SIMULTANEOUS ACTION OF TWO GLYCOSIDASES ON TWO SUBSTRATES HELD IN CLOSE SPATIAL PROXIMITY[1]

A. S. Perlin

Prairie Regional Laboratory
National Research Council
Saskatoon, Saskatchewan
Canada

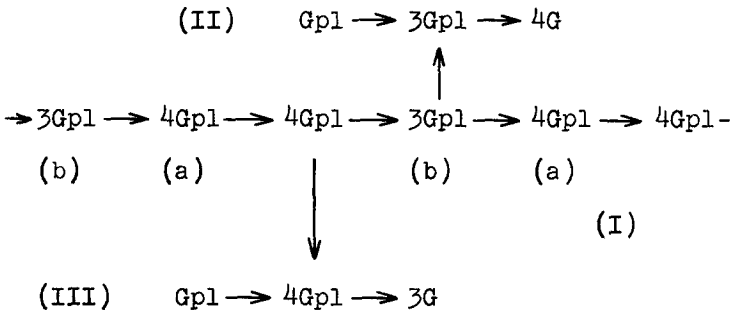
Received January 4, 1965

Recent studies on the enzymolysis of polyglucans (6) have provided the opportunity of examining a relatively novel type of enzyme system - i.e., one in which two hydrolytic enzymes are added simultaneously to a macromolecule that contains a substrate site for each enzyme, the two sites being close to each other in space. Another property of the system is that as one substrate is hydrolyzed by its specific enzyme, the supply of the second substrate available to its enzyme is depleted proportionately. This report describes some preliminary observations on such a system.

It was reported earlier that lichenin and related β-D-glucans of cereals are attacked in highly selective fashion by two different polyglycosidases (4, 5, 8). The β-D-glucopyranosyl units of these polysaccharides form an ordered sequence in which an isolated 1,3-linkage alternates with two consecutive 1,4-linkages along most of the linear chain (I). A cellulase from Streptomyces sp. QMB814 (11) degrades I by cleavage at the 4-substituted β-D-glucosyl

[1] Issued as N.R.C. No. 8339.

unit (a) with the formation of trisaccharide II (O-β-D-glucopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-D-glucose). The point of cleavage in I for a laminarinase from Rhizopus arrhizus QM1032 (9) is the 3-substituted β-D-glucosyl unit (b), and the hydrolysis product in this reaction is trisaccharide III (O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→3)-D-glucose). Although both enzymes attack the same



polymeric molecule (I), in reality two distinct substrates are involved. These substrates can be regarded as bound together in close proximity, with their focal points for enzymic attack at the adjacent D-glucosyl units (a) and (b). Most probably, the two different substrate sites involved comprise more than these single D-glucosyl units (1, 6, 7, 10) and overlap appreciably. If the cellulase and laminarinase are added together and then can compete with one another, such competition should be readily evident in the composition of the products, for as the polymer is hydrolyzed by either enzyme the amount of substrate available to the second enzyme is progressively depleted.

The experimental methods were essentially those used earlier in determining the mode of action of the cellulase and laminarinase on the polyglucans (5, 8). Under the

conditions used, trisaccharide II or III accounts for about 60% of the original polymer, and disaccharide, tetrasaccharide and polymeric residue are minor hydrolyzate constituents. Lichenin was used as substrate in the current experiments because the minor oligosaccharides are formed in lesser proportion from it than from the cereal glucans. Qualitative analysis of the hydrolyzates was readily carried out by paper chromatography, since trisaccharides II and III are cleanly separated from each other and from other products by using ethyl acetate-acetic acid-water (2) as solvent. A comparison of the kinetic properties of the two enzymic reactions has been narrowly restricted by a necessity to use low substrate concentrations (solutions containing more than 0.6% lichenin were too viscous), which complicates the estimation of initial hydrolytic rates, as well as by the limited quantity of purified laminarinase available. However, characteristics such as K_m or V_{max} for each enzyme acting on lichenin are probably of the same order of magnitude. This is suggested by reaction velocity measurements during intermediate stages (25-50%) of the hydrolysis. Thus, the observed rates of formation of reducing sugar by a given weight of the cellulase preparation closely paralleled those by an equal weight of the laminarinase preparation over a 6-fold range of lichenin concentration, with the latter enzyme being approximately 20% less active.

