Metabolite Control of L-Fucose Utilization*

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SUMMARY

Thyroid fucokinase is responsive to a number of metabolites which might serve in a regulatory capacity. In addition to inhibition by ADP and stimulation by GMP, fucokinase responds selectively to a series of nucleotide sugars. Of those studied, only guanine nucleotide sugars moderate the activity of the enzyme. GDP-α-D-mannose, GDP-α-D-glucose, GDP-α-D-rhamnose, and GDP-α-L-rhamnose all stimulate fucokinase activity. GDP-β-L-fucose on the other hand is strongly inhibitory. In the case of GDP-α-D-mannose stimulation, a physiological role seems possible, but the rationale is not entirely clear. The effects of GDP-β-L-fucose, on the other hand, may represent physiological control effects through feedback inhibition by an end product.

L-Fucose has been found to be a terminal sugar residue in a surprisingly large number of mammalian glycoproteins. Although its specific role in these glycoproteins has not been precisely defined, it appears to be of considerable importance in mediating cell interactions and may figure significantly in neoplastic transformations (1, 2).

Despite its significance in mammalian systems, little research has been conducted into control mechanisms affecting L-fucose utilization. This communication reports such studies which we have recently undertaken with the enzyme fucokinase (ATP:6-deoxy-L-galactose-1-phosphotransferase, EC 2.7.1.52). This enzyme catalyzes the phosphorylation of L-fucose to form β-L-fucose 1-phosphate, a first step in utilization of free fucose in glycoprotein synthesis. It has been detected in porcine liver (3, 4), porcine submaxillary glands (5), and, more recently in this laboratory, in canine thyroid tissue (6). It has been suggested that this enzyme is widespread and indeed that it may be found in any tissue which synthesizes an L-fucose-containing glycoprotein (3, 6). In most of these tissues, it must serve as the primary step in a salvage process to introduce dietary or recycled free L-fucose into the metabolic scheme leading ultimately to the production of guanosine diphospho-β-L-fucose (GDP-β-L-fucose), the nucleotide sugar donor for fucose incorporation into oligosaccharides (7, 8). In thyroid tissue, fucokinase may be important because of the extensive degradation of thyroglobulin, an L-fucose-containing glycoprotein, to release its stored hormone thyroxine (with consequent formation of free fucose). As such, it would be appropriate that the enzyme be under regulatory control. This report deals with metabolite control of fucokinase exerted by nucleotides and nucleotide sugars.

MATERIALS AND METHODS

The fucokinase preparations used in these studies were derived from canine or porcine thyroid tissue. The porcine thyroid enzyme has recently been purified 23,000-fold (6, 9).

Guanosine diphospho-β-L-fucose was a generous gift from Drs. R. Barker and P. Rosevear of the Department of Biochemistry at Michigan State University. α-D- and α-L-Guanosine diphosphohamnose, guanosine diphospho-α-D-glucose, and cytidine diphospho-α-D-glucose were generous gifts from Dr. G. Barber of the Department of Biochemistry at Ohio State University. All other materials were obtained from commercial sources.

The enzymatic assay for fucokinase was reported in a previous publication (6). Radioactive L-fucose is converted to β-L-fucose 1-phosphate, trapped on an ion exchange paper disc, and counted by liquid scintillation techniques.

RESULTS AND DISCUSSION

Initial observations of metabolite control of fucokinase were made with a partially purified preparation from canine thyroid tissue. While establishing the specificity of this enzyme for ATP, it was observed that additions of GTP, GDP, and GMP preparations stimulated fucokinase activity whereas all other nucleotide mono-, di-, and triphosphates, with the exception of ADP, were without effect. ADP is a potent inhibitor of the enzyme. Careful examination of the guanine derivatives by paper chromatography and paper electrophoresis have subsequently established that the GTP and GDP were contaminated with GMP. Removal of this component from the GTP and GDP preparations renders them ineffective as fucokinase stimulators. Thus, GDP is apparently the responsible stimulatory metabolite. Mindful of metabolite control of other carbohydrate pathways by nucleotide sugars, e.g. hexosamine biosynthesis (10) and deoxysugar formation in bacterial systems (11), we tested the effects of those nucleotide sugars available to us at the time. A summary of the pertinent data from these studies is presented in Fig. 1. Guanosine diphospho-α-D-mannose is seen to be a potent stimulator of fucokinase affecting a 2.5-fold activation of the enzyme above control values with greater stimulatory responses registered at lower concentrations than observed for GMP.

