FEBS Letters 359 (1995) 110–112 FEBS 15090

New and specific nucleoside diphosphate glucose substrates for glycogenin

Miriam D. Alonso, Erik J. Lagzdins, Joseph Lomako, Wieslawa M. Lomako, William J. Whelan*

Department of Biochemistry and Molecular Biology, University of Miami School of Medicine (M823), PO Box 016129, Miami, FL 33101, USA

Received 22 December 1994

Abstract Glycogenin, the autocatalytic, self-glucosylating primer for glycogen synthesis by glycogen synthase, is presumed, in vivo, to use UDP-glucose as the source of the glucose residues it adds to itself. When we tested its ability to utilize other nucleoside diphosphate glucoses, it emerged that purine nucleotides are not utilized but two pyrimidine nucleotides are used, in addition to UDP-glucose. These are CDP-glucose and TDP-glucose. CDP-glucose is utilized at 70% of the rate of UDP-glucose. While there is no evidence that CDP-glucose is a natural substrate for glycogenin, it has the advantage over UDP-glucose in that it can be used specifically to detect and assay glycogenin in the presence of glycogen synthase because CDP-glucose, unlike UDP-glucose, is not a substrate for the synthase.

Key words: Glycogenin; Apo-glycogenin; CDP-glucose; TDP-glucose

1. Introduction

When the self-glucosylating action of glycogenin was first detected in muscle and heart extracts, the glucose donor substrate used was UDP-glucose [1], the same as is used by glycogen synthase to extend the maltosaccharide primer chain that glycogenin creates on itself. When working with tissue extracts or partly purified preparations containing both glycogenin and synthase, it has been possible to distinguish between the two enzymes by virtue of the fact that the K_m values of glycogenin and synthase for UDP-glucose differ by three orders of magnitude [2–4], although the form of synthase we have named pro-glycogen synthase is active at the same micromolar UDP-glucose concentration as is optimal for glycogenin [5]. Another distinction is possible because glycogenin is activated by Mn^{2+}, while the synthases are activated by glucose 6P, and not vice versa [6–8].

It would, however, be useful if a specific glucose donor substrate could be found for glycogenin so as to permit a study of its behavior in presence of synthase, and to be able sequentially first to synthesize the primer and then, by the addition of UDP-glucose, to initiate glycogen synthesis proper. This would facilitate the study of the properties and function of the complex between glycogenin and synthase that Cohen and his colleagues consider is necessary for glycogen synthesis [8,9].

A specific glycogenin substrate was reported by Meezan et al. [10]. This is UDPxylose. However, glycogenin will add only one xylose to itself. No further chain growth, either with UDP-xylose or UDP-glucose, takes place [11].

We have tested the commercially available range of nucleoside diphosphate glucoses and now report that TDP-glucose, and especially CDP-glucose, are substrates for glycogenin, the latter being specific, not utilized by glycogen synthase.

2. Materials and methods

2.1. Materials

All biochemicals were from Sigma or Fischer Scientific Co. Recombinant rabbit muscle wild-type, Phe^{194} mutant and apo-glycogenins were prepared as by Alonso et al. [12–14].

2.2. Methods

The experimental conditions employed in the incubation of glycogenins with potential glucose donors are described in the legends to the figures. The HPLC fractionation procedure for the products of the intermolecular transglucosylation of *p*-nitrophenyl α-maltoside (Fig. 1) is described in [13]. The self-glucosylating activity of recombinant wild-type glycogenin in the presence and absence of CDP was measured as in [13].

3. Results and discussion

We were led to the experiments reported here by considering our own results, and the results of others, on the inhibition of glycogenin by nucleotides. ATP at physiological concentration is a powerful inhibitor of native muscle glycogenin [11], but less so of recombinant muscle glycogenin [13,15]. UTP and UDP are more powerful inhibitors of recombinant glycogenin than is ATP [15]. These inhibitions occur at millimolar concentrations of nucleotide. CDP, by contrast, almost completely (93%) inhibits native kidney glycogenin at 25 μM concentration [16] and we now report that this is about the same (90%) for recombinant muscle glycogenin.

