HHS Public Access

Are there errors in glycogen biosynthesis and is laforin a repair enzyme?

Peter J. Roach

Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, USA

Peter J. Roach: proach@iupui.edu

Abstract

Glycogen, a branched polymer of glucose, is well known as a cellular reserve of metabolic energy and/or biosynthetic precursors. Besides glucose, however, glycogen contains small amounts of covalent phosphate, present as C2 and C3 phosphomonoesters. Current evidence suggests that the phosphate is introduced by the biosynthetic enzyme glycogen synthase as a rare alternative to its normal catalytic addition of glucose units. The phosphate can be removed by the laforin phosphatase, whose mutation causes a fatal myoclonus epilepsy called Lafora disease. The hypothesis is that glycogen phosphorylation can be considered a catalytic error and laforin a repair enzyme.

1. Introduction

Glycogen synthase (EC 2.4.1.11) transfers glucose moieties from its substrate UDP-glucose to form α-1,4-glycosidic linkages at the growing, non-reducing ends of a glycogen molecule ([1]; Fig. 1). The ideas presented in this article had their origins in recent work from my laboratory that is described in three papers [2–4]. We showed that glycogen synthase rarely incorporates the β-phosphate of UDP-glucose into glycogen [4]. We also showed that the phosphate could be released by the action of the laforin phosphatase [2]. The hypothesis to be explored is that the incorporation of phosphate into glycogen is a catalytic error and that laforin acts as a repair or damage control enzyme.

2. Enzyme specificity and side-reactions

In organic chemistry, yields can be low and side reactions problematic. In nature, enzymes have evolved to accelerate chemical reactions so as to permit a coherent pattern of chemical interconversions, fast enough and accurate enough, to fulfill the needs of the cell [5]. The kinetic properties of enzymes ensure proper fluxes through complex metabolic networks. How good must an enzyme be, in terms of its specificity and its ability to avoid unwanted side reactions? This question needs to be elaborated before addressing the specifics of glycogen phosphorylation and the hypothesis formulated above.

Discussion of enzyme specificity is often directed at selectivity for substrates, the degree of promiscuity, and immediately distinction must be made between enzymes that have seemingly evolved to catalyze one particular reaction, of which glycogen synthase might be considered an example, and enzymes, such as cytochrome P450s [6] or alcohol

dehydrogenases [7] whose evolved function is to act on a variety of substrates. The latter enzymes are not promiscuous and rather are multispecific or broad-specificity enzymes [6]. It may be difficult or impossible to define unique physiological substrates and products. However, even enzymes viewed as having evolved to mediate a single reaction can be capable of catalyzing other interconversions. There is indeed a substantial literature on this concept of catalytic promiscuity which is by no means a rare phenomenon [6]. Such alternate reactions may even be physiologically significant, and maintained through evolution, or alternatively they can simply be incidental due to the chemistry of the catalysis. Enzymatic promiscuity is viewed as critical for the evolution of new activities.

In a cell, the promiscuous activity of an enzyme towards an alternate substrate can be considered a side reaction. Another example of a side reaction, more in keeping with experimental organic synthesis, would be the generation of alternative products from the same substrate. For example, many phosphokinase enzymes are capable of hydrolyzing their co-substrate, usually ATP, to generate ADP and inorganic phosphate [8]. Similarly, the glucosyltransferase glycogenin, whose function is to transfer glucose from UDP-glucose to form a priming oligosaccharide for glycogen synthesis, can hydrolyze its substrate UDP-glucose to generate UDP and glucose [9]. As explained below, the introduction of phosphate into glycogen may fall into this category of side reaction.

3. Catalytic errors

When is a side reaction an error? Semantics as well as science enter into answering this question. The idea of an "error" requires that there is a "correct" outcome. Much discussion of catalytic errors has addressed the biosynthesis of information-rich biopolymers, like DNA [10], RNA [11] and proteins [12], for which a "correct" product can be defined. Errors occur when an incongruent base or amino acid, not directed by the coding polymer, is incorporated. Such events can be considered promiscuous substrate selection, a side-reaction, even though all three of these syntheses are mediated by especially complex enzymatic machines. For all three processes, sophisticated editing and/or repair mechanisms exist to improve the fidelity of polymer production. For DNA synthesis, the rationale is especially compelling: misincorporation of a base in DNA can change the genetic blue-print of the cell and would be perpetuated through cell division. Special mechanisms have evolved to ensure accuracy in DNA synthesis and elaborate repair processes also exist. In this example, most people would agree that production of a new DNA molecule not faithfully reproducing the sequence of the priming strand is indeed an error, that there is an identifiable "correct" reaction. Compared with errors in DNA, mistakes in the synthesis of individual RNA or protein molecules obviously have a less deleterious impact but, even so, mechanisms exist to minimize their occurrence. How does glycogen phosphorylation compare with such processes?

