LETTERS TO THE EDITORS

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Synthesis of Amylopectin

NATURAL starch is a mixture of two components, which can be separated in a number of ways although it is doubtful if complete separation has yet been attained. The names, amylose and amylopectin, used earlier in a different sense, may be conveniently retained to describe the two components. Amylose, forming about 25 per cent of whole starch, is constituted of unbranched chains of glucose residues, the members being mutually linked by α-1 : 4-glycosidic linkages. Amylopectin possesses for the most part the same 1 : 4 glucose linkages and forms about 75 per cent of natural starch, but its chain structure is branched or laminated through the lateral linking of its shorter lengths of 1 : 4 glucose chains by certain α-1 : 6-glycosidic bonds.

C. S. Hanes[1] isolated from the potato (and from the pea) a phosphorylase which effected, in the presence of phosphate ion, the conversion of whole starch into glucose-1-phosphate, and demonstrated that this reaction was, in a sense, reversible *in vitro*. The polysaccharide produced in this reverse reaction was not, however, identical with whole starch but with only one of its components. At least 85 per cent of the Hanes' synthetic starch had the properties of an amylose. Thus, (1) it retrograded rapidly from solution, (2) it gave a pure blue colour with iodine, and (3) it was completely hydrolysed to maltose by β-amylase. End-group assay showed its continuous chain-length to be 80–90 glucose members[2] whereas that of whole starch averages 24–30 units. Furthermore, the molecular weight of the methylated product determined osmotically corresponded to a particle size of 80–100 glucose units, thus providing clear evidence of the absence of branching in this synthetic amylose.

We now have to report the separation from the potato of an enzyme system which catalyses the conversion of glucose-1-phosphate into a polysaccharide which is not amylose but which is probably identical with the amylopectin component of natural starch. This new polysaccharide is constituted entirely of *d*-glucose units, it gives a purple-red colour with iodine, it is soluble in water and does not retrograde from solution. In these properties it is not to be distinguished from the amylopectin fraction of natural starch. Furthermore, it is attacked by β-amylase and maltose is liberated, but, as with natural amylopectin, the hydrolysis is arrested before the conversion into maltose is complete. This behaviour is characteristic of the branched-chain type and distinguishes it sharply from the unbranched, amylose type which is entirely converted to maltose by β-amylase. Under identical conditions and by the same preparation of β-amylase, synthetic amylopectin and natural amylopectin were converted to maltose to the extent of 45 and 49 per cent respectively. These figures represent resting points in the hydrolysis and were attained after about four hours in each case.

In an exploratory experiment, a few grams of the synthetic amylopectin have been methylated and the proportion of end-group assayed. The result quite definitely indicates that the substance is not amylose, inasmuch as the number of glucose residues in the unit chain is 20. We lay no stress on the actual numerical value given for the chain-length because this preliminary assay was carried out on too small a quantity of methylated product to conform to the usual standard of accuracy, and, moreover, the sample submitted to assay contained only 41 per cent methoxyl as compared with the usual 44–45 per cent. The margin of error is not, however, greater than ± 10 per cent. Although much dubiety is attached to the estimation of molecular weight by viscosity measurement, it may nevertheless be of significance that our viscosity measurements indicate a molecular size corresponding to about 100 hexose units when the K_m constant for methylated whole starch is used. With this reservation in mind, the particle size is seen to be some five times the size of the unit chain, a fact which supports the evidence of branching given by the observation of the cessation of β-amylase activity at 45 per cent conversion.

Hassid and McCready[3] find for separated natural amylopectin, by end-group assay, a unit chain-length of 25 glucose residues and a molecular weight (by viscosity measurement) corresponding to 450 glucose units. The same experimental error margin (± 10 per cent) will apply to this assay figure as to ours for the reason that less than 0·5 gm. of end-group was estimated.

It is our intention to repeat the end-group assay and also to determine the molecular weight by the more trustworthy osmotic method when adequate quantities of synthetic amylopectin become available.

It is too early to discuss the bearing of these observations on the question of starch synthesis in the plant; but we have some reason to believe that Hanes' amylose-synthesizing enzyme participates also in the enzyme system responsible for the synthesis of our amylopectin. It would appear that an additional factor—the *Q* factor—is present in our enzyme preparation which modifies the normal synthetic activity of the phosphorylase (*P* enzyme) and enables 1 : 6-glycosidic cross-linkages to be formed.

It is perhaps important to mention that our synthetic amylopectin cannot be an amylolytic degradation product of an amylose synthesized in the normal way by phosphorylase. We have taken the greatest care to ensure the absence of both α- and β-amylase from our enzyme preparations.

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[1] Hanes, C. S., *Proc. Roy. Soc.*, B, **128**, 421; **129**, 174 (1940).
[2] Haworth, W. N., Heath, R. L., and Peat, S., *J. Chem. Soc.*, 55 (1942).
[3] Hassid, W. Z., and McCready, R. M., *J. Amer. Chem. Soc.*, **65**, 1159 (1943).

Penicillinase from *B. subtilis*

IN the bacteriological examination of blood or exudates of patients under treatment with penicillin, it is necessary to inactivate any penicillin present if viable penicillin-sensitive organisms are to grow. The routine testing of penicillin products for sterility also requires a means of neutralizing the antibacterial effect of penicillin. The coli-penicillinase method[1] has the disadvantages that the preparation is turbid and the activity of the final product varies.