Earlier we had observed that maltose inhibits the self-glucosylation of glycogenin [17]. Further study revealed that the 'inhibition' was because the added sugar competes for the glucose transferred from UDP-glucose. The *p*-nitrophenyl α-maltosaccharides and the α-glucoside are even more potent than maltose [17], while, more recently, dodecyl β-maltoside has been shown to be better still as an acceptor [18]. It was with the use of these acceptors that we were able to learn that the Phe^{194} and Thr^{194} mutant forms of glycogenin which, lacking Tyr^{194}not self-glucosylate, are nevertheless fully active in intermolecular transglucosylation to these small molecule acceptors [12,13].

The inhibition of glycogenin by UTP and UDP [15] might be explained by their competing for the UDP-glucose binding site. Reasoning this, we asked whether CDP might be bound by glycogenin at the same site and, in turn, whether CDP-glucose might be a donor substrate. We decided to test all commercially available nucleoside diphosphate glucoses.

We tested recombinant wild-type and Phe^{194} glycogenins with ADP-glucose, CDP-glucose, GDP-glucose, TDP-glucose and UDP-glucose. Since these are not all available labelled with a radioisotope, we tested them as substrates for intermolecular

*Corresponding author. Fax: (1) (305) 324 5665.
E-mail: wwhelan@mednet.med.miami.edu

SSDI 0014-5793(95)00018-6

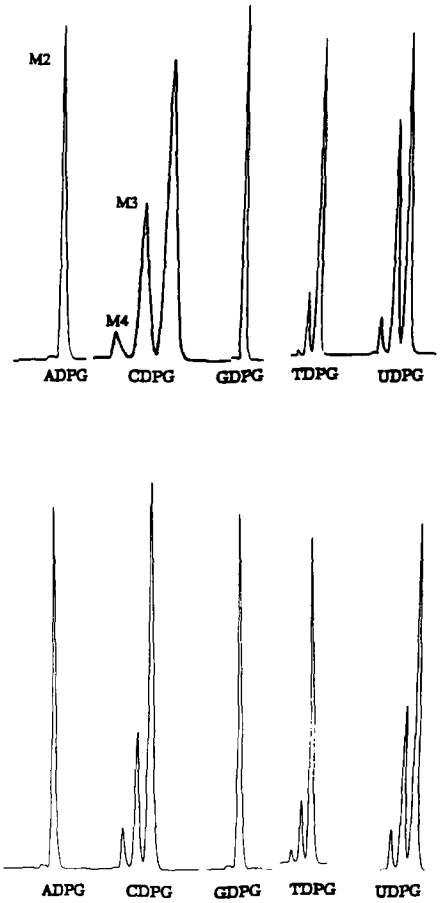

Fig. 1. Illustrating the nucleotide sugar specificity of glycogenin. The digests (100 μl each) contained 1 μM recombinant enzyme (wild-type in upper panel, Phe^{194} mutant in lower panel) together with 10 mM nucleoside diphosphate glucose, 10 mM *p*-nitrophenyl α-maltoside, 5 mM $MnCl_2$ and 50 mM Tris-HCl buffer, pH 7.4. After incubation for 18 h at room temperature, a 10 μl portion of digest was loaded onto a C18 column and fractionated by reverse-phase HPLC using an ascending gradient of acetonitrile (10–20%; 1 ml/min for 20 min). The sole, or right-hand, peak, in each case corresponds to the *p*-nitrophenyl α-maltoside substrate (M2) and, to the left of it the products of transglucosylation, *p*-nitrophenyl α-maltotrioside (M3) and α-maltotetraoside (M4). They were detected by absorbance at 320 nm.

transglucosylation to *p*-nitrophenyl α-maltoside and to assay the products by HPLC. The results are shown in Fig. 1.