4. Glycogen, glycogen phosphorylation and Lafora disease

Glycogen, a branched polymer of glucose, serves as a reservoir of glucose, synthesized in times of nutritional plenty for use in times of need [1]. Glycogen isolated from biological sources is polydisperse and does not have a unique structure. A popular model of the

glycogen molecule envisions successive tiers of chains with an average length of 13 glucose residues [13,14]. Internal chains would have on average two branch points while the outermost tier would consist of unbranched chains (Fig. 1). The bulk synthesis of glycogen requires the action of two enzymes, glycogen synthase, mentioned above, and the branching enzyme which introduces the α-1,6-glycosidic linkages that form the branch points. The structure of glycogen is dictated by the kinetic properties of these two enzymes but in a stochastic rather than a precise way – not every chain is 13 glucoses long and branch points are not necessarily in identical relative positions in every chain.

Thus, the synthesis of glycogen is profoundly different from the mechanism by which nucleic acids and proteins are assembled with a strict conservation of informational content. Can we then assert that there is a "correct" glycogen product? Obviously not in the same way as is possible for nucleic acids and proteins but a number of genetic diseases of glycogen metabolism are informative on this point. Though rare, mutations in several different genes cause a series of glycogen storage diseases or glycogenoses [15]. In some cases, aberrant glycogen structures are formed that contribute to the pathology of the disease. For example, mutations in the branching enzyme gene cause Andersen or adult polyglucosan body diseases in which, as could be predicted, the glycogen formed has fewer branches than normal, leading to a less soluble polymer (sometimes called polyglucosan) that forms insoluble deposits. It would seem reasonable to consider polyglucosan to be an imperfect or even incorrect product of the glycogen synthetic pathway.

The most important constituent of glycogen is glucose which of course underlies its metabolic function. Since the 1980s, however, it has been recognized that glycogen contains trace amounts of phosphate [16–18], 1 phosphate per 500 to 1500 glucoses in muscle glycogen [2,3]. In mice, this level of phosphorylation is invariant with age [3]. From analyses of rabbit muscle glycogen, the phosphate was found to exist as phosphomonoesters at C2 and C3 of glucose residues [4]. For many years, the origin and function of the phosphate was obscure. Recently, however, Tagliabracci et al. [4] reported that *in vitro* the normal biosynthetic enzyme, glycogen synthase, was capable of introducing phosphate that originated as the β-phosphate of its substrate UDP-glucose. Phosphate incorporation was infrequent compared with the normal addition of glucose residues, 1 phosphate for every ~10,000 glucoses. It was proposed that phosphate was introduced as glucose-phosphate in a side reaction mediated by glycogen synthase, with concomitant formation of UMP rather than UDP. The reason for the disparity between the rate of phosphate incorporation by glycogen synthase and the steady state glycogen phosphorylation state is likely explained by the fact that individual glycogen molecules undergo multiple rounds of synthesis and degradation, and phosphate can accumulate. Phosphate content depends not only on the synthetic rate but also the relative rates of glucose and phosphate removal.

