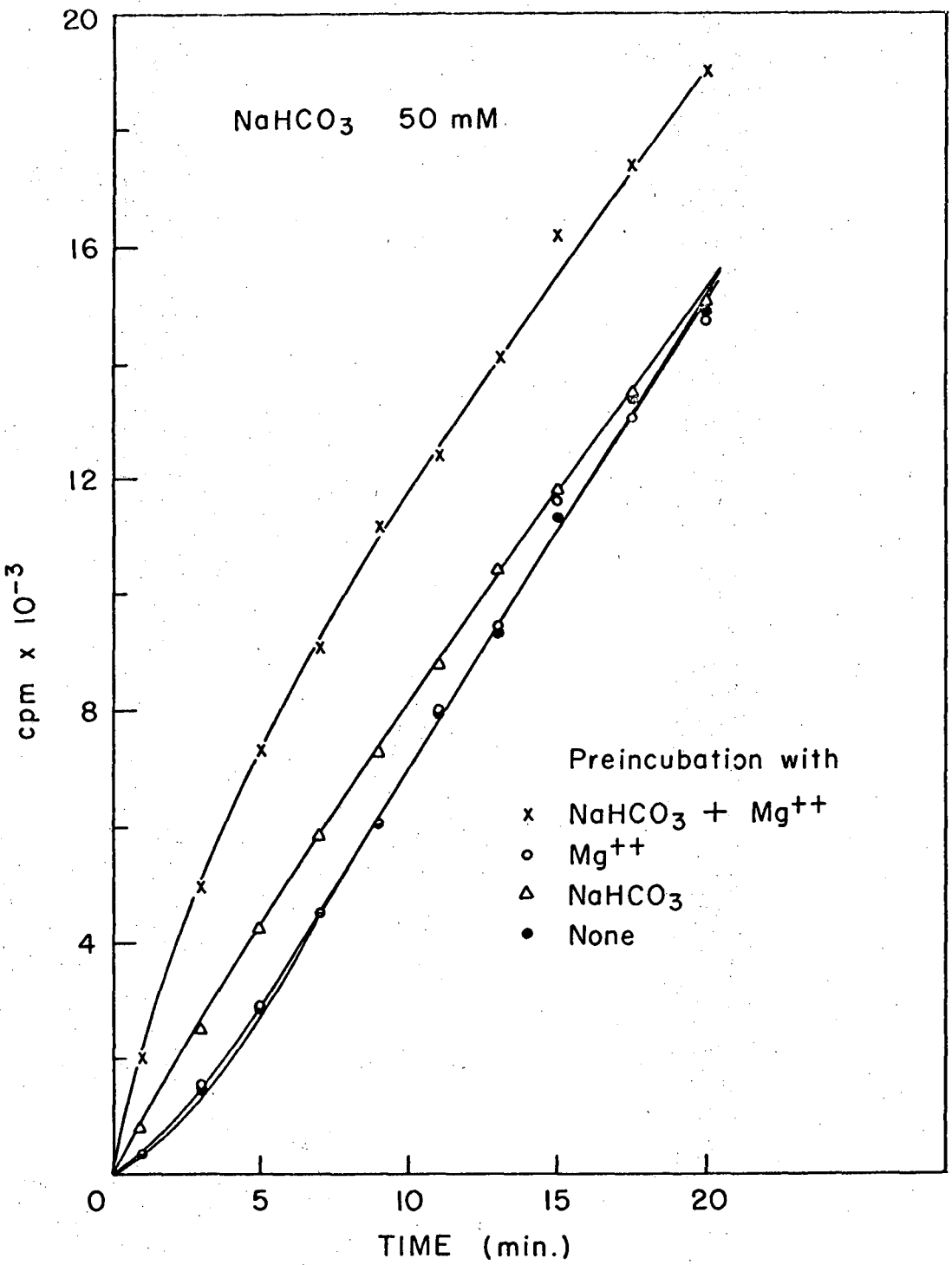

Fig. 2

XBL732-4665