All three pyrimidine nucleoside diphosphate glucoses, but neither of the purine forms, acted as donor substrates. The formation of *p*-nitrophenyl α-maltotrioside was seen as a product of transfer and in each case this was utilized for the formation of *p*-nitrophenyl α-maltotetraoside. Measuring the relative percentage utilization of the three glucose donors showed that CDP-glucose was used to 71% of the extent of UDP-glucose and TDP-glucose 33%. The results with the Phe^{194} mutant were essentially identical (Fig. 1). The experiments were conducted with 10 mM glucose donors, incubated for 18 h, in order to form sufficient mass of product to quantitate by HPLC.

Through the kindness of Dr. L. Roden, we obtained a sample of CDP-[^{14}C]glucose which we compared with UDP-[^{14}C]glucose at the usual 2 μM concentration, using the wild-type enzyme [13]. The relative initial-rate utilization of the CDP-glucose for self-glucosylation was the same 71% as observed at 10 mM concentration for glucosylation of *p*-nitrophenyl α-maltoside.

As an added proof that glucose could be transferred to glycogenin from these new donor substrates, we incubated them with recombinant glucose-free, apo-glycogenin [14] and examined the products by SDS-PAGE (Fig. 2). The average of 8 glucose residues added in self-glucosylation [14] is sufficient to cause the glucosylated protein to migrate at a distinctly lower rate than the glucose-free protein. Apo-glycogenin is compared before and after incubation with ADP-glucose and GDP-glucose in Fig. 2, (lanes 1,2,4, respectively). All three protein samples comigrated. When the same protein was incubated with CDP-glucose, TDP-glucose or UDP-glucose (lanes 3,5,6, respectively) they migrated more slowly than did apo-glycogenin (lane 1).

These experiments suggest that glycogenin has a UDP-glucose binding site that can accommodate any of the three pyrimidine nucleotides tested. The inability to utilize ADP-glucose or GDP-glucose, while undergoing inhibition by ATP (see above), suggests that the latter binds elsewhere. Our findings prompt an examination of the hypothesis with which we began, that CDP itself can compete with UDP-glucose for the latter's binding site.

So far as we are aware, CDP-glucose and TDP-glucose are not recognized as glucose donor substrates in mammalian metabolism. In Salmonella, CDP-glucose is the precursor of the 3,6-dideoxyhexoses, paratose and ascarylose [19]. We do not therefore suggest that either of these glucose donors is a native substrate for mammalian glycogenin. The importance of the finding that CDP-glucose is an effective alternative to UDP-glucose is because CDP-glucose is not utilized by muscle glycogen synthase [20]. (TDP-glucose is used by synthase, at 5% of the rate of UDP-glucose [21].) That glycogenin can now be assayed with this specific substrate means that it will be possible, either in tissue extracts, or in deliberate mixtures of homogeneous glycogenin and synthase, to study glycogen synthesis sequentially by first creating the primer, with CDP-glucose, and then glycogen, with UDP-glucose. Such studies are necessary in order to understand what is required for the efficient initiation of glycogen synthesis. Cohen et al. [8,9] have claimed that a 'tight' 1:1 complex of glycogenin and synthase is required. In experiments in which they had dissociated native glycogenin and synthase, several hours were required for reassociation and the extent of utilization of glycogenin increased as reassociation took place. Other workers, by contrast, fail to notice any requirement of recombinant glycogenin for time-dependent

