Monte-Carlo Investigation of Nearest-neighbour Auto-inhibitory Effects in the Oxidation of Amylose by Periodate Ion

OLAV SMIDSRØD, BJØRN LARSEN and TERENCE PAINTER

Norwegian Institute of Seaweed Research, N.T.H., N-7034 Trondheim, Norway

At any stage in its oxidation by periodate, a molecule of amylose can be regarded as a linear sequence of unoxidised glucose residues (A) and oxidised glucose residues (B). The unoxidised units (A) can therefore exist at the centre of four different kinds of triplet, AAA, AAB, BAA and BAB. It was shown that the kinetics of the reaction can be explained if these four different possible states of unreacted units are distinguished, and a separate, second-order rate-constant is assigned to each. This was done by showing that an experimental curve could be closely matched with a curve generated in the digital computer by the following Monte-Carlo procedure.

A population of n amylose molecules, each M units in length, was represented by an array of n rows and M columns of zeros. Random oxidation of the units was simulated by the supply of random integers (μ) in the interval from unity to nM. The success of an attack was recorded by the introduction of an arbitrary integer (1) into the appropriate position in the array, and other integers (2 and 3, respectively) into the preceding and following positions in the same row. Superimposition of a 2 on to a 3, or of a 3 on to a 2, was recorded by conversion into a 4. The probability of the success of an attack on a 2, a 3, or a 4 was determined by the contingency of other random numbers (σ) falling within a specified fraction (α_L, α_R, or α_{LR}, respectively) of the designated interval. All attacks on zeros were recorded as successful, while subsequent attacks on positions occupied by 1's were not recorded. The passage of time was recorded as the number of random integers (μ) supplied, this being progressively weighted to represent the consumption of periodate.

The results were interpreted as evidence for the rapid establishment of equilibria between the aldehyde groups of oxidised units and the hemiacetals that they may form with the hydroxyl groups on neighbouring, unoxidised units. The positions of the equilibria were given by the appropriate values of α_L, α_R, and α_{LR}.

The kinetics of the reactions of polymers are often very complex, because reaction of one unit or linkage in a given chain can modify the reactivities of other units or linkages in the same chain. In flexible coils, the effect is

likely to be short-ranged, and the reactive sites immediately adjacent to those attacked should be most affected. An example is the acid-hydrolysis of proteins, in which scission of a peptide bond generates a protonated, N-terminal amino-group, which then exerts an electrostatic shielding effect over the adjacent peptide bond in the fragment containing it.[1-3] Such an effect may be described as auto-inhibitory. Another example, in which both linkages adjacent to those attacked are greatly reduced in reactivity, is the hydrolysis of amylose by α-amylase.[4,5]

In this laboratory, it was recently observed that the oxidation of 1,4-linked polysaccharides by periodate ion is also strongly auto-inhibitory, and this was traced to the formation of 6-membered hemiacetal rings between the aldehyde groups of oxidised sugar residues and the closest hydroxyl groups on the two nearest-neighbouring, unoxidised residues.[6-9] In the case of one polysaccharide, sodium alginate, these hemiacetals are so stable that reaction virtually ceases when every unoxidised unit has at least one oxidised unit as a nearest neighbour, and it was possible to predict the observed oxidation-limit on the assumption that hemiacetal formation was very rapid compared to the rate of oxidation.[6,7]

With other polysaccharides, however, oxidation proceeds ultimately to completion, the ratio of the initial and final rates being about 50:1 for xylan [8] and 28:1 for amylose.[9] In these cases, it was suggested that an equilibrium is set up between the inter-unit hemiacetals and the corresponding aldehydic forms, but the idea that a true equilibrium is rapidly established could be only tentatively advanced, and was based upon comparison with alginate, and with simple theoretical models that assumed complete protection of the nearest neighbours of oxidised units from subsequent oxidation.

It was evident that a definitive answer to this question should, in principle, be accessible from a sufficiently detailed theoretical analysis of the kinetic data, and that such an analysis would be greatly simplified because inhibition of the nearest neighbours of oxidised units only could be confidently assumed. Furthermore, although the conformation of amylose in solution is currently the subject of much controversy, it is at least clear that it behaves in aqueous solution as a random coil,[10-12] so that the assumption of random attack of the oxidant on the chains should also be well justified.