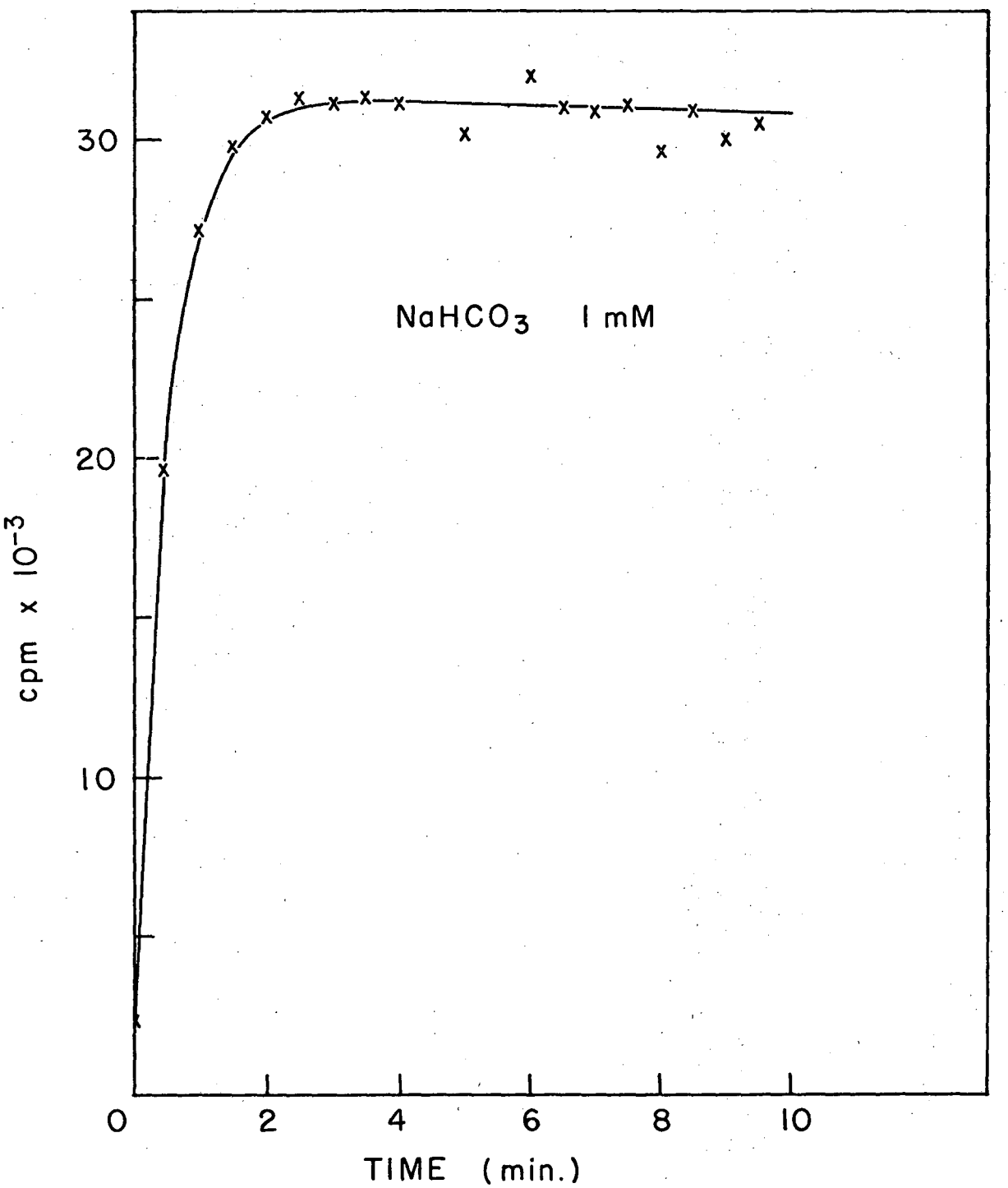

Fig. 3

XBL732-4668