Worby et al. [19] first reported that the laforin phosphatase could hydrolyze phosphate from amylopectin, a branched polysaccharide from plant starch which has a similar chemical structure to glycogen and which contains C3 and C6 phosphomonoesters [20,21]. They were unable to demonstrate dephosphorylation of liver glycogen. Subsequently, Tagliabracci et al. [2] demonstrated release of the phosphate from muscle glycogen by laforin. Laforin is, by amino acid sequence, a member of the dual specificity family of protein phosphatases (DSP;

[22]). Laforin is also the only phosphatase in mammalian genomes that, in addition to a DSP domain, has an integral carbohydrate binding module, of the CBM20 subfamily [23]. Mutation of the CBM20 abolishes the ability of laforin to bind to and to dephosphorylate glycogen [2]. Laforin, which is encoded by the *EPM2A* gene, was first identified in connection with a neurological disorder called Lafora disease, a fatal progressive myoclonus epilepsy with typical onset in the teenage years [24–27]. Roughly half of the cases of Lafora disease result from mutations in the *EPM2A* gene. A characteristic of Lafora disease is the presence of insoluble deposits, called Lafora bodies, in many tissues, including muscle, liver, heart and brain. The major constituent of Lafora bodies is poorly branched glycogen, polyglucosan. In a mouse model of Lafora disease in which the *Epm2a* gene is disrupted, an increase in glycogen phosphorylation was observed, suggesting that laforin dephosphorylates glycogen also *in vivo* [2]. As the *Epm2a*–/– mice aged, the increased glycogen phosphate correlated with disturbances in glycogen structure, decreased branching frequency, and decreased solubility in water, paralleling the formation of Lafora bodies in the mice [3]. Increased phosphate content has also been reported in Lafora bodies from Lafora patients [28]. Not understood currently is the exact relationship between the increased glycogen phosphorylation and the decreased branching frequency. There is, though, an emerging sense that Lafora disease might be a type of glycogenosis.

5. Is laforin a repair or damage control enzyme?

Excessive accumulation of phosphate in glycogen is associated with structurally malformed glycogen, and so perhaps the side reaction of glycogen synthase responsible for the introduction of phosphate can be considered a catalytic error. There is a parallel with errors in nucleic acid synthesis in that infrequent side reactions are involved. Interestingly, for glycogen synthase, the error rate of $\sim 10^{-4}$ is within the range of 10^{-3}–10^{-6} reported for single base substitutions by proofreading-defective DNA polymerases [10]. If hyperphosphorylated glycogen is an aberrant biosynthetic product, can the enzyme that normally limits glycogen phosphorylation be considered a repair enzyme? Certainly, the absence of laforin activity leads to a serious pathological outcome in the form of Lafora disease. The phosphorylation of glycogen, however, differs from the misincorporation of bases into nucleic acids acids in that normal glycogen contains a low level of phosphate. Phosphate removal by laforin in vivo is not 100% efficient and a basal level of glycogen phosphorylation appears to be tolerated. It is as though there is a phosphorylation threshold below which glycogen structure can remain in an acceptable range. Without laforin activity, though, the result is devastating and I would argue that laforin normally acts in a repair or damage control function.

Acknowledgments

References

1. Roach PJ. Glycogen and its metabolism. Curr. Mol. Med. 2002; 2:101–120. [PubMed: 11949930]

2. Tagliabracci VS, et al. Laforin is a glycogen phosphatase, deficiency of which leads to elevated phosphorylation of glycogen in vivo. Proc. Natl. Acad. Sci. USA. 2007; 104:19262–19266. [PubMed: 18040046]

3. Tagliabracci VS, et al. Abnormal metabolism of glycogen phosphate as a cause for Lafora disease. J. Biol. Chem. 2008; 283:33816–33825. [PubMed: 18852261]

4. Tagliabracci VS, et al. Phosphate incorporation during glycogen synthesis and Lafora disease. Cell. Metab. 2011; 13:274–282. [PubMed: 21356517]

5. Atkinson, DE. Cellular Energy Metabolism and its Regulation. New York: Academic Press; 1977.

6. Khersonsky O, Tawfik DS. Enzyme promiscuity: a mechanistic and evolutionary perspective. Annu. Rev. Biochem. 2010; 79:471–505. [PubMed: 20235827]

7. Persson B, Hedlund J, Jornvall H. Medium- and short-chain dehydrogenase/reductase gene and protein families: the MDR superfamily. Cell. Mol. Life Sci. 2008; 65:3879–3894. [PubMed: 19011751]

8. Knowles JR. Enzyme-catalyzed phosphoryl transfer reactions. Annu. Rev. Biochem. 1980; 49:877–919. [PubMed: 6250450]

9. Hurley TD, Stout S, Miner E, Zhou J, Roach PJ. Requirements for catalysis in mammalian glycogenin. J. Biol. Chem. 2005; 280:23892–23899. [PubMed: 15849187]

