BRANCHING ACTION OF AMYLO-1,6-GLUCOSIDASE/OLIGO-1,4→1,4-GLUCANTRANSFERASE

F.HUIJING, E.Y.C.LEE, J.H.CARTER and W.J.WHELAN
Department of Biochemistry, University of Miami School of Medicine, Miami, Florida, 33152, USA

Received 12 February 1970

1. Introduction

Glycogen-storage disease type IV is characterized by the total absence of branching enzyme (EC 2.4.1.18, α-1,4-glucan: α-1,4-glucan 6-glycosyltransferase) [1, 2]. The disease is also characterized by the accumulation, in all tissues investigated, of an amylopectin-like glycogen with an average chain length of 16-22 [1, 3, 4] as compared with 10-14 for normal glycogen. The enigmatic accumulation of a branched polysaccharide in the absence of branching enzyme, rather than a linear polysaccharide, has never been satisfactorily explained.

We propose the hypothesis that the branch linkages in the polysaccharide isolated from glycogen-storage disease type IV tissues are synthesized through the reversible action [5] of the debranching enzyme system, amylo-1,6-glucosidase/oligo-1,4→1,4-glucantransferase* (EC 3.2.1.33; EC 2.4.1.25). Evidence for the hypothesis is that in the presence of a physiological concentration of glucose, the rabbit-muscle debranching enzyme system behaves as a branching enzyme when assayed for branching activity as by Brown and Brown [1].

2. Methods

Rabbit-muscle debranching enzyme was prepared by a modification of the method of Brown and Brown [6]. The major differences were the use of repeated DEAE-cellulose column chromatography to remove phosphorylase *b*, which has been shown to be the major contaminant of glucosidase/transferase preparations [7], and fractionation on a Sephadex G-200 column as the final stage of purification. The latter step removed a small amount of branching enzyme which would otherwise have interfered with our experiments. A preparation with a final specific activity of 7.6 IU/mg was obtained, which was free from α-amylase, branching enzyme and phosphorylase activities.

Baker's yeast glucosidase/transferase was prepared by the method of Lee et al. [8]. The preparation had a specific activity of 4.8 IU/mg, and was free from α-amylase and branching enzyme activities.

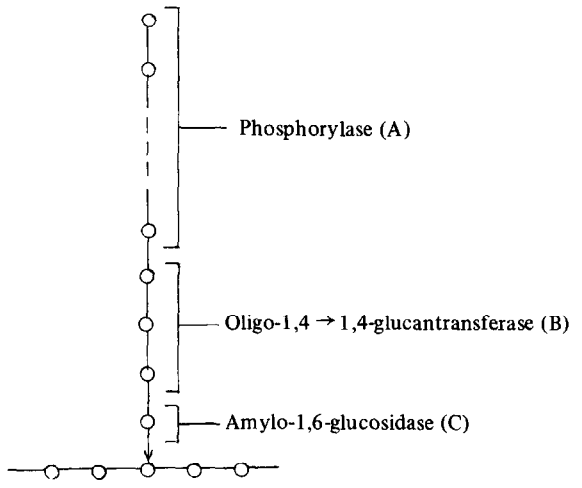
Phosphorylase *b* was isolated from rabbit muscle as by Fischer et al. [9]. The enzyme was converted into phosphorylase *a* with phosphorylase kinase, and freed from traces of branching enzyme by DEAE-cellulose chromatography.

3. Results and discussion

The splitting of a glycogen branch linkage is envisioned as a three-stage process [10, 11]. The linkage in question must be the sole connection of the chain to the rest of the molecule. First the chain is reduced in length to 4 glucose units by phosphorolysis. Then a maltotriosyl unit is transferred by oligo-1,4→1,4-glucantransferase to another chain end. Finally the remaining 1→6-linked glucose unit is removed by hydrolysis. This is shown in Scheme 1.

The slight reversibility of the hydrolytic action of amylo-1,6-glucosidase is known [5], and indeed is

* Abbreviation: Amylo-1,6-glucosidase/oligo-1,4→1,4-glucantransferase to glucosidase/transferase.



Scheme 1. Showing the stepwise degradation (A→C) of a unit chain of glycogen, and the possible re-creation of a chain by the sequence C→A. (o = combined α-glucose; — = 1→4-bond; ↓ = 1→6-bond).

used in a convenient assay of the enzyme (e.g. see [12]). The transferase action is freely reversible [13], as, of course, is that of phosphorylase. Type IV patients are not deficient in the two-component debranching system. Therefore, there exists the potential to create branch linkages, even though branching enzyme is missing.