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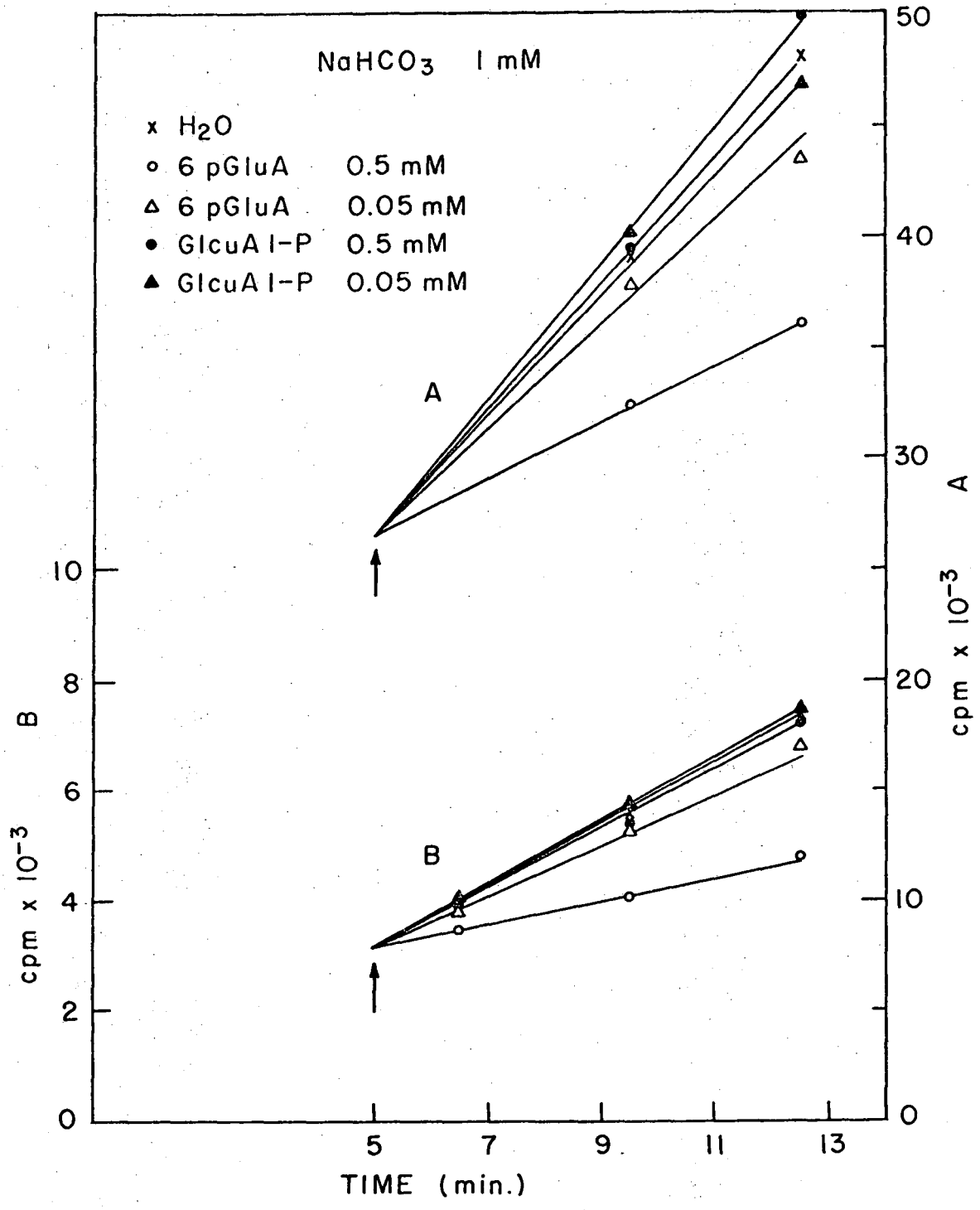
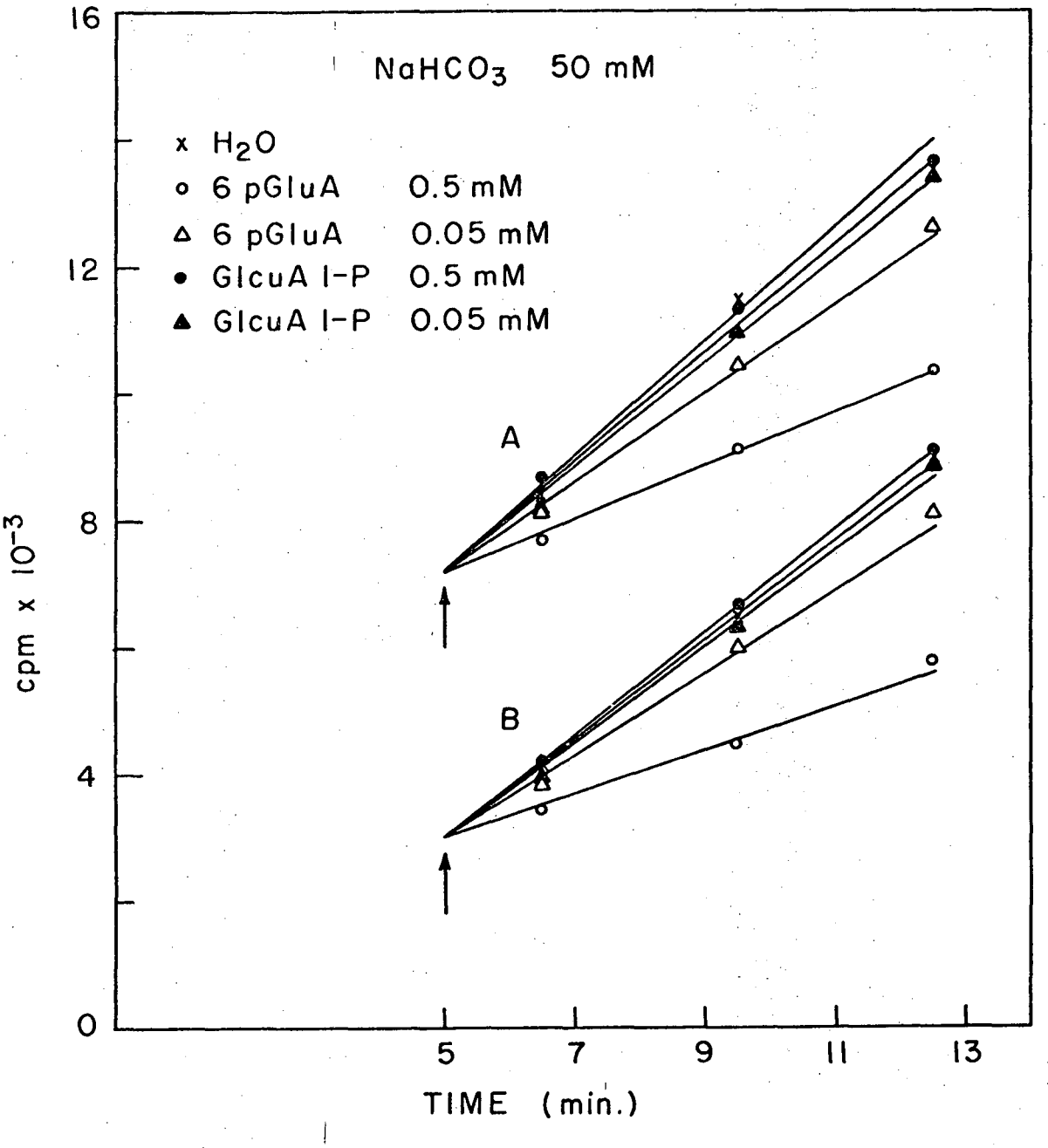