While attempting to arrive at an analytical solution to the problem, however, it became clear that the differential rate equation would contain a highly complex statistical term that probably could not be integrated without recourse to approximations of doubtful validity. Similar difficulties are met in the analogous case of auto-activatory reactions, such as the thermal depolymerisation of synthetic addition polymers.[13-15]

Recently, Monte-Carlo computer simulation-techniques have been adapted for the treatment of complex problems in chemical kinetics,[16,17] and it was evident that they would be ideally suited for work on the reactions of polymers. The procedure now described is very simple and accurate, is reasonably economical in computer time, and could be readily adapted for the study of nearest-neighbour auto-inhibitory and auto-activatory reactions generally.

PROCEDURE

The general outline of the programme, given in the abstract of this paper, will be readily followed with the assistance of the following, additional details.

Size of the array. The array is a two-dimensional matrix of integers, and is regarded as a physical representation of a population of polymer molecules. The n rows of the matrix were arbitrarily regarded as separate polymer molecules, while M, the number of monomeric units in each molecule, determined the number of columns.

The number (M) of columns should, in principle, be the degree of polymerisation of the material studied experimentally. That material was, in the present case,[9] amylose having a viscosity-average degree of polymerisation of 2.7×10^3, corresponding to a number-average degree of polymerisation of about 1350 on the assumption of the so-called "most probable" molecular-weight distribution. Whereas it would have been technically feasible to simulate just such a population of chains, given a sufficiently large computer, this was unnecessary, because it was found that, for all values of M greater than 50, the kinetics were identical within the required limits of error. Most of the runs were done with $M = 80$.

The number n of molecules in each sample must be large enough to be regarded as representative of the vastly greater number in the experimental sample, and is determined by the accuracy required. This accuracy is not independent of M because, the larger the value of M, the smaller will be differences between the degrees of reaction of the individual molecules. The value of n required for good accuracy is also dependent upon the fundamental nature of the process under study. In the present work, the auto-inhibitory effect upon nearest neighbours of reacted units only gave rise to very narrow distributions in the degrees of conversion of the individual molecules in the sample. With a value for M of 80, it was possible to obtain the required accuracy with a value of n also of 80.

The accuracy required in the Monte-Carlo results is clearly determined by the accuracy of the experimental data with which they must be matched. The accuracy of the experimental data considered here was such that the standard deviation in a single reading was 0.5 % of the total amount of oxidant consumed at the end of the reaction.[9] With $M = n = 80$, the standard deviation in the Monte-Carlo results varied during the "reaction" in a manner to be indicated, but was less than 0.5 % throughout.

Generation of random numbers. This was done with a congruential (power-residue) [18] generator, namely:

$$I_k = 5^{13} \cdot I_{k-1} \ (\text{mod } 2^{35})$$
$$U_k = \text{FLOAT} \ (I_k)/2^{35}$$

Generators of this kind have been shown to give high performance in statistical tests for randomness.[19] In the Univac 1108 computer, delivery of one random number with this generator required 24 μsec.

The numbers were supplied initially in the interval from zero to unity. The random integers (μ) in the interval from unity to nM inclusive were obtained by multiplication of these by nM, followed by correction to the next

highest integer. The random numbers (σ) were kept as real numbers in the interval from zero to unity.

Simulation of the reaction. At the outset, all the monomeric units were represented in the array as zeros. The position of each unit in the array was marked by an integer in the range from unity to nM inclusive. Thus, the units in the first row were numbered consecutively from 1 to M, those in the second row from $(M+1)$ to $2M$, and so on. Upon delivery of the first random integer (μ), the zero in the appropriate position in the array was changed to a 1, the zero in the preceding position in the same row was changed to a 2, and the zero in the following position in the same row was changed to a 3. If there was no preceding or no following position in the same row, that is, if the random number corresponded to a terminal unit in a polymer molecule, then only the one neighbouring unit in the row was modified in the appropriate way.