10. Kunkel TA, Bebenek K. DNA replication fidelity. Annu. Rev. Biochem. 2000; 69:497–529. [PubMed: 10966467]

11. Thomas MJ, Platas AA, Hawley DK. Transcriptional fidelity and proofreading by RNA polymerase II. Cell. 1998; 93:627–637. [PubMed: 9604937]

12. Zaher HS, Green R. Fidelity at the molecular level: lessons from protein synthesis. Cell. 2009; 136:746–762. [PubMed: 19239893]

13. Gunja-Smith Z, Marshall JJ, Mercier C, Smith EE, Whelan WJ. A revision of the Meyer-Bernfeld model of glycogen and amylopectin. FEBS Lett. 1970; 12:101–104. [PubMed: 11945551]

14. Melendez-Hevia E, Waddell TG, Shelton ED. Optimization of molecular design in the evolution of metabolism: the glycogen molecule. Biochem. J. 1993; 295:477–483. [PubMed: 8240246]

15. Chen, Y-T.; Burchell, A. Glycogen storage diseases. In: Scriver, CR.; Beaudet, AL.; Sly, WS.; Valle, D., editors. The Metabolic and and Molecular Bases of Inherited Disease. New York: McGraw-Hill; 1995. p. 935-965.

16. Fontana JD. The presence of phosphate in glycogen. FEBS Lett. 1980; 109:85–92. [PubMed: 6153366]

17. Lomako J, Lomako WM, Kirkman BR, Whelan WJ. The role of phosphate in muscle glycogen. Biofactors. 1994; 4:167–171. [PubMed: 7916962]

18. Lomako J, Lomako WM, Whelan WJ, Marchase RB. Glycogen contains phosphodiester groups that can be introduced by UDP glucose: glycogen glucose 1-phosphotransferase. FEBS Lett. 1993; 329:263–267. [PubMed: 8396041]

19. Worby CA, Gentry MS, Dixon JE. Laforin: a dual specificity phosphatase that dephosphorylates complex carbohydrates. J. Biol. Chem. 2006; 281:30412–30418. [PubMed: 16901901]

20. Ritte G, Heydenreich M, Mahlow S, Haebel S, Kotting O, Steup M. Phosphorylation of C6- and C3-positions of glucosyl residues in starch is catalysed by distinct dikinases. FEBS Lett. 2006; 580:4872–4876. [PubMed: 16914145]

21. Zeeman SC, Smith SM, Smith AM. The diurnal metabolism of leaf starch. Biochem. J. 2007; 401:13–28. [PubMed: 17150041]

22. Minassian BA, et al. Mutations in a gene encoding a novel protein tyrosine phosphatase cause progressive myoclonus epilepsy. Nature Genetics. 1998; 20:171–174. [PubMed: 9771710]

23. Wang J, Stuckey JA, Wishart MJ, Dixon JE. A unique carbohydrate binding domain targets the lafora disease phosphatase to glycogen. J. Biol. Chem. 2002; 277:2377–2380. [PubMed: 11739371]

24. Ramachandran N, Girard JM, Turnbull J, Minassian BA. The autosomal recessively inherited progressive myoclonus epilepsies and their genes. Epilepsia. 2009; 50(Suppl. 5):29–36. [PubMed: 19469843]

25. Gentry MS, Dixon JE, Worby CA. Lafora disease: insights into neurodegeneration from plant metabolism. Trends Biochem. Sci. 2009; 34:628–639. [PubMed: 19818631]

26. Delgado-Escueta AV. Advances in lafora progressive myoclonus epilepsy. Curr. Neurol. Neurosci. Rep. 2007; 7:428–433. [PubMed: 17764634]

27. Andrade DM, Turnbull J, Minassian BA. Lafora disease, seizures and sugars. Acta Myol. 2007; 26:83–86. [PubMed: 17915579]