Fig. 4

XBL732-4666

Chu & Bassham



XBL732-4667

Fig. 5

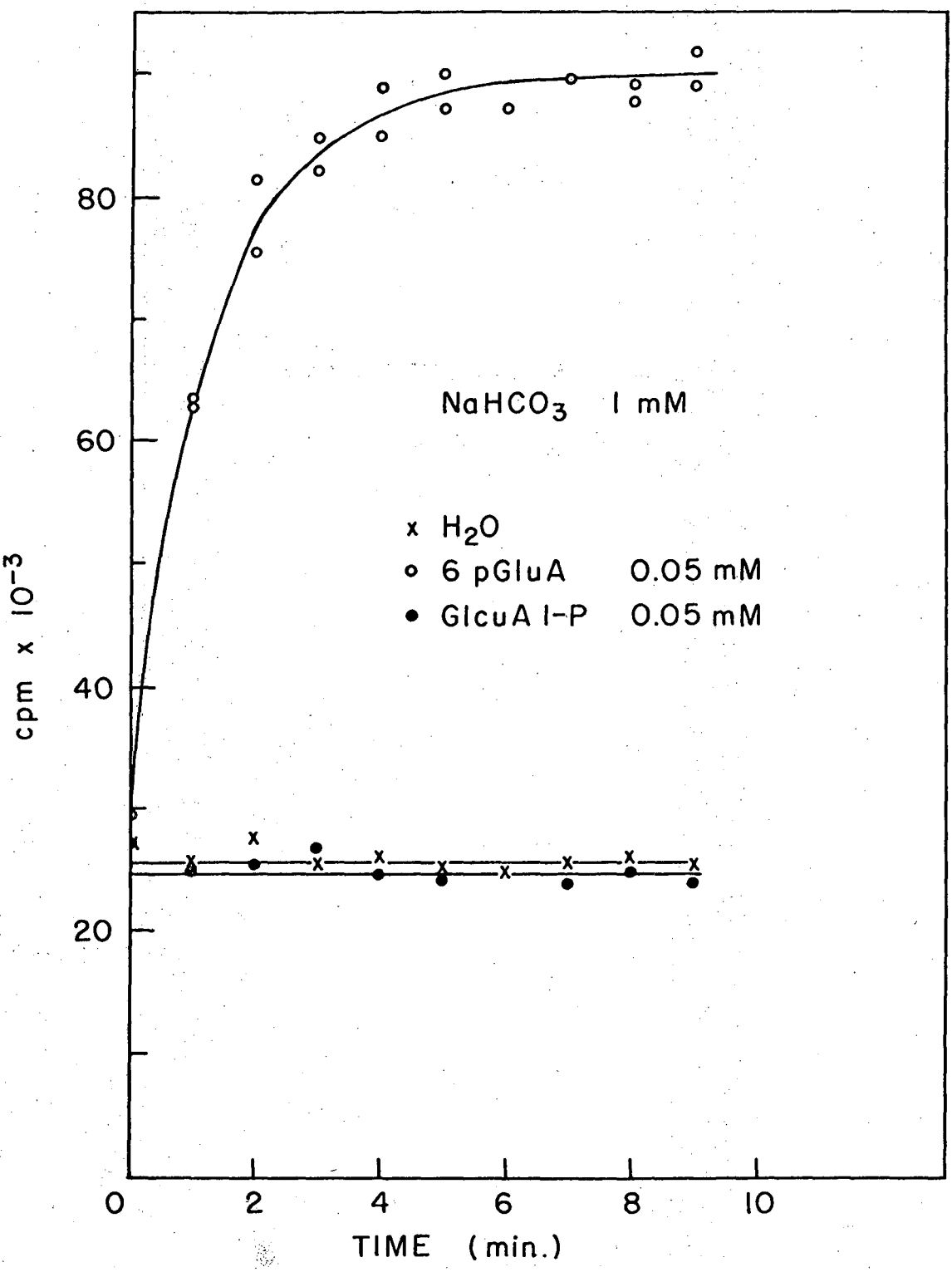


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Fig. 6

Chu & Bassham

