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An Approximate Estimation of the Number Average DP of Amylose and Amylopectin in Moderately Degraded Whole (Unfractionated) Starch by the Periodate Method

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Introduction

The well-known difficulties[1] in determining the true average degree of polymerization (DP) of the amylose and amylopectin fractions of starch can be ultimately reduced to two basic causes: first, the imperfect fractionating techniques, which make it virtually impossible to obtain the separated fractions complete, pure, and undegraded; and second, the unknown degree of association of the starch molecules in solution. Whereas the former difficulty is merely technical, the latter is of a fundamental character, and it is almost impossible to obtain even an approximate idea of the true DP of the fractions.

This state of things is illustrated by the discrepancies found between the experimental data of various authors.[2] Thus, for instance, an amylose having an intrinsic viscosity of 1.23 was found to have an osmotic pressure DP of 800, whereas another sample, having an intrinsic viscosity of 1.76 gave a DP of 770 by the same method in the same solvent. For one sample of potato amylose the intrinsic viscosity and the osmotic pressure DP found were 4.06 and 3,800, respectively; these figures are three to four times higher than those met with as a rule. The position with respect to amylopectin is even more chaotic. Thus, a sample of tapioca amylopectin acetate, dissolved in chloroform, gave an osmotic pressure DP of 1,300 for an intrinsic viscosity of 1.35, whereas another sample, which had an intrinsic viscosity of 1.27 (i.e., somewhat lower than the former), gave an osmotic pressure DP of no less than 21,000 by the same method in the same solvent. In one light scattering determination, the weight average DP of an amylopectin sample was found to be no less than 1,450,000 (molecular weight, 420 million); the sample had an intrinsic viscosity of 1.26.

These data are being quoted not to cast a reflection on the quality or usefulness of the work on which they are based, but merely to show that

with our present knowledge of the physical chemistry of starch, any determination of *true* DP, however crudely approximate, is preferable to any determination, however precise, where the solution effects remain unknown.

In view of the above, an ideal method for the determination of true molecular weights of amylose and amylopectin should meet two conditions: fractionation should not be required, and the method itself should be independent of associative effects in solution. Now the periodate oxidation method[3] is known to meet the second condition.

It will be shown in what follows that, when applied to whole native starches, the periodate method cannot by itself give any information as to the DP of the two fractions; when the starch has been randomly degraded to a very moderate extent, however, the results can be used to deduce the number average DP of each molecular species in that degraded starch with a fair degree of approximation.

Theory

It is well known that on quantitative periodate oxidation of starch, two and one molecules of formic acid are evolved from each reducing and nonreducing end, respectively.

Consider an amylose molecule having a DP of $N_0{}^A$. Let $F_0{}^A$ be the average number of moles of formic acid evolved per mole-AGU (equal to 162 g.) of that molecule on quantitative periodate oxidation. Then it is seen at once that

$$F_0{}^A = 3/N_0{}^A \tag{1}$$

The substances dealt with in practice are always polymer-homologous mixtures of $m_1{}^A$ molecules of DP = 1, $m_2{}^A$ molecules of DP = 2 . . . $m_p{}^A$ molecules of DP = p. Thus the total number of molecules is $\sum_i m_i{}^A$, the total number of AGU's is $\Sigma m_i{}^A N_i{}^A$, and the number average DP will be given by $\bar{N}_0{}^A = \sum_i m_i{}^A N_i{}^A / \sum_i m_i{}^A$. Three molecules of formic acid will be evolved by each molecule, and $3\sum_i m_i{}^A$ molecules of formic acid by all the molecules. The average amount of formic acid $F_0{}^A$ evolved per AGU will therefore be

$$F_0{}^A = 3\sum_i m_i / \sum_i m_i{}^A N_i{}^A = 3/\bar{N}_0{}^A$$

It will thus be seen that the formic acid actually evolved is equal to that which would be evolved if the amylose sample consisted of molecules of an uniform DP equal to $N_0{}^A$. Formula (1) is therefore valid for any mixture of amylose molecules whose number average DP is equal to $\bar{N}_0{}^A$.

Consider an amylopectin molecule, whose DP is equal to $N_0{}^B$ and whose repeating chain length is n AGU's. Then the number of molecules of formic acid evolved on quantitative periodate oxidation will be

$$F_0{}^B = 2/N_0{}^B + 1/n \tag{2}$$

A reasoning exactly similar to the foregoing will show that for a polymer-homologous mixture of amylopectin molecules, the number average DP $\bar{N}_0^B$ will be given by

$$F_0^B = 2/\bar{N}_0^B + 1/n$$

It will be seen that, just as before, formula (2) is valid for any polymer-homologous mixture of amylopectin molecules of a number average DP of $\bar{N}_0^B$.

Let F_0 be the number of moles of formic acid evolved per AGU-mole of whole starch on quantitative periodate oxidation. Then

$$F_0 = wF_0^A + (1 - w)F_0^B = \frac{3w}{\bar{N}_0^A} + \frac{2(1 - w)}{\bar{N}_0^B} + \frac{1 - w}{n} \qquad (3)$$

where w is the weight fraction of amylose in the granule.

