PRELIMINARY NOTES

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Presence in liver of a 3′:5′-cyclic AMP stimulated kinase for the *I* form of UDPG–glycogen glucosyltransferase

The existence of a kinase to convert the *I* form of liver glycogen transferase (UDPG:α-1,4-glucan α-4-glucosyltransferase, EC 2.4.1.11) to the *D* form was predicted from the rapid occurrence of this change in transferase activity promoted by glucagon administration to the insulin-treated normal dog[1]. Such a kinase might be analogous to the transferase *I* kinase of muscle both in its action[2] and in its activation by 3′:5′-cyclic AMP[3,4], according to the concept that liver phosphorylase activation occurs because glucagon promotes an accumulation of 3′:5′-cyclic AMP in liver which activates dephosphophosphorylase kinase[5]. Such an activation of phosphorylase was seen in these previous experiments, as well as a 2–3-fold increase in 3′:5′-cyclic AMP concentration in liver (unpublished results). We now report that a kinase enzyme activity is present in transferase prepared from particulate glycogen[6], and also that transferase can be prepared free of kinase and kinase free of transferase. When the two preparations are added together in the presence of ATP and Mg^{2+}, the *I* form of transferase is converted to the *D* form. This conversion is stimulated by added 3′:5′-cyclic AMP.

Extracts of liver, from normal dogs fed 50 g glucose 90 min prior to sacrifice, were prepared by the centrifugation at 12 000 × *g* for 20 min of a 1:3.5 homogenate in 0.05 M Tris–HCl, 0.005 M EDTA, 0.010 M Na_2SO_3 (pH 8.2). The proportion of "total" transferse, assayed (+ Glc-6-*P*), in the *I* form, assayed (− Glc-6-*P*), was increased to more than 90% by incubation of this extract at 30° for 60 min following addition of $MgCl_2$ to a 0.010 M concentration[7]. After a second centrifugation at 12 000 × *g* to remove denatured protein, the extract was centrifuged at 78 000 × *g* for 75 min. Using the homogenizing buffer, the particulate glycogen was gently rinsed free of the overlying microsomal layer, and either was resuspended in 0.40 M sucrose, 0.005 M EDTA (1/7 the original volume) and recentrifuged, or was resuspended (without this washing step) directly in 0.40 M sucrose without EDTA (1/7 the original volume). About 20% of the transferase in the pellet was separated from the particulate glycogen by a 10-min incubation at 37° and concentrated with addition of solid ammonium sulfate, according to STEINER, YOUNGER AND KING[6]. Similar preparations by this method had varying amounts of kinase relative to the transferase.

Greater recovery of transferase *I* from the particulate glycogen as well as removal of the kinase has been achieved by DEAE-cellulose column chromatography which also separates the transferase from phosphorylase. Furthermore, the kinase, essentially free of transferase *I*, has been prepared from the 150 000 × *g* supernatant of the above extract by concentration with 30% methanol and then ammonium sulfate fractionation.

Recovery studies, after centrifugation at 78 000 × *g*, indicate that the kinase in the 12 000 × *g* incubated extract is mainly in the soluble fraction but is also present in the microsomal sediment.

For assay of transferase *I* kinase the reaction mixture in 0.050 M Tris–HCl (pH 8.2), 0.005 M EDTA, 0.010 M Na_2SO_4, 0.005 M dithiothreitol contains the kinase sample sufficiently diluted so that no more than 50% of the transferase *I* is converted during the incubation. Transferase *I* (preincubated 30 min in the above buffer) is added and the kinase incubation, at 30°, is begun by the transfer of the above enzymes to tubes containing sufficient substrate and activator additions to obtain a final effective concentration of 0.010 M $MgCl_2$, 0.008 M ATP, and up to $4 \cdot 10^{-6}$ M 3′:5′-cyclic AMP. Caffeine (0.005 M) is included in all incubations to inhibit the phosphodiesterase which destroys the 3′:5′-cyclic AMP. After 2–10 min of incubation, transferase is assayed (+ and — Glc-6-*P*)[8] by transferring aliquots of the kinase reaction mixture into 2 vol. of transferase test mixture, which contains 0.020 M EDTA and 0.025 M KF to prevent further kinase action and to inhibit the conversion of *D* to *I*. One unit of kinase converts 0.010 unit of transferase *I* to transferase *D* per ml per min of incubation, when the initial transferase *I* concentration is 0.10 unit/ml kinase reaction mixture*.

Using the more highly purified transferase and kinase preparations the apparent K_m for transferase *I* of the kinase has been found to be about 0.7 unit/ml, under the specified conditions. Furthermore, within the limits of 0.4 to 2.0 units of kinase per ml, the initial rate of the kinase reaction is linear with respect to the kinase concentration.