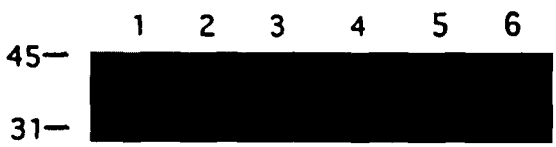

Fig. 2. Depicting the ability of apo-glycogenin to self-glucosylate with pyrimidine but not purine nucleoside diphosphate glucoses. The digests (100 μl each) contained 1.35 μM recombinant apo-glycogenin [14], 100 μM nucleoside diphosphate glucose, plus $MnCl_2$ and buffer as in Fig. 1. After incubation for 1 h at room temperature, an equal volume of 20% trichloroacetic acid was added and the precipitates examined by SDS-PAGE, the protein bands being revealed by Coomassie blue. The molecular masses of the standards (kDa) are shown on the left. Lane 1, untreated apo-glycogenin; 2, incubated with ADP-glucose; 3, CDP-glucose; 4, GDP-glucose; 5, TDP-glucose; 6, UDP-glucose.

association with synthase in order for the former to prime the latter efficiently [15]. The use of glucose-free recombinant glycogenin (apo-glycogenin) [14], with CDP- and UDP-glucoses, and glycogen synthase, should enable the necessity, and the requirements, for complex formation to be explored.

Acknowledgements: We thank Dr. L. Roden for a gift of CDP-[^{14}C]glucose and Ms. Johanna Epstein for technical assistance. This work was supported by a grant from the National Institutes of Health (DK 37500).

References

[1] Whelan, W.J. (1986) BioEssays 5, 136–140.
[2] Pitcher, J., Smythe, C., Campbell, D.G. and Cohen, P. (1987) Eur. J. Biochem. 169, 497–502.
[3] Lomako, J., Lomako, W.M. and Whelan, W.J. (1988) FASEB J. 2,3097–3103.
[4] Rodriguez, I.R. and Fliesler, S.J. (1988) Arch. Biochem. Biophys. 260, 628–637.
[5] Lomako, J., Lomako, W.M., Whelan, W.J., Dombro, R.S., Neary, J.T. and Norenberg, M.D. (1993) FASEB J. 7, 1386–1393.
[6] Lomako, J., Lomako, W.M. and Whelan, W.J. (1990) FEBS Lett. 268, 8–12.
[7] Lomako, J., Lomako, W.M. and Whelan, W.J. (1991) FEBS Lett. 279, 223–228.
[8] Smythe, C. and Cohen, P. (1991) Eur. J. Biochem. 200, 625–631.
[9] Smythe, C., Watt, P. and Cohen, P. (1990) Eur. J. Biochem. 189, 199–204.
[10] Meezan, E., Ananth, S., Manzella, S., Campbell, P., Siegal, S., Dillion, D.J. and Roden, L. (1994) J. Biol. Chem. 269, 11503–11508.
[11] Lomako, J., Lomako, W.M. and Whelan, W.J. (1990) BioFactors 2, 251–254.
[12] Alonso, M.D., Lomako, J., Lomako, W.M. and Whelan, W.J. (1994) FEBS Lett. 342, 38–42.
[13] Alonso, M.D., Lomako, J., Lomako, W.M. and Whelan, W.J. (1995) J. Biol. Chem., in press.
[14] Alonso, M.D., Lomako, J., Lomako, W.M., Whelan, W.J. and Preiss, J. (1994) FEBS Lett. 353, 222–226.
[15] Cao, Y., Mahrenholz, A.M., DePaoli-Roach, A.A. and Roach, P.J. (1993) J. Biol. Chem. 268, 14687–14693.
[16] Roden, L., Ananth, S., Campbell, P., Manzella, S. and Meezan, E. (1994) J. Biol. Chem. 269, 11509–11513.
[17] Lomako, J., Lomako, W.M. and Whelan, W.J. (1990) FEBS Lett. 264, 13–16.
[18] Manzella, S.M., Roden, L. and Meezan, E. (1994) Anal. Biochem. 216, 383–391.
[19] Mayer, R.M. and Ginsburg, V. (1965) J. Biol. Chem. 240, 1900–1904.
[20] Goldemberg, S.H. (1962) Biochim. Biophys. Acta 56, 357–359.
[21] Kornfeld, R. and Brown, D.H. (1961) J. Biol. Chem. 237, 1772–1777.