In eq. (3) F_0, w, and hence n may be experimentally determined (see below) but since neither N_0^A nor N_0^B are known, the equation cannot be solved.

It will now be assumed that the starch granule has been randomly degraded, the degradation (proportion of the links broken to the total number of links) being equal for amylose and amylopectin, the maltose and isomaltose links in the starch being indistinguishable in that they are equally subject to degradation.

Let S bonds be broken at random in an amylose molecule having an initial DP equal to N_0^A. Then the degree of degradation α will equal $S/(N_0^A - 1) \approx S/N_0^A$ and the resulting fragment mixture will have a number average DP N_α^A given by

$$\bar{N}_\alpha^A = \frac{N_0^A}{S + 1} = \frac{1}{\alpha + (1/N_0^A)} \qquad (4)$$

If a polymer-homologous mixture of amylose molecules is considered as before, its total number of bonds will be $\sum_i m_i^A(N_i^A - 1)$ and if α is to retain its former value, the number of bonds broken σ will be given by

$$\alpha = \frac{\sigma}{\sum_i m_i^A(N_i^A - 1)} \qquad \text{or} \qquad \sigma = \alpha\sum_i m_i^A(N_i^A - 1)$$

The number average DP of the resulting mixture of molecules will be the ratio of the total number of AGU's to the total number of molecules present in the mixture, or

$$\bar{N}_\alpha^A = \frac{\sum_i m_i^A N_i^A}{\sum_i m_i^A + \sigma} = \frac{1}{(1/N_0^A) + \alpha}$$

very approximately, since $1/\bar{N}_0^A \ll 1$.

Thus, formula (4) is seen to be applicable to any polymer-homologous amylose mixture having a number average DP equal to $\bar{N}_\alpha^A$.

For the branched amylopectin molecule, having a DP equal to N_0^B, the number of bonds equals $N_0^B - 1$, just as in amylose, and an exactly similar reasoning will lead to the formula

$$\bar{N}_\alpha^B = [1/(1/\bar{N}_0^B) + \alpha] \tag{5}$$

The quantity of the moles of formic acid evolved per AGU-mole of whole starch, degraded to the extent α, will be, in analogy with formula (3),

$$F_\alpha = \frac{3w}{\bar{N}_\alpha^A} + \frac{2(1-w)}{\bar{N}_\alpha^B} + \frac{1-w}{n} \tag{6}$$

Since neither $\bar{N}_\alpha^A$ nor $\bar{N}_\alpha^B$ are known, this equation cannot be solved either. However, *approximate* values of $\bar{N}_\alpha^A$ and $\bar{N}_\alpha^B$ may be deduced from it as follows.

It is known from experiment that

$$\bar{N}_0^A \leqslant \bar{N}_0^B \tag{7}$$

Let us now limit our considerations to the values of α above a minimum given by

$$\alpha \bar{N}_0^A \geqslant 1 \tag{8}$$

which amounts to saying that the extent of degradation is such that, on the average, at least one link is broken per amylose molecule. If we now define $K_\alpha = \bar{N}_\alpha^B / \bar{N}_\alpha^A$, then

$$K_\alpha = \frac{\alpha + (1/\bar{N}_0^A)}{\alpha + (1/\bar{N}_0^B)} \tag{9}$$

in view of eqs. (4) and (5).

Then, the analysis of eq. (9) while assuming eqs. (7)and (8) will show that

$$1 \leqslant K_\alpha \leqslant 2 \tag{10}$$

It may thus be deduced from eqs. (6), (9), and (10) that the value of $\bar{N}_\alpha^A$ is comprised between the limits

$$\frac{3w + \dfrac{2(1-w)}{2}}{F_\alpha - \dfrac{1-w}{n}} \leqslant \bar{N}_\alpha^A \leqslant \frac{3w + 2(1-w)}{F_\alpha - \dfrac{1-w}{n}}$$

and that of $\bar{N}_\alpha^B$ between the limits

$$\frac{w+2}{F_\alpha - \dfrac{1-w}{n}} \leqslant \bar{N}_\alpha^B \leqslant \frac{4w+2}{F_\alpha - \dfrac{1-w}{n}}$$

The respective arithmetic means $\bar{N}_\alpha{}^{A*}$ and $\bar{N}_\alpha{}^{B*}$ of the extreme values are

$$\bar{N}_\alpha{}^{A*} = \frac{3(w+1)}{2\left(F_\alpha - \frac{1-w}{n}\right)} \tag{11}$$

$$N_\alpha{}^{B*} = \frac{5w+4}{2\left(F_\alpha - \frac{1-w}{n}\right)} \tag{12}$$

The respective relative deviations from the mean value are given by

$$\delta_A = \frac{\left|\bar{N}_\alpha{}^{A} - \bar{N}_\alpha{}^{A*}\right|}{\bar{N}_\alpha{}^{A*}} \leqslant \frac{1-w}{3(1+w)} \tag{13}$$

$$\delta_B = \frac{\left|\bar{N}_\alpha{}^{B} - \bar{N}_\alpha{}^{B*}\right|}{\bar{N}_\alpha{}^{B*}} \leqslant \frac{3w}{5w+4} \tag{14}$$

Numerical Example*

An *undegraded* starch sample was titrated potentiometrically with iodine.[4] From the proportion of iodine absorbed, the amylose content was calculated to be 20% (i.e., $w = 0.2$).