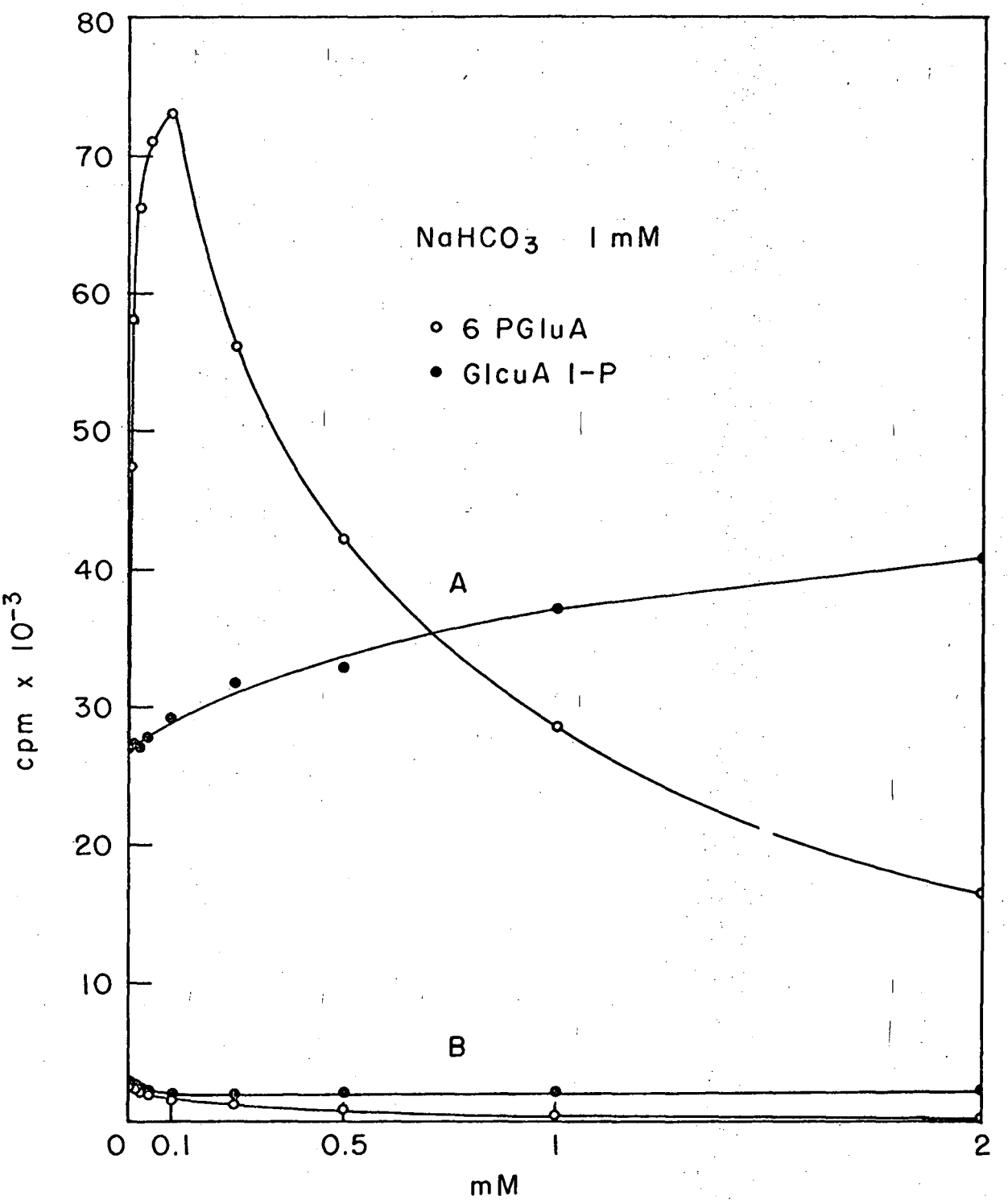

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Fig. 7

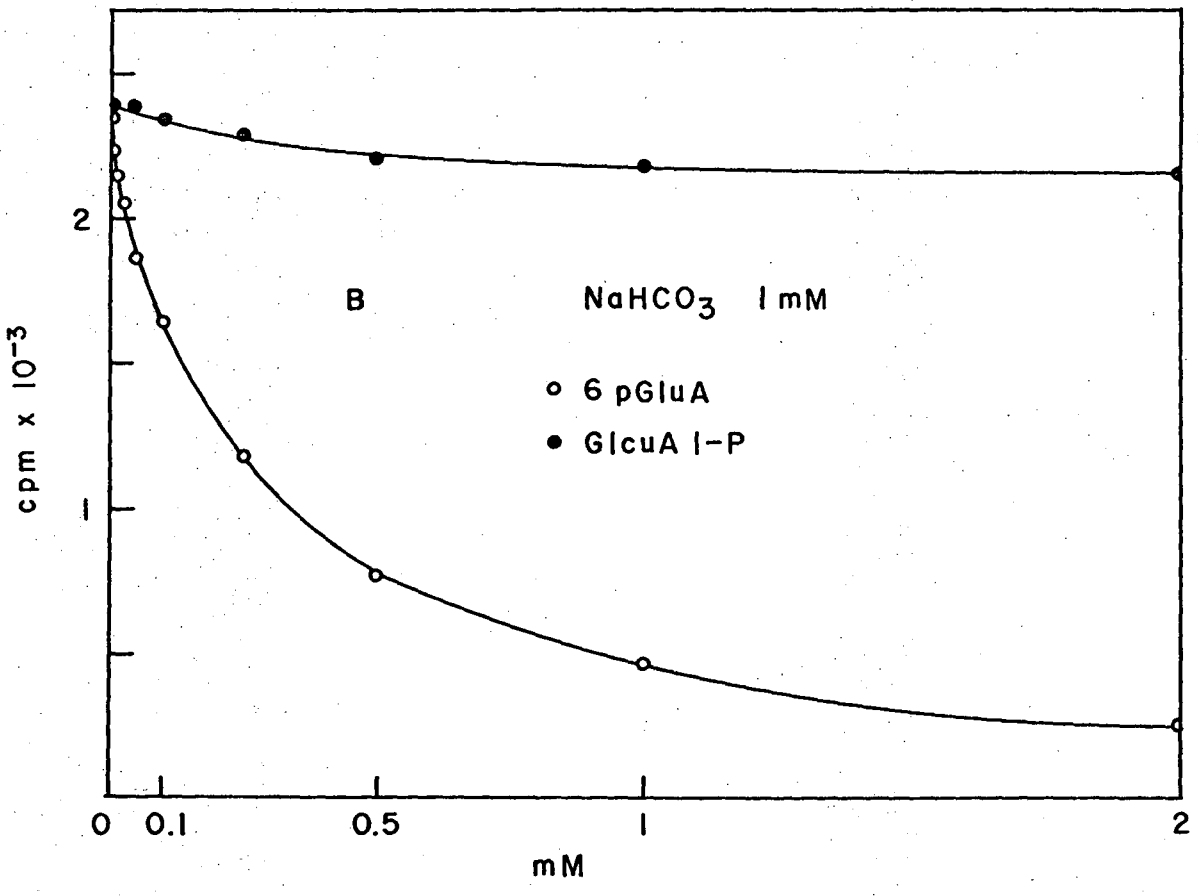

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Fig. 8

