SHORT COMMUNICATIONS

Effects of Adenosine 5'-Monophosphate, Glucose 1-Phosphate and Nucleotide Sugars on the Activity of Glycogen Phosphorylase from *Dictyostelium discoideum*

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INTRODUCTION

The control of glycogen degradation in animal systems involves the regulation of glycogen phosphorylase (1,4- α -D-glucan:orthophosphate α -glucosyltransferase; EC 2.4.1.1) through a complex sequence of enzymic interconversions between AMP-dependent and AMP-independent forms of the enzyme (Fischer *et al.*, 1970). Rosness *et al.* (1971) have reported that addition of cyclic AMP during development in the cellular slime mould *Dictyostelium discoideum* stimulated the rate of glycogen degradation and proposed that interconversion was occurring between two forms of the phosphorylase. However, studies with crude cell-free extracts did not support this hypothesis.

Glycogen phosphorylase from *D. discoideum* has recently been purified (Thomas & Wright, 1976) and it is similar to phosphorylases from other microbial and plant systems in which only one form exists. To understand more clearly the factors which might regulate its activity, the effects of several activators and inhibitors have now been investigated.

METHODS

Growth of organism. *Dictyostelium discoideum* strain NC-4 (ATCC 24697) was grown with *Escherichia coli* on nutrient agar sheets as previously described (Marshall *et al.*, 1970).

Enzyme assays. Glycogen phosphorylase activity was measured both in the direction of glycogen synthesis and of degradation. The assay for glycogen degradation was similar to that described previously (Thomas & Wright, 1976) except that the product of the reaction, glucose 1-phosphate, was assayed discontinuously after 10 to 30 min incubation of enzyme extract with glycogen. The enzyme showed a linear rate of activity up to 40 min incubation. Glucose 1-phosphate was assayed by adding 0.9 ml of the original reaction mixture (initially 1.0 ml) to a cuvette containing (in 0.1 ml) 50 μ mol imidazole/HCl buffer (pH 7.1), 5 μ mol MgCl_2 , 0.1 unit phosphoglucomutase, 0.3 unit glucose-6-phosphate dehydrogenase and 1.0 nmol glucose 1,6-bisphosphate; A_{340} was then determined before and 15 min after adding NADP^+ (1.0 μ mol in 10 μ l). A change in A_{340} of 0.62 was equivalent to 0.1 μ mol product.

Phosphorylase activity in the direction of glycogen synthesis was determined by following the rate of incorporation of [^{14}C]glucose 1-phosphate into ethanol-precipitated polysaccharide. The reaction mixture contained (in 0.1 ml): 2.0 μ mol [^{14}C]glucose 1-phosphate (0.5 to 1.0 μCi), 1.0 mg oyster glycogen, 5.0 μ mol β -glycerol phosphate buffer (pH 6.8), 0.5 μ mol sodium EDTA and enzyme extract. After 10 min at 23 °C the reaction was stopped by adding 0.2 ml 10% (w/v) trichloroacetic acid and 50 μ l 5% (w/v) glycogen as carrier. The radioactive glycogen was precipitated and counted following the procedure of Rosness *et al.* (1971). One unit of enzyme activity was defined as that amount of enzyme catalysing the incorporation of 1.0 μ mol glucose 1-phosphate into glycogen per min.

Protein determination. Protein was determined by the method of Lowry (1957) using bovine serum albumin as the standard.

Fig. 1. Effect of 5'-AMP and glucose 1-phosphate on the activity of glycogen phosphorylase at various concentrations of phosphate. Enzyme (0.030 units mg^{-1}) prepared from the culmination stage of development was assayed in the presence or absence of 1 mM-5'-AMP or 0.1 mM-glucose 1-phosphate (G-1-P). Phosphorylase activity was determined in the direction of glycogen degradation.

RESULTS AND DISCUSSION

Most microbial and plant phosphorylases are reported to be active in the absence of 5'-AMP, but the enzyme may or may not be activated by this nucleotide. Rosness *et al.* (1971) indicated that glycogen phosphorylase activity of *D. discoideum* was stimulated by 5'-AMP though the investigations of Jones & Wright (1970) and Firtel & Bonner (1972) did not confirm this. When the enzyme was now assayed using the conditions of Jones & Wright (1970), no requirement for any nucleotide was exhibited. However, if assays were carried out under conditions which allow for product accumulation, i.e. by using a discontinuous assay, 5'-AMP (1 mM) stimulated the phosphorylase activity of crude extracts about 25 % over the control (Fig. 1). Other nucleotides (ADP, ATP, 2'-AMP, 3'-AMP, cyclic AMP, adenosine) were without effect. 5'-AMP appears to act by altering the $V_{\max}$ of the reaction (Fig. 1): at a low concentration (1 mM) the $V_{\max}$ increased from 0.130 to 0.162 without appreciably affecting the K_m for the substrate phosphate (P_i). The non-competitive nature of 5'-AMP activation suggests that the activity of the nucleotide does not involve binding at the substrate site. The ability of 5'-AMP to stimulate glycogen phosphorylase activity was also apparent when assays were performed in the direction of glycogen synthesis. Maximal stimulation occurred at 5 mM-5'-AMP and represented about a 40 % increase in activity over that of the control. The inhibition by glucose 1-phosphate could be reversed by 5'-AMP (Fig. 1). Similar results were noted for the inhibition of phosphorylase by nucleotide sugars (GDPglucose and UDPglucose), though only a partial reversal of GDPglucose inhibition occurred even at high 5'-AMP concentrations (20 mM). These results are similar to the observations that ATP inhibition of phosphorylase b can be relieved by 5'-AMP (Parmeggiana & Morgan, 1962).

As has been previously reported (Jones & Wright, 1970; Firtel & Bonner, 1972), the slime mould phosphorylase, when assayed in either direction, is competitively inhibited by nucleotide sugars (GDPglucose, UDPglucose, ADPglucose). GDPglucose was the most effective inhibitor and UDPglucose was the least. These observations are in contrast to other phosphorylases which are, in general, more sensitive to inhibition by UDPglucose (Chen & Segel, 1968). In *D. discoideum*, the concentration of UDPglucose increases about threefold (0.1 mM to 0.3 mM based on cell volume) from aggregation to culmination

(Wright *et al.*, 1968). These values appear to be somewhat lower than the measured K_i of the enzyme for UDPglucose. If compartmentalization is not a factor, then UDPglucose may have minimal effect *in vivo* on glycogen phosphorylase activity.

When the enzyme was assayed under the present conditions, which allow for product accumulation, glucose 1-phosphate inhibited activity by about 50 % at 0.2 mM. Glucose 1-phosphate is probably a competitive inhibitor with respect to phosphate (P_i), increasing the K_m for the substrate from 5 to 10 mM (Fig. 1). Glucose 6-phosphate and glucose, competitive inhibitors of the phosphorylase from *Neurospora crassa* (Shepherd & Segel, 1969), had no effect on the *D. discoideum* enzyme. During differentiation, the concentration of glucose 1-phosphate has been reported (Pannbacker, 1967) to vary threefold, reaching a maximum of 0.01 mM based on cell volume at culmination. This is somewhat below the concentration (0.2 mM) of glucose 1-phosphate which causes significant inhibition (50 %) of the phosphorylase reaction. However, it is possible that local concentrations of glucose 1-phosphate within a cell undergoing phosphorolysis may be somewhat higher. In this situation, the degree of phosphorylase inhibition would be determined by the relative concentration of 5'-AMP. Recently, E. Coe (personal communication) has found evidence which indicates that those cells located in the head of migrating pseudoplasmodia contain higher glucose 1-phosphate concentrations.

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