In considering whether the reverse reaction of glucosidase/transferase can be implicated as the branching agent in the actual formation and accumulation of a branched polysaccharide, the influence of an active chain-elongating system must also be taken into account. Given a minimal amount of primer, any new chains formed by the action of the glucosidase/transferase would be the focus of rapid chain elongation; the branch point in the rapidly lengthening side chain would become progressively protected from hydrolysis by the debranching enzyme system. This may be inferred from studies which show that the ability of glucosidase/transferase to debranch glycogen decreases with increasing chain length [7, 14]. Thus, while equilibrium considerations of the reverse reaction of amylo-1,6-glucosidase alone would not favour any major degree of branch-point synthesis, the opposite might be true in the case where there is a concomitant action of oligo-1,4→1,4-glucantransferase coupled to an actively synthesizing chain-elongating system.

Glucosidase/transferase was therefore tested for its ability to synthesize branch linkages in conjunction with phosphorylase and α-glucose-1-phosphate as the chain-elongating system. The experimental conditions employed were the assay method of Brown and Brown [1] for branching enzyme, which utilizes the observation that very little phosphate release from α-glucose-1-phosphate is catalyzed by rabbit-muscle phosphorylase in the absence of added primer. The presence of traces of endogenous primer may be relied on, and on addition of a branching enzyme, new non-reducing ends are formed and an increasingly rapid synthesis takes place, as evidenced by the release of inorganic phosphate. When the debranching enzyme system was added to phosphorylase/glucose-1-phosphate, no reaction took place, indicating freedom from branching enzyme (fig. 1). However, when this system was supplemented by glucose, a rapid release of inorganic phosphate was observed after an initial lag period. Glucose without debranching enzyme was ineffective. Thus, by the criteria of this assay procedure, rabbit-muscle glucosidase/transferase displays branching activity in the obligatory presence of glucose. The incorporation of ^{14}C-glucose into 67% ethanol-insoluble material (polysaccharide) was also measured and was seen to parallel the inorganic phosphate release.

It should be noted that the glucose concentration in this experiment (5 mM) was in the range of its concentration in blood, while the polysaccharide concentration was many times lower than that encountered *in vivo*. Halving the glucose concentration decreased the rate of phosphate release by about one-third. It should be noted too that the concentration of debranching enzyme used in these experiments was many times that *in vivo*.

A similar experiment was performed using glucosidase/transferase from yeast. No release of inorganic phosphate, or of incorporation of ^{14}C-glucose into 67% ethanol-insoluble material, was observed. In the presence of a small amount of added primer (12.5 μg amylose/ml) no increase in the rate of release of phosphate was observed, although a limited amount of ^{14}C-glucose was now incorporated into 67% ethanol-insoluble material. The explanation for these