This process was continued, all "attacks" on positions occupied by zeros being recorded by conversion into 1's, and all subsequent attacks on 1's being rejected. Where a 1 was introduced next to a position occupied by a 2 or a 3, that integer was changed to a 4.

When an attack was made on a position occupied by a 2, a 3 or a 4, it was recorded as successful (by conversion of that integer to a 1) only with a certain, specified probability, this being α_L for a 2, α_R for a 3, and α_{LR} for a 4. These probabilities were kept constant throughout each run, and were determined by the contingency of a second random number (σ) falling within fractions, α_L, α_R, or α_{LR}, respectively, of unity.

Thus, when an attack was made on a position occupied, for example, by a 2, a second random number (σ) was called for, in the interval from zero to unity, and, if it was α_L or any number less than α_L, the 2 was changed to a 1. At the same time, the integer in the preceding position was changed to a 2 if it was a zero, or to a 4 if it was a 3.

If, on the other hand, the random number (σ) was higher than α_L, no change was made. This allowed the same position to be attacked again, any number of times, later in the reaction, until an attack was accepted as successful. On each occasion, a new random number (σ) was called for, to determine the success or failure of the attack. It was, of course, possible for the 2 to change to a 4 before being successfully attacked, and when that happened, the probability of the success of an attack was changed automatically to α_{LR}.

The time-scale of the reaction. Throughout the reaction, all the random integers (μ) that were supplied were counted, regardless of whether they resulted in the introduction of a 1 or not, and the total number of 1's that were successfully introduced into the array was also counted. When exactly $0.01\,nM$ 1's had been introduced, the number of random integers (μ) that had been required to introduce them was noted and stored in the memory of the computer. The number of random numbers (σ) that had been used in the selection procedures was not counted among these. This process was continued, the total number of random integers (μ) that had been supplied being noted every time that the degree of reaction had increased by an increment of 1 %. The number of random integers thus constituted an arbitrary time-scale of the reaction.

In the early stages of the reaction, when only a small number of random integers (μ) had been supplied, this time-scale was not as accurate as could be desired, and an alternative system was adopted. After each successive increment of 1 % of the total reaction had been completed, a count was made of the total number (N_0) of remaining zeros in the array, and of the total numbers (N_2, N_3, and N_4) of 2's, 3's, and 4's, respectively. As an approximation, it could be assumed that the rate of reaction was virtually constant during each individual increment of 1 % of the total reaction. This rate (r) would then be proportional to $N_0 + N_2(\alpha_L) + N_3(\alpha_R) + N_4(\alpha_{LR})$, and this quantity was calculated for each increment. The time required to complete each increment was then proportional to $(1/r)$, and summation of all the values of $(1/r)$ then provided a measure of the time required to achieve any given degree of reaction.

Correction of the time-scale for consumption of the attacking reagent. The measures of the passage of time just described take no account of the decrease in rate that, in a bimolecular reaction, would result from depletion of the attacking reagent. This effect was corrected for by dividing the measures of the time required for completion of each successive 1 % of the reaction, by the fraction of the original amount of attacking reagent remaining, halfway through each increment.

For example, in the experimental study considered here, amylose that was 3 mM with respect to *vic*-diol groups was oxidised in 5 mM periodate. The molar excess of the oxidant was therefore 1.667 initially, and, halfway through the first 1 % of the reaction, it was $(1.667 - 0.005) = 1.662$. The arbitrary measure of time required for completion of the first 1 % of the reaction was therefore divided by 1.662. That for the second 1 % was divided by $(1.667 - 0.015)$, that for the third by $(1.667 - 0.025)$, and so on. These corrected increments of the arbitrary time-scale were then summed as before.

Calibration of the corrected time-scale. From a special study of the early stages of the reaction,[9] the initial rate was known with fair accuracy, and, expressed as a second-order rate-constant, it was 225 ± 12 1 mole^{-1} h^{-1}. Since the initial slopes of the theoretical curves were quite insensitive [9] to the specified values of α_L, α_R, and α_{LR}, a first approximation for the factor needed to convert the arbitrary time-scale into hours was readily obtained with one such curve.

