The Size of Polysaccharide Molecules

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By the chemical assay of the end group in a methylated cellulose it was found by HAWORTH und MACHEMER[1] that this derivative existed as a long open-chain structure and that the chain length of this product corresponded to about 200 glucose members or groups or a molecular weight of about 40000. It was then pointed out in that paper that we regarded this value as the average lower limit of the unit of the macro molecule. It was added that although it is of interest it is of no vital concern to the study of the intimate structure of cellulose whether this value is to be increased to 100000 for native cellulose. At the same time it is of immense general importance that the approximate limiting figure for the molecular size of all such polysaccharides should be investigated and experiments which have been continued in my laboratories have confirmed the above results. Using high viscosity cellulose from cotton linters, methylated celluloses have been prepared having viscosities corresponding to 250 and 340 glucose units, based on the STAUDINGER factor. Higher viscosities than these are obtained if the cellulose is not fully methylated. In all cases it has been observed that the end group method of measurement shows a chain length of about 200 glucose units. Specimens of very much higher viscosity are also under examination and will be reported on shortly.

It is possible that some common fundamental factor is operating to determine the very large molecular size of polysaccharide molecules. Consequently it would seem undesirable to limit the investigation of this problem to the study of a single polysaccharide. The factors underlying these questions can best be investigated by a general study of many types of carbohydrate and this has been attempted. The application of the chemical end group method to starches is facilitated both by the relative ease of methylation in this series and by the comparatively high

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[1] J. chem. Soc. London **1932**, 2270.

proportion of tetramethyl glucose liberated on hydrolysis of the fully methylated derivatives. An extensive examination has therefore been undertaken with the object of investigating the relationships between the values for the particle size and molecular weight determined by viscosity, osmotic pressure, and the end group method.

Firstly, the standard procedure was adopted. A non-reducing starch acetate was formed under carefully controlled conditions and this was subjected to simultaneous deacetylation and methylation by methyl sulphate and sodium hydroxide. In this way it was found that for potato starch, soluble starch (commercial A. R. sample), maize starch, and waxy maize starch the percentage of tetramethyl glucose given on hydrolysis was 4·5 % corresponding to a chemical chain length of 24 to 26 units of glucose or a molecular weight of approximately 4000. The conditions were then varied in that acetates of different viscosities were prepared from a given sample of starch. In every instance it was ascertained that the reducing power of the acetate was negligible, the iodine number being the same as for the original sample of unacetylated starch and the copper number showed only slight variation, the acetates of lower viscosity having the higher copper number. It should be noted that the copper numbers even for the least viscous acetate are of the same order and indeed less than those recorded by RICHARDSON, HIGGINBOTHAM, and FARROW[2] for unmodified starches. On methylation, viscous methyl derivatives were obtained from the viscous acetates and less viscous methyl derivatives from the less viscous acetates. The observed chemical chain length was in all cases 24—26 glucose units. In other experiments the starches were methylated directly, without preliminary transformation to the acetate, and by modification of the experimental conditions methylated starches of very varied viscosities were obtained corresponding, on the basis of the older calculation used by STAUDINGER for cellulose, to an apparent molecular weight of 7000 rising to 100000. In these experiments no acid reagent came into contact with the starch during the whole of the procedure by which the fully methylated derivatives were obtained. On hydrolysis of this series of methylated starches the observed chemical chain length calculated from the yield of tetramethyl glucopyranose was again 24—26 glucose units. Osmotic

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[2] SHIRLEY Inst. Mem. **14** (1935) 63.

pressure measurements determined for these products seemed to indicate that the particle weight corresponds to approximately 5 to 6 times the values derived from the viscosity data.