The same sample of *undegraded* starch was quantitatively oxidized with periodate and one mole of formic acid per 25 AGU's was evolved. Since this was contributed, for all practical purposes, by amylopectin only,† $n = (1 - 0.2) \times 25 = 20$.

The starch was then randomly degraded, and the periodate determination repeated on the degraded sample, when one mole of formic acid per 18.2 AGU's was evolved, so that $F_\alpha = 1/18.2 = 0.055$. Now, $\bar{N}_\alpha{}^{A}$ and $\bar{N}_\alpha{}^{B}$ can be calculated directly from formulas (11) and (12) and the maximum error from formulas (13) and (14), respectively.

$$\bar{N}_\alpha{}^{A} = \frac{3(0.2+1)}{2\left(0.055 - \frac{1-0.2}{20}\right)} = 120 \pm 22\%$$

$$\bar{N}_\alpha{}^{B} = \frac{5 \times 0.2 + 4}{2\left(0.055 - \frac{1-0.2}{20}\right)} = 167 \pm 12\%$$

* Taken from actual experimental data.

† This amounts to saying that in eq. (3) the expression $3/N_0{}^A$ may be neglected. For an *undegraded* cereal starch, the error thus introduced will be less than 5%, and so not more than that introduced by any known fractionation procedure.

References

1. See, e.g., F. C. Cleveland and R. W. Kerr, *J. Am. Chem. Soc.*, **71**, 16 (1949).
2. Greenwood, C. T., *Advances in Carbohydrate Chem.*, **11**, 363 and 373 (1956).
3. Bobbitt, I. M., *Advances in Carbohydrate Chem.*, **11**, 11–41 (1956).
4. Lansky, S., M. Kooi, and T. J. Schoch, *J. Am. Chem. Soc.*, **71**, 4066 (1949).

Synopsis

A method, based on periodate oxidation is proposed for the determination of approximate true number average DP of amylose and amylopectin fractions in whole starch which has been randomly degraded to a moderate extent. The method is approximate only. This is compensated for by the fact that it is not affected by associative phenomena in solution, and that no fractionation is required. A numerical example is given, showing the application of the theoretical formulas to experimental data.

Résumé

1. Une méthode basée sur l'oxydation au periodate est proposée pour la détermination d'une valeur approchée du DP moyen de l'amylose et de fractions d'amylopectine de l'amidon complet dégradé au hasard à un degré de conversion modéré. *2.* La méthode n'est qu'approximative. Ceci est compensé par le fait qu'elle n'est pas affectée par des phénomènes d'association en solution et qu'aucun fractionnement n'est requis. *3.* Un exemple numérique est donné, montrant l'application de la formulie théorique aux données expérimentales.

Zusammenfassung

Eine auf der Perjodatoxydation beruhende Methode zur Bestimmung von angenäherten, wahren Zahlenmittelwerten von DP für Amylose- und Amylopektinfraktionen in statistisch zu einem mässigen Grade abgebauten Gesamtstärke wird angegeben. Die Methode liefert nur angenäherte Werte. Das wird durch den Unstand kompensiert, dass sie nicht durch Assoziationserscheinungen in Lösung beeinträchtigt wird und dass keine Fraktionierung erforderlich ist. Ein Zahlenbeispiel, das die Anwendung der theoretischen Formeln auf die Versuchsdaten zeigt, wird gegeben.

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An approximate determination of number average DP of amylose and amylopectin in degraded starch without recourse to fractionation*

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The true molecular weights of both amylose and amylopectin are unknown, since, in the first place, the two components cannot be separated from each other without degradation, and, more important, their degrees of association in solvents, organic or inorganic, are unknown. For this reason it would be advantageous to obviate the necessity for fractionation, and to determine the degree of polymerization (DP) by a chemical, rather than a physical method, the former method not being affected by the solute being associated.

Such a method is provided for by the use of sodium periodate, which is a specific and stoichiometric reagent of vic-hydroxy groups (glycols). If the polysacharide is of the 1-4′-polyanhydrohexopyranose type, then, on being quantitatively oxidized with periodate, it will consume a number of moles of periodate and it will evolve a certain number of moles of formic acid.

If the starch is randomly degraded to a certain extent — say 1 glucosidic bond scission in 500 or less AGUs — the amount of formic acid produced will be a measurable function of the molecular weight.

Mathematical formulae are deduced which allow the number-average DP of *each* molecular species to be determined with a fair degree of approximation for a randomly degraded *unfractionated* starch sample, if the amount of formic acid evolved by the sample is known.

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