Subsequently, when the values of these reactivity coefficients that were required to give a good fit with the experimental curve were known more precisely, a small correction to the conversion factor was made. After this, all theoretical curves of practical interest matched the experimental data for the first 10 % of the reaction perfectly, within the known limits of error.

Standard deviation in the Monte-Carlo results. The standard deviation in the mean of the individual degrees of reaction of the n chains in the sample was calculated at regular intervals throughout the reaction. This standard deviation therefore corresponded to that in a single point on the theoretical curve.

RESULTS

Experimental details of the oxidation of amylose (3 mM with respect to *vic*-diol groups) in 5 mM sodium metaperiodate at 20°, and a presentation of

the results in graphical form, are given elsewhere.[9] In the present analysis, the same data will be reported, more accurately, in tabular form, but a part of it is also shown graphically, to illustrate the first stage of the matching procedure.

Coarse matching. After having determined the correct time-scale, the object was to select values of the reactivity coefficients, α_L, α_R, and α_{LR}, to give the closest possible fit with the experimental data. From the results of earlier work,[8,9] it was expected that α_{LR} would be smaller than either α_L or α_R, and it seemed logical to begin with the assumption that it was identical with the product of α_L and α_R.
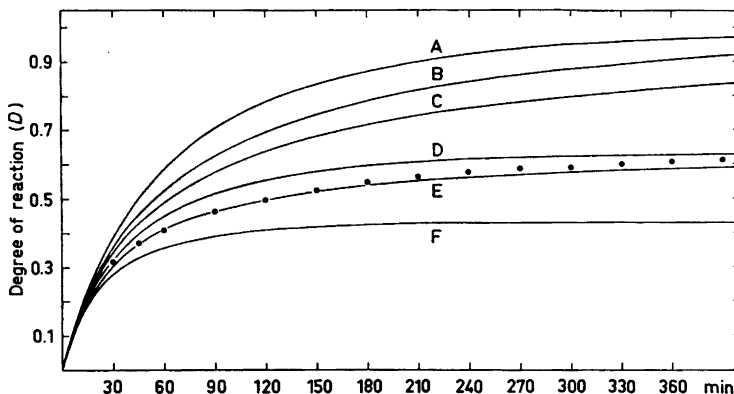

Fig. 1. Family of theoretical curves, obtained by the Monte-Carlo procedure, for nearest-neighbour auto-inhibitory reactions, in which the reactivities of the two units adjacent to reacted units in a linear polymer chain are fractions (α_L and α_R, respectively) of the reactivity of units not having reacted units as nearest neighbours. The reactivity of units having reacted units as both nearest neighbours is assumed to be $\alpha_L\alpha_R$ throughout. Curve A: $\alpha_L=\alpha_R=1.00$; curve B: $\alpha_L=0.50$, $\alpha_R=1.00$; curve C: $\alpha_L=0.25$, $\alpha_R=1.00$; curve D: $\alpha_L=0.00$, $\alpha_R=1.00$; curve E: $\alpha_L=0.00$, $\alpha_R=0.50$; curve F: $\alpha_L=0.00$, $\alpha_R=0.00$.

The time-scale in every case is such that the initial rates are identical with that of the oxidation of amylose (3 mM with respect to *vic*-diol groups) in 5 mM sodium metaperiodate at 20°, and the inserted points ● are the experimental ones for that reaction.

Fig. 1 shows a family of theoretical curves, obtained with different values of α_L and α_R, and with $\alpha_{LR}=(\alpha_L)(\alpha_R)$ in every case. In this figure, the values of α_L and α_R that give any curve are, of course, interchangeable, for example, curve C would also be obtained with $\alpha_L=1.00$ and $\alpha_R=0.25$. No attempt has been made to depict the statistical nature of the Monte-Carlo results by showing the scatter in the numerous points, because the scatter was so small that all the points could be accommodated within the thickness of the lines drawn.

Curve A was obtained with $\alpha_L=\alpha_R=1.0$, and is therefore a typical curve for an ordinary, second-order reaction. This curve was identical with one obtained by simple integration of the appropriate, second-order rate-equation,[9] which provided a useful check that the Monte-Carlo procedure was operating correctly.