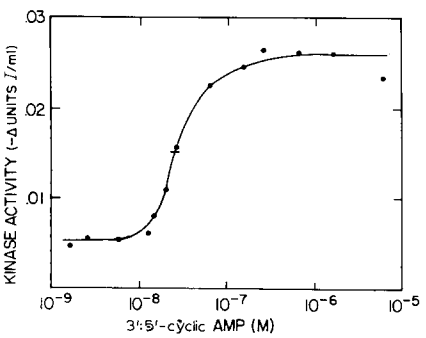


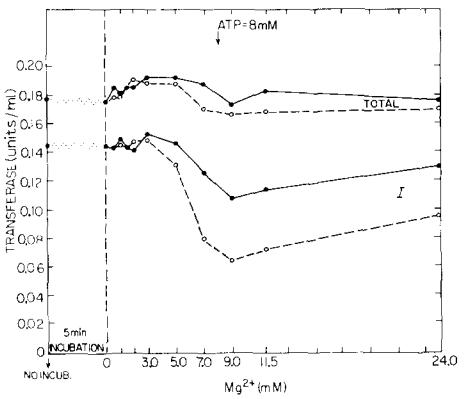
Fig. 1. Transferase *I* kinase activity (decrease in units of transferase *I* per ml kinase reaction mixture) as influenced by concentration of 3′:5′-cyclic AMP, during 10-min incubation at 30° (see text). Half maximal stimulation of the kinase, indicated (- -), occurs with $4 \cdot 10^{-8}$ M 3′:5′-cyclic AMP.

The addition of $4 \cdot 10^{-6}$ M 3′:5′-cyclic AMP (in the presence of 0.005 M caffeine) increases kinase activity 0.5–4.0-fold, depending on the kinase fraction tested. Fig. 1 shows the activation of kinase with increasing concentrations of 3′:5′-cyclic AMP in a preparation[6] which is essentially without activity in the absence of 3′:5′-cyclic

* This unit of kinase activity equals about 5 of the units designated for muscle transferase *I* kinase[2].

AMP, and shows a 4-fold activation by this nucleotide with a concentration of $4 \cdot 10^{-8}$ M being required for half maximal activation. This inactivity of the kinase without 3′:5′-cyclic AMP clearly explains why these authors[6] were unable to detect kinase activity in the presence of ATP and Mg^{2+} alone.

The Mg^{2+} and the ATP concentration dependencies of the kinase reaction are represented in Figs. 2 and 3. In these studies the more highly purified preparations of kinase free of transferase and transferase *I* free of kinase were combined. It is apparent



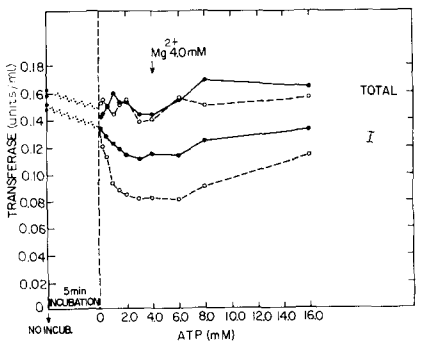


Fig. 2. Mg^{2+} dependance of transferase *I* kinase reaction with concentration of ATP kept constant at 8.0 mM. Transferase activity (+ and −Glc-6-*P*) was measured before and after a 5-min incubation in the kinase reaction mixture (see text) with (○ - - - ○) and without (●—●) added 3′:5′-cyclic AMP ($4 \cdot 10^{-6}$ M).

Fig. 3. ATP dependence of transferase *I* kinase reaction with Mg^{2+} concentration kept constant at 4.0 mM. Other conditions same as in Fig. 2.

that maximal kinase activity, whether in the presence or in the absence of excess 3′:5′-cyclic AMP, is obtained when Mg^{2+} and ATP are present in approximately equal concentrations. It is seen that high concentrations of either ATP or Mg^{2+} each inhibit the kinase. The inhibition with excess ATP is similar to that observed with transferase *I* kinase of muscle, but no inhibition by Mg^{2+} was observed in the case of the muscle enzyme[4].

It is of interest that incubation of purified transferase *I* and transferase *I* kinase, without added Mg^{2+}, leads to no decrease in "total" transferase (+ Glc-6-*P*) (Fig. 2) whereas in crude systems the existence of an inactive form of transferase is readily demonstrable[7] by such an incubation followed by a second incubation in the presence of Mg^{2+} and Na_2SO_3. This indicates that the "indirect" pathway of the *D* to *I* conversion, through an inactive form of transferase, is a separate one and is not required for the reverse conversion of transferase *I* to *D*.

The above findings of a transferase *I* kinase in normal liver completes the enzymic system for interconversion of the two forms (*I* and *D*) of liver transferase. It is seen that the liver system resembles that in muscle, but differs in several aspects. The existence of such a complete set of interconverting enzymes provides opportunity for rapid regulation of the activity of transferase *I*, through the mediation of a hormonal effect on the activity of the interconverting enzymes.

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