Although the two enzymes exhibit little difference in hydrolytic power when used independently, the laminarinase was totally ineffective on lichenin in the presence of only half its weight of cellulase. Periodic chromatographic examination of digests in which cellulase and laminarinase were admixed in a proportion of 0.5:1, 1:1 or 1:2, showed the

ready formation of trisaccharide II, the product of cellulolytic breakdown; but trisaccharide III could not be detected at any stage. Only when the proportion of cellulase was reduced 4-5 fold or the proportion of laminarinase increased 4-5 fold was the laminarinase able to function in the mixed system. Under these disparate conditions trisaccharide III was now produced in an amount about equal to that of II. Appreciable quantities of cellobiose and laminaribiose also were found in these latter digests, derived possibly by competitive hydrolysis of relatively long-chain intermediates. The inhibitory effect of cellulase was equally marked when the enzyme was added well after the degradation of lichenin had been initiated by laminarinase. This was shown by treating the polysaccharide with laminarinase until the degree of hydrolysis reached a value of ca 25% and then adding an equal, or one-half, proportion of cellulase. Subsequent hydrolysis of the residual substrate was found to have yielded trisaccharide II but little, if any, additional III.

This striking interference by cellulase with the normal action of laminarinase does not appear to be due to irreversible inactivation of the latter enzyme by the former, to the presence of selective inhibitors in the cellulase preparation, nor to products of cellulase hydrolysis. Thus, degradation of the polysaccharide laminarin by laminarinase was unaffected (both in rate of hydrolysis and the nature of the products formed) by the presence in the digest of five equivalent proportions of cellulase. Also, lichenin was hydrolyzed by the laminarinase in normal fashion when the admixed cellulase was first heat-denatured; and hydrolysis was not retarded by addition of trisaccharide II to the

digest. Further, when lichenin was first treated with cellulase to effect about a 25% degree of hydrolysis and the cellulase then was deactivated by heat, the partially degraded residue was readily attacked further by added laminarinase.

The marked difference in relative effectiveness of the two enzymes when mixed, in contrast with their similar hydrolytic activities individually, does not find a ready explanation in the data available. The results are not at variance with the probability noted above that the two substrate sites in lichenin overlap appreciably. Something akin to competitive inhibition may occur, in that the formation of one enzyme-substrate complex prevents ready access of the second enzyme to its substrate. However, this is likely to account for only part of the inhibition observed since there probably is more than adequate substrate present initially to permit appreciable independent action by each enzyme. For this reason also, unless the concentration of enzyme-substrate complexes is unusually high in these systems, factors such as the relative stability of the complexes or as the equilibria governing their formation would not be expected to favor one enzyme so heavily in the presence of the other.

Another possibility is that cellulase causes inhibition by reversing the formation of the laminarinase-lichenin complex. Thus, the sudden increase in yield of III (from nil to 50%) caused by a relatively small change in the laminarinase:cellulase ratio (from 2:1 to 4:1) is reminiscent of a desorption process. Some type of interaction between the two enzymes themselves also might be visualized. In a

complexed form the effectiveness of the laminarinase could conceivably be impaired, but not necessarily that of the cellulase. However, when the proportion of cellulase in the mixture is lowered sufficiently there would be an excess of laminarinase, which then could function independently. Although no interference is apparent when laminarin is the substrate for the enzyme mixture, the cellulase presumably would have little affinity for this polysaccharide, and also cannot remove it from the system as it does with lichenin.

There is widespread interest in mechanisms whereby enzyme-catalyzed reactions are controlled *in vivo*. It is well recognized (3) that results obtained with isolated enzyme systems may not be extrapolated with confidence to an interrelated network of systems. The present observations provide yet another example of how complexities might be multiplied, even when only two individual systems are brought together.

ACKNOWLEDGMENTS

The author expresses thanks to Dr. E. T. Reese for generous supplies of the enzymes used, and to Dr. W. B. McConnell for very helpful discussion.

REFERENCES

1. Gottschalk, A. Advances in Carbohydrate Chem. 5, 49 (1950).
2. Jermyn, M. A. and Isherwood, F. A. Biochem. J. 44, 402 (1949).

3. Pardee, A. B. In The Enzymes. Academic Press, New York. Second Edition (1959). Vol. 1, p. 681.

4. Parrish, F. and Perlin, A. S. Nature 187, 1110 (1960).

5. Parrish, F., Perlin, A. S., and Reese, E. T. Can. J. Chem. 38, 2094 (1960).

6. Perlin, A. S. In Enzymic Hydrolysis of Cellulose and Related Materials. Pergamon Press, New York (1963). p. 185.

7. Perlin, A. S. and Reese, E. T. Can. J. Biochem. Physiol. 41, 1842 (1963).

8. Perlin, A. S. and Suzuki, S. Can. J. Chem. 40, 50 (1962).

9. Reese, E. T. and Mandels, M. Can. J. Microbiol. 5, 173 (1959).

10. Reese, E. T. and Mandels, M. Can. J. Microbiol. 10, 103 (1964).

11. Reese, E. T., Smakula, E., and Perlin, A. S. Arch. Biochem. Biophys. 85, 171 (1959).