Curve D shows the behaviour of a system in which reaction of any unit leads to immediate and complete protection of only one adjacent unit from subsequent attack, the protected unit always having the same directional relationship to the reacted unit that protects it, throughout the length of the chain. In this case, reaction clearly cannot proceed to completion, and it is noteworthy that the limiting degree of reaction (0.635) is identical with that calculated analytically [9] for chains 80 units in length.

Curve F is the corresponding one for complete protection of both nearest neighbours of reacted units from subsequent attack, and, again, the limiting degree of reaction (0.435) agrees with that calculated analytically.[8] This curve should correspond quite closely to the behaviour of systems such as the periodate oxidation of a homopolymeric alginate,[7] and perhaps also α-amylolysis.[4,5]

The points in Fig. 1 are experimental ones for the first 6.5 h of the reaction of amylose with periodate,[9] and it is seen that they are all quite close to curve E, for which $\alpha_L = 0.00$ and $\alpha_R = 0.50$.

Fine matching. Attempts to obtain a closer fit with the experimental data than that provided by curve E in Fig. 1 were guided by two considerations. The first was the recognition that, with $\alpha_L = 0$, $\alpha_R = 0.5$ and $\alpha_{LR} = 0$, the reaction represented by curve E could never proceed to completion, and that α_L would therefore have to be given a value greater than zero. The second was that the ratio of the final to the initial rates of reaction must in every case be numerically identical with α_{LR}; this ratio could be measured with fair accuracy [9] from the experimental curve, and was known to be approximately 1:28.

Starting with the assumption that α_{LR} was exactly $1/28$ ($= 0.0357$), the value of α_L was increased systematically by small increments above zero, the value of α_R being calculated in every case from the assumed relationship, $\alpha_{LR} = (\alpha_L)(\alpha_R) = 0.0357$. In this way, it was quickly found that a good fit with the experimental data was obtained with $\alpha_L = 0.102$ and $\alpha_R = 0.350$. With these values, however, all theoretical points for degrees of reaction over 0.58 were consistently higher than the experimental ones by about $0.5-1.0$ %. This was clearly significant, and it was evident that the value of α_{LR} was actually a little lower than 0.0357. Further investigation showed that all significant differences between the theoretical and experimental points disappeared when α_{LR} was set equal to 0.0330, with $\alpha_L = 0.097$ and $\alpha_R = 0.340$.

Table 1 compares the experimental data with the theoretical results obtained with the two sets of reactivity coefficients just described. The third significant figure given for the theoretical degrees of reaction was obtained by graphical interpolation. Two experimental points, namely those at 15 and 30 min, respectively, are clearly erroneous. The agreement between the remaining 36 experimental points and the best set of theoretical results is virtually absolute.

It may be noted that the reactivity coefficients could be evaluated with higher accuracy by using the computer to carry out a systematic, statistical matching of the theoretical with the experimental data. This would be a relatively routine exercise in computer programming, but it is not of particular chemical interest to seek such an improvement in accuracy at the present time.

Table 1. Oxidation of amylose (3 mM with respect to *vic*-diol groups) in 5 mM sodium metaperiodate at 20°. Experimental degrees of oxidation (D_{expt}) are compared with those (D_{calc}) calculated with two different sets (1 and 2) of reactivity coefficients (α_L, α_R, and α_{LR}). Coefficients 1 are 0.350, 0.102, and 0.0357, respectively, and coefficients 2 are 0.340, 0.097, and 0.0330, respectively. The differences (Diff.) between the experimental and calculated figures are given as ($D_{expt} - D_{calc}$). The theoretical standard deviation refers to D_{calc}, and is common to both sets of coefficients.