Precisely similar results have been obtained with potato starch, and with both this and maize starch it has been observed that when direct methylations are carried out in the cold without passing through the acetate, or when viscous acetates are methylated at low temperatures, the resulting methylated derivatives have a high viscosity. It is possible to obtain in this way methylated starches of apparent molecular weight 100000 (from the older viscosity data) and with a methoxyl content of only 43—44 %. On further methylation even under the same mild conditions it is invariably found that as the methoxyl content rises the viscosity falls. It follows therefore that beyond a certain methoxyl content further methylation is impossible without simplification of the molecular aggregate. This is to be regarded as significant evidence in favour of the aggregation hypothesis which has already been advanced. On this view starch consists of comparatively short chains which are capable of undergoing aggregation to very large units of enormous particle size and conversely can undergo disaggregation at least partially. There can be no question of chemical degradation during the final phase of the methylation process, since the yield of tetramethyl glucose obtained from the viscous methylated starch of methoxyl content 43 %, is almost identical with that from the less viscous fully methylated derivative having methoxyl content 45 %.

There is thus abundant evidence that under various conditions starch can be acetylated without appreciable rise in the reducing power. The acetates can possess all degrees of viscosity. By direct methylation without passing through the acetate, or by methylation of the above acetates, derivatives of varying degrees of viscosity can be obtained. It would appear that there is no relationship subsisting between viscosity and the observed chemical chain length, which remains invariable for specimens of undegraded starch derivatives. As soon as hydrolytic degradation of starch into dextrins is deliberately attempted the value for the chemical assay of the end group diminishes progressively.

Facts of similar interest have been obtained by the study of glycogen from several sources, namely, from rabbit liver, fish liver and fish muscle. From some of these sources the chemical end group method of investigation has given values corresponding to

12 glucose units in the chain and in other cases to 18 glucose units. Viscosity measurements, using again the STAUDINGER formula developed for cellulose, are in agreement with these values. On the other hand the osmotic pressure measurements obtained by using a cellophane membrane show very much higher particle sizes. This method has been developed in my laboratory by CARTER and RECORD[3]. The values vary between 3400 and 5400 glucose units in the methylated glycogens from the rabbit, haddock, hake and dogfish. Still higher values were found for the acetylated specimens. These values are so large that they show no comparison with the corresponding derivatives of starch or with the viscosity values. Two samples of methylated starch showed osmotic pressures corresponding in particle sizes to 630 and 140 glucose units. In these instances the sample which gave solutions of higher viscosity exerted the lower osmotic pressure. On the other hand two samples of methylated starch dextrins of short chain length exhibited osmotic pressures indicating the presence of 23 and 24 glucose units and these results are not far removed from the end group value of 12 and 9 determined by chemical methods.

With the polysaccharide inulin it has already been shown[4] by the chemical end group method of assay that methylated inulin corresponds to a chain of about 30 fructose units. The osmotic pressure determinations have confirmed exactly this estimate of chain length. On the other hand the viscosity value in this case is exceedingly low and if the same factor as was used by STAUDINGER for cellulose is employed these viscosity data point to the presence of only 9 fructose units. The probable reason for this wide disparity is commented upon by the present writer in his Presidential Address to the British Association for the Advancement of Science, Chemistry Section, 1935, page 32. It was there pointed out that configurational or spatial arrangements of a model must affect very widely the factors underlying viscosity measurements and in the case of inulin the spacing of the chemical structure demands that the molecule is arranged as a laminated series of fructofuranose rings which are not joined end to end as in the case of cellulose or starch.

It has never been claimed by the present author that the chemical end group method of determination of chain length

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[3] J. Soc. chem. Ind. **1936**, 218.

[4] HAWORTH, HIRST, and PERCIVAL, J. chem. Soc. London **1932**, 2384.

corresponds with the particle size. The view advanced in earlier papers has been that there is an aggregation of such chains which are joined by physical links or by co-ordination end to end. A measurement of particle size must take account of the physical molecule and the molecular aggregate. This may involve not only aggregation to increase the length of the chain but also the lateral combination between the chains. These factors must be recognised in any comparison of the molecular weights of cellulose, starch, glycogen, or other polysaccharides. A striking example is afforded in the case of the polysaccharide xylan. Here it was shown[5] that the chemical unit of methylated xylan is composed of about 18 pentose residues. It was there suggested in view of physical measurements that four or more of these chains were grouped together by co-ordination or other type of union between the reducing end of the chain and an intermediate hydroxyl position of an adjoining chain.

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[5] HAWORTH, HIRST, and OLIVER, J. chem. Soc. London **1934**, 1917.