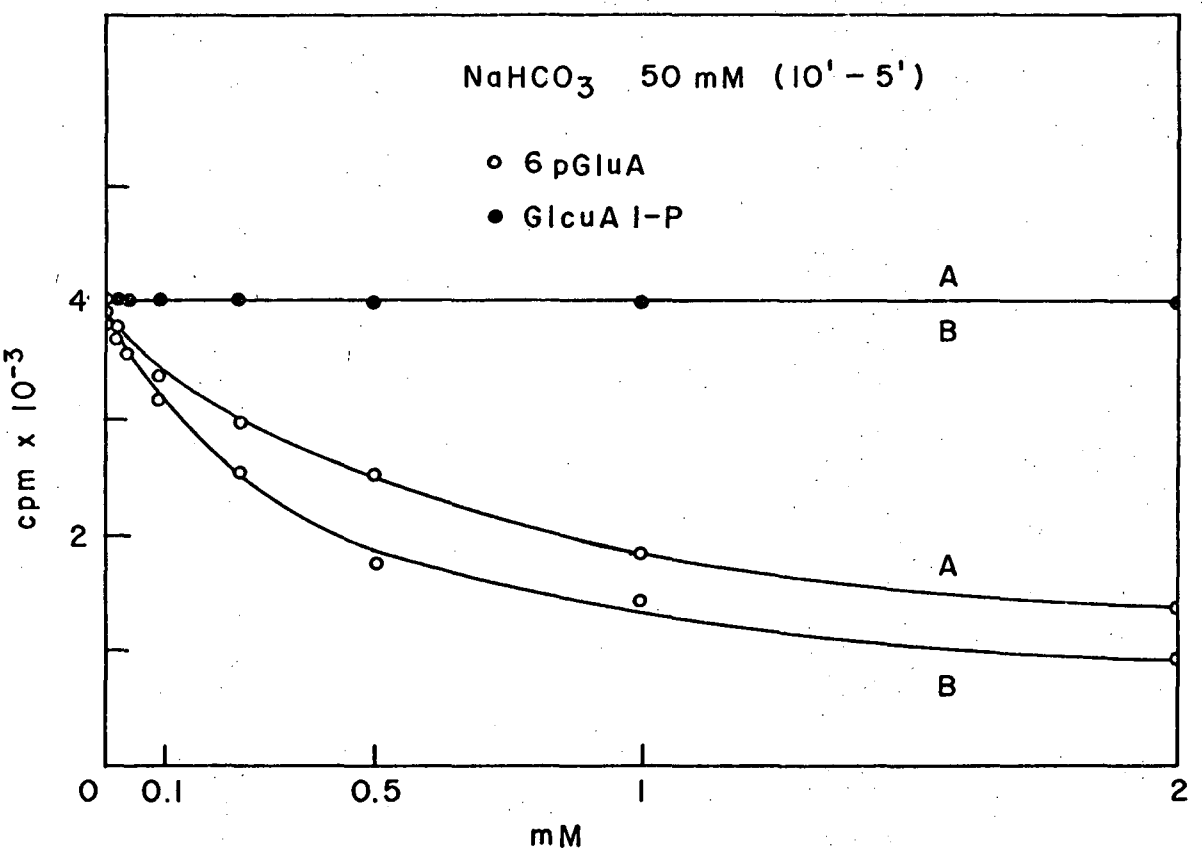

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Fig. 9

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