28. Sakai M, Austin J, Witmer F, Trueb L. Studies in myoclonus epilepsy (Lafora body form). II. Polyglucosans in the systemic deposits of myoclonus epilepsy and in corpora amylacea. Neurology. 1970; 20:160–176. [PubMed: 4188951]
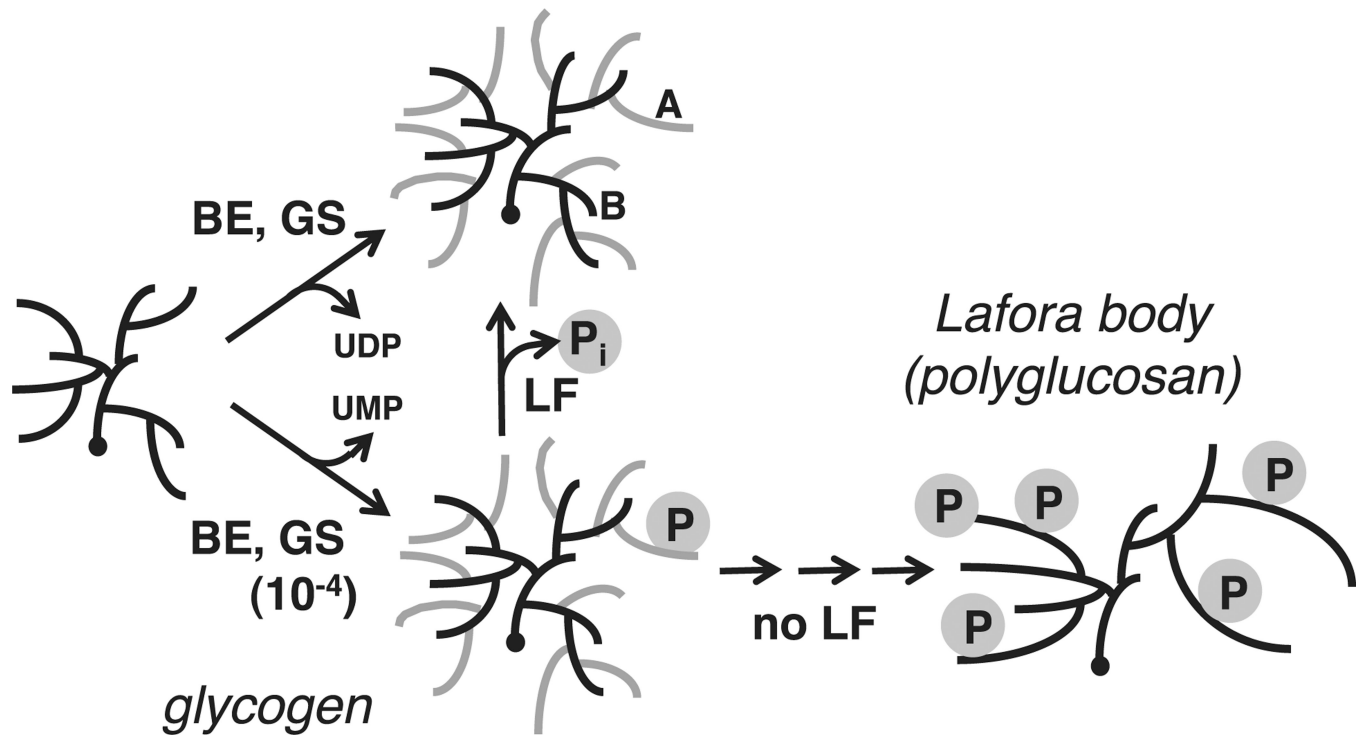
Fig. 1.
Model of glycogen structure and polyglucosan formation. Glycogen is depicted as being composed of successive tiers of polyglucose chains of average length 13 residues. The inner B chains would have on average two branches while the outer A chains would be unbranched. Normal glycogen synthesis, mediated by glycogen synthase (GS) and branching enzyme (BE), adds glucose moieties from UDP-glucose to the non-reducing ends of the molecule and introduces the branchpoints (grey chains; upper arrow). In the proposed rare side reaction (lower arrow), glucose-phosphate is transferred instead of glucose with the formation of UMP. The accumulation of covalent phosphate in glycogen is normally opposed by the action of the laforin phosphatase (LF). In the absence of active laforin, as in Lafora disease, phosphate accumulates in the glycogen leading ultimately to structural disturbances, the formation of poorly branched polyglucosan and the insoluble deposits of Lafora bodies.