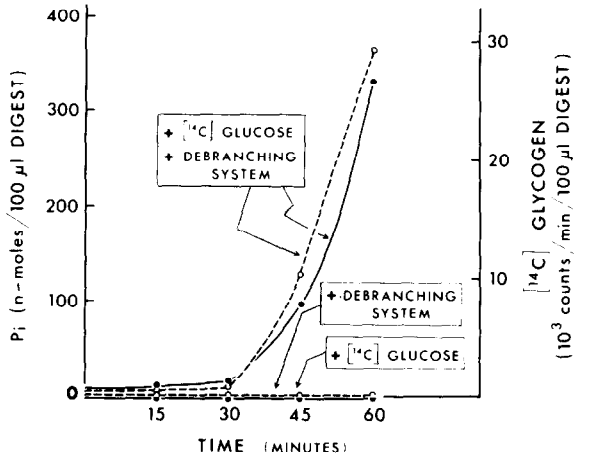


Fig. 1. Branching activity of rabbit-muscle glucosidase/transferase. The system (" + ^{14}C-glucose + debranching system") contained 13 mM Na maleate, pH 6.2, 25 mM 2-mercaptoethanol, 3 mM AMP, 0.65 IU rabbit-muscle phosphorylase *a*, 0.5 IU rabbit-muscle glucosidase/transferase, 5 mM ^{14}C-glucose (total cpm, 45 X 10^6); 53 mM glucose-1-phosphate, in a total volume of 0.75 ml. Phosphate production (- o - - - o -) was measured after stopping the reaction by mixing 0.1 ml of the incubation mixture with 0.9 ml of 0.72N H_2SO_4. To this was added 1 ml of a solution containing 4% ferrous sulphate and 1% ammonium molybdate in 1 N H_2SO_4. The absorbance of the solution was measured at 700 nm after an exactly timed period of 2 min. Incorporation of ^{14}C-glucose into 67% ethanol-insoluble material (- • —— • -) was measured on 0.05 ml samples by the method of Thomas et al. [17]. Three control digests were incubated in parallel. The first contained no debranching system (i.e. " + ^{14}C-glucose"), the second no ^{14}C-glucose (i.e. "+ debranching system") while the third contained neither glucose nor debranching system. Release of inorganic phosphate and/or incorporation of ^{14}C-glucose was measured as appropriate. No changes in these parameters took place in any of the control digests during 60 min.

results comes from the observation that the transferase of yeast has a preference for the transfer of maltosyl units [8]. The resultant side chains would be three units long, and these would not be expected to serve as primers for the phosphorylase reaction [15].

In conclusion, it can be said that the mammalian glycogen debranching system is capable of synthesizing 1→6-bonded side chains which can be elongated by phosphorylase, and that in the presence of phosphorylase and glucose-1-phosphate it is capable of acting effectively as a branching enzyme. Under *in vivo* conditions, the chain-lengthening enzyme might be expected to be glycogen synthetase. This has similar specificity requirements to phosphorylase as regards primer [15, 16] and should therefore cooperate equally well with the debranching enzyme system.

Acknowledgements

This work was supported by grants from The National Institutes of Health (AM 13359 and AM 12532). The authors also thank Miss D.Wolter for her assistance. E.Y.C.Lee is an Investigator of the Howard Hughes Medical Institute.

References

[1] D.H.Brown and B.I.Brown, Proc. Natl. Acad. Sci. U.S. 56 (1966) 725.
[2] J.Fernandes and F.Huijing, Arch. Diseases Childhood 43 (1968) 347.
[3] B.Illingworth and G.T.Cori, J. Biol. Chem. 199 (1952) 653.
[4] L.W.J.Holleman, J.A.van de Haar and G.A.M.de Vaan, Lab. Invest. 15 (1966) 357.
[5] J.Larner and L.H.Schliselfeld, Biochim. Biophys. Acta 20 (1956) 53.
[6] D.H.Brown and B.I.Brown, in: Methods in Enzymology, Vol. 8, eds. E.F.Neufeld and V.Ginsburg (Academic Press, New York, 1966) p. 515.
[7] T.E.Nelson, E.Kolb and J.Larner, Biochemistry 8 (1969) 1419.
[8] E.Y.C.Lee, J.H.Carter, L.D.Nielsen and E.H.Fischer, Biochemistry, in the press.
[9] E.H.Fischer, E.G.Krebs and A.B.Kent, Biochem. Prepn. 6 (1958) 68.
[10] G.J.Walker and W.J.Whelan, Biochem. J. 76 (1960) 264; M.Abdullah, P.M.Taylor and W.J.Whelan, in: Control of Glycogen Metabolism, eds. W.J.Whelan and M.P. Cameron (Churchill, London, 1964) p. 123.
[11] D.H.Brown and B.Illingworth, in: Control of Glycogen Metabolism, eds. W.J.Whelan and M.P.Cameron (Churchill, London, 1964) p. 139.
[12] H.G. Hers, W.Verhue and F.Van Hoof, European J. Biochem. 2 (1967) 257.
[13] H.G.Hers, W.Verhue and M.Mathieu, in: Control of Glycogen Metabolism, eds. W.J.Whelan and M.P. Cameron (Churchill, London, 1964) p. 151.
[14] D.H.Brown, B.I.Brown and C.F.Cori, Arch. Biochem. Biophys. 116 (1966) 479.

[15] D.H.Brown and C.F.Cori, in: The Enzymes, Vol. 5, eds. P.D.Boyer, H.Lardy and K.Myrback (Academic Press, New York, 1961) p. 207.

[16] D.H.Brown, B.Illingworth and R.Kornfeld, Biochemistry 4 (1965) 486.

[17] J.A.Thomas, K.K.Schlender and J.Larner, Anal. Biochem. 25 (1968) 486.