Time in min	D_{expt}	Coefficients 1		Coefficients 2		Theoretical standard deviation
		D_{calc}	Diff.	D_{calc}	Diff.	
1.00	0.018	0.018	0.000	0.018	0.000	
2.00	0.036	0.035	0.001	0.035	0.001	
3.25	0.057	0.056	0.001	0.056	0.001	
4.00	0.068	0.068	0.000	0.068	0.000	
5.00	0.082	0.082	0.000	0.082	0.000	
6.00	0.097	0.096	0.001	0.096	0.001	0.0025
8.00	0.124	0.122	0.002	0.122	0.002	
9.00	0.135	0.134	0.001	0.134	0.001	0.0033
15.0	0.217	0.193	0.026	0.196	0.023	0.0040
30.0	0.316	0.298	0.018	0.300	0.016	0.0048
45.0	0.373	0.362	0.011	0.364	0.009	0.0044
60.0	0.409	0.407	0.002	0.408	0.001	0.0044
90.0	0.465	0.462	0.003	0.467	−0.002	0.0039
120	0.496	0.502	−0.006	0.502	−0.006	0.0034
150	0.528	0.529	−0.001	0.529	−0.001	
180	0.550	0.550	0.000	0.550	0.000	0.0034
210	0.566	0.566	0.000	0.566	0.000	
240	0.580	0.580	0.000	0.580	0.000	
270	0.591	0.592	−0.001	0.592	−0.001	
300	0.594	0.602	−0.008	0.602	−0.008	
330	0.602	0.610	−0.008	0.611	−0.009	0.0028
360	0.612	0.619	−0.007	0.620	−0.008	
390	0.618	0.627	−0.009	0.628	−0.010	
420	0.625	0.635	−0.010	0.635	−0.010	
450	0.630	0.641	−0.011	0.641	−0.011	
555	0.653	0.662	−0.009	0.661	−0.008	0.0028
630	0.670	0.677	−0.007	0.673	−0.003	
690	0.679	0.687	−0.008	0.683	−0.004	
750	0.689	0.695	−0.006	0.690	−0.001	
810	0.698	0.703	−0.005	0.698	0.000	0.0028
870	0.707	0.711	−0.005	0.705	0.002	
930	0.712	0.718	−0.006	0.712	0.000	
990	0.723	0.724	−0.001	0.719	0.004	
1200	0.743	0.746	−0.003	0.739	0.004	
1320	0.751	0.755	−0.004	0.750	0.001	0.0034
1440	0.753	0.765	−0.012	0.760	−0.007	
1560	0.766	0.777	−0.011	0.770	−0.004	
1680	0.778	0.789	−0.011	0.778	0.000	0.0040

For the present purpose, it seems to be both safe and sufficient to conclude that $\alpha_L = 0.100 \pm 0.010$, that $\alpha_R = 0.340 \pm 0.020$, and that $\alpha_{LR} = (\alpha_L)(\alpha_R)$. The kinetics of the oxidation of amylose under the stated conditions can therefore be described in terms of four second-order rate-constants, namely, k, $(\alpha_L)k$, $(\alpha_R)k$ and $(\alpha_{LR})k$, where k is the initial rate, and has a value of 225 ± 12 l mole^{-1} h^{-1}.

Other characteristics of the reaction. In addition to accounting for the kinetics of the consumption of periodate by amylose, the Monte-Carlo programme was able to predict the changes in other experimentally measurable quantities occurring during the reaction. Among these was the average sequence-length of the groups of contiguous, unoxidised glucose residues in the chains, and the size-distribution of these groups. The changes occurring in these quantities with increasing degree of oxidation had been studied by means of the Barry degradation, and, although this method could not be readily adapted for precise, quantitative work, a semi-quantitative description of the results was published.[9]

It has been mentioned that one of the methods used for obtaining an index of the passage of time in the simulated reaction consisted in counting the numbers, N_0, N_2, N_3, and N_4, of the subscripted integers in the array at 1 % intervals throughout the process. The sum $(N_0 + N_2 + N_3 + N_4)$ is clearly the number of unreacted monomeric units, and, for an array of 80×80 integers, it is $6400(1 - D)$, where D is the degree of reaction.

