ON THE NON-IDENTITY OF PHOSPHORYLASE b KINASE AND UDPG:α-1,4-GLUCAN α-4-GLUCOSYLTRANSFERASE*

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From the work of Appleman et al. (1964, 1965) it is clear that muscle UDPG:α-1,4-glucan α-4-glucosyltransferase (EC 2.4.1.11) and phosphorylase b kinase (EC 2.1.7.38) are very similar. The activity of both enzymes is affected by incubation with ATP and Mg^{2+} (in the presence or absence of adenosine 3',5' cyclo-phosphate), or by incubation with Ca^{2+} plus a protein factor, or by incubation with trypsin. Furthermore, the two enzymes show similar behavior in heat inactivation studies (Appleman et al. 1965).

The experiments of Craig and Larner (1964) on the other hand demonstrated that when rat diaphragms were incubated with insulin, UDPG α-glucan glucosyltransferase was activated with no detectable effect on the activity of phosphorylase (α-1,4 glucan:orthophosphate glucosyltransferase, EC 2.4.1.1), and therefore by inference, no effect on phosphorylase b kinase. In addition Danforth (1965) demonstrated the presence of UDPG α-glucan transferase in muscle of the strain of mice described by Lyon and Porter (1963) (Strain I_{FnLn}) in which no phosphorylase b kinase is detectable.

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It seemed therefore desirable to provide direct proof that the two different activities are not contained in one protein. We assayed both activities in a purified phosphorylase b kinase preparation and in a purified (UDPG:α-glucan) transferase preparation. The phosphorylase b kinase was prepared according to Krebs et al. (1964) through the "40,000 rev/min" step and stored for 3 months at -20°C. The transferase was isolated and purified as described by Rosell-Perez and Larner (1964) with an additional ammonium sulfate precipitation and high speed centrifugation (1 hr. 105,000 x g) after the DEAE-cellulose column chromatography. The enzyme was stored for a month at -80°C. The transferase preparation contained 42% transferase I (Friedman and Larner 1963), was free of transferase D phosphatase, but still contained transferase I kinase (Friedman and Larner 1963) and the protein factor necessary for inactivation of the transferase I in the presence of high concentrations (10^{-3}M) Ca^{2+} (Appleman et al. 1965).

Table 1

	Protein mg/ml*	UDPG-glycogen glucosyl transferase activity** mμ moles/min/ mg protein	Phosphorylase b kinase to activity*** units/mg****	Phosphorylase b kinase to transferase ratio
UDPG-glycogen glucosyl transferase preparation	8	324	18	0.056
Phosphorylase b kinase preparation	2.5	35	26,400	753

*Estimated by the method of Warburg and Christian (1941)

**In the presence of glucose-6-phosphate

***at pH 8.2

****One unit is the amount of phosphorylase b kinase that converts 100 Cori units phosphorylase b to a per 5 min. in the test mixture described by Krebs et al (1964).

Phosphorylase b kinase was assayed as described by Krebs et al. (1964). Transferase was assayed as described by Hizukuri and Larner (1964). However, it was found that the assay procedure could be shortened by decreasing the time necessary for glycogen precipitation to 15 minutes. As can be seen from Table 1 the transferase preparation contained phosphorylase b kinase, and the phosphorylase b kinase contained a trace of transferase activity. Therefore both enzymes were slightly crosscontaminated. From the table it can be seen that the ratio of phosphorylase b kinase to transferase activities varied from 0.056 to 753 (about a 13,000 fold difference) in the two preparations.

After further storage of the phosphorylase b kinase preparation at -20°C for three weeks and subsequent chromatography on DEAE-cellulose the phosphorylase b kinase to transferase activity ratio was further increased to 22,000.

From these results it is concluded that the two enzyme activities are contained in separate proteins rather than in one protein moiety. It is not known whether the phosphorylase b kinase activity in the transferase preparation is due to real contamination of transferase with phosphorylase b kinase or whether transferase I kinase has some activity towards phosphorylase b. The fact that transferase is present in the phosphorylase b kinase preparation is not surprising in view of the fact that the acid precipitation step in the isolation procedure is known to concentrate transferase as well as phosphorylase b kinase.

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