Since the canine thyroid preparation contained substantial quantities of protein impurities, we re-examined the stimulatory effects of GDP-α-D-mannose in preparations of porcine thyroid fucokinase which we have subsequently isolated in a highly purified form (9). With this preparation, it was observed that GDP-α-D-mannose and GMP also exhibited stimulatory effects on the porcine enzyme with the nucleotide sugar effecting a one-half maximal stimulation of activity at a concentration 1 order of magnitude lower (1 X 10^{-7} M) than observed with the canine system. Surprisingly, a number of guanosine nucleotide sugars are also seen to be effectors of fucokinase (Table I), even though some of these might not be expected to be found in mammalian systems. One exception to this stimulatory effect is the inhibitory effect of GDP-β-L-fucose (Table I). This effect was examined over an extended concentration range (Fig. 2) with the result that GDP-β-L-fucose is seen to induce a 50% inhibition of fucokinase activity at a concentration of 5 X 10^{-4} M. Inhibition cannot be the result of simple dilution of labeled fucose in the assay mixture by...
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FIG. 1. Effect of GMP, GDP-β-D-mannose, ADP, and other metabolites on canine thyroid fucokinase activity. The numbered points in this figure are: 1 and 7, guanosine; 2, α-D-mannose 1-phosphate; 3, inorganic phosphate; 4, UDP-N-acetyl-α-D-glucosamine; 5, UDP-α-D-glucose; 6, CDP-choline or UDP-α-D-galactose; 8, GTP; 9, GDP; 10, guanosine.

Table I
Influence of nucleotide sugars on porcine thyroid fucokinase

The nucleotide sugars, with the exception of GDP-β-L-fucose, were present in the assay mixture at a concentration of 2 mM with an additional 2 mM magnesium ion added to the 6 mM magnesium ion concentration of the existing assay mixture. GDP-β-L-fucose was present at a 1.25 mM concentration with 1.25 mM added magnesium ion.

<table>
<thead>
<tr>
<th>Component added</th>
<th>% control activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>UDP-α-D-xylose</td>
<td>103</td>
</tr>
<tr>
<td>UDP-α-D-glucose</td>
<td>133</td>
</tr>
<tr>
<td>UDP N-acetyl α-D-glucosamine</td>
<td>136</td>
</tr>
<tr>
<td>UDP-α-L-rhamnose</td>
<td>102</td>
</tr>
<tr>
<td>CDP-α-D-glucose</td>
<td>93</td>
</tr>
<tr>
<td>ADP-α-D-glucose</td>
<td>96</td>
</tr>
<tr>
<td>ADP-α-D-mannose</td>
<td>93</td>
</tr>
<tr>
<td>GDP-α-D-glucose</td>
<td>332</td>
</tr>
<tr>
<td>GDP-α-L-rhamnose</td>
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</tr>
<tr>
<td>GDP-α-D-rhamnose</td>
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</tr>
<tr>
<td>GDP-α-L-rhamnose</td>
<td>337</td>
</tr>
<tr>
<td>GDP-β-L-fucose</td>
<td>0</td>
</tr>
</tbody>
</table>

fucose hydrolyzed from the nucleotide sugar since the concentration of fucose to be obtained by complete hydrolysis of the nucleotide is approximately 1/100 of the fucose concentration in the assay mixture.

The rationale for the observed in vitro stimulatory action of GDP-α-D-mannose on fucokinase is not completely clear. Certainly, GDP-mannose is intimately connected to fucose metabolism. It has been shown to be the precursor for GDP-β-L-fucose in bacteria and in some animal tissues (12). Additionally, both GDP-α-D-mannose and GDP-β-L-fucose serve as precursors for some of the same polysaccharides, e.g., polysaccharide B of thyroglobulin (13). Thus, the possibility of a regulatory function in vivo exists. The fact that a variety of GDP-sugar derivatives stimulates fucokinase is puzzling, but may simply reflect the fact that the stimulation site is more specific for the guanosine portion of the nucleotide sugar than for the saccharide moiety. Partial stimulation of the enzyme by guanosine monophosphate may support this idea.

GDP-β-L-fucose inhibition of fucokinase is again consistent with a metabolite-controlled enzyme. Elevation of GDP-β-L-fucose may occur when glycoprotein synthesis has been reduced in the tissue for physiological reasons. Feedback control of fucokinase would curb GDP-β-L-fucose production.

Preliminary studies with crude porcine liver fucokinase preparations show similar effects with GDP-α-D-mannose and GDP-β-L-fucose. Thus, the regulatory effects may be common to fucokinase regardless of the tissue type and reflect a general control mechanism for fucose utilization in mammalian systems.

REFERENCES

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W L Richards, R D Kilker and G S Serif