It is evident that the weight-fraction, F_1, of the total, unreacted units that have reacted units as both nearest neighbours is $N_4/(N_0 + N_2 + N_3 + N_4)$, and in Fig. 2, this quantity is plotted as a function in the degree of reaction, D. From this curve, it is now readily understood why, when the degree of oxidation had reached 0.70, Barry degradation no longer yielded the phenylosazone of any fragment larger than glucose, because at that stage, 97.5 % of all unoxidised residues had oxidised residues as both nearest neighbours. It is
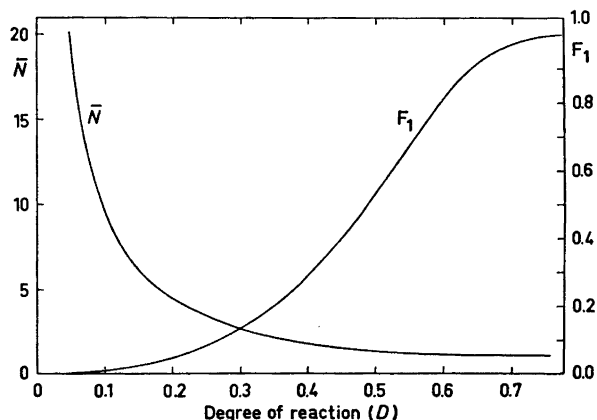

Fig. 2. Theoretical (Monte-Carlo) results for the changes in the number-average sequence-length $(\bar{N})$ of the groups of contiguous, unreacted monomeric units, and in the weight-fraction (F_1) of the total unreacted units having reacted units as both nearest neighbours, as a function in the degree of reaction (D). The reactivity coefficients (*cf.* Table 1) are $\alpha_L = 0.097$, $\alpha_R = 0.340$, and $\alpha_{LR} = 0.033$.

also clear why the last 36 % of the reaction was completed in close accordance with second-order kinetics, because it is seen that, from $D = 0.64$ onwards, more than 90 % of the remaining reactive sites are structurally identical.

Fig. 2 also shows the changes in the number-average sequence-length $(\bar{N})$ of the groups of contiguous, unreacted glucose residues with increasing degree of reaction (D). This quantity was calculated as follows. It was reasoned that, in any row of the array, the number of 2's must be equal to, or be greater or less by one, than the number of 3's. In the whole array of 80×80 integers, therefore, $N_2 = N_3 \pm 80$, a condition which was, of course, found to be correct. Furthermore, it was evident that, to a good approximation, either N_2 or N_3 would be the total number of groups of consecutive, unreacted glucose residues in the array. Addition to this of N_4, which is similarly a close approximation for the total number of isolated, unreacted glucose residues, then gives the total number of unreacted sequences, $(N_2 + N_4)$ or $(N_3 + N_4)$. The number-average sequence-length $(\bar{N})$ was calculated by dividing the total number of unreacted units, $(N_0 + N_2 + N_3 + N_4)$, by $[(N_2 + N_3)(\frac{1}{2}) + N_4]$. The curve obtained (Fig. 2) further illustrates the capacity of the Monte-Carlo model to account for the results of the Barry degradations.[9]

DISCUSSION

At any stage in its reaction, a uniformly linked, homopolymeric chain can be regarded as a linear sequence of unreacted units (A) and reacted units (B). If the two ends of the chain are non-identical, non-terminal, unreacted units (A) can exist at the centre of four different kinds of triplet, AAA, AAB, BAA, and BAB. The present analysis has shown that the kinetics of the periodate oxidation of amylose can be accounted for if these four different possible states of unreacted units are distinguished, and a separate, second-order rate-constant is assigned to each.

The necessity of distinguishing between these four different kinds of reactive site is fully consistent with the evidence obtained earlier, that the auto-inhibitory effect is due to the formation of 6-membered hemiacetal rings between the aldehyde groups of oxidised units and the hydroxyl groups at positions 2 and 3 of unoxidised units.[6-9]

The constancy in the reactivities of the four sites throughout the reaction now permits the further conclusion, that a true equilibrium is very rapidly established, after oxidation of any glucose residue, between the liberated aldehyde groups and the inter-residue hemiacetal forms. The positions of these two equilibria are known with some accuracy from the given values of the reactivity coefficients, and they substantiate the earlier conclusion [9] that one of the two equilibria lies farther on the side of the inter-residue hemiacetal form than does the other. The difference between the positions of the two equilibria is, however, not quite so large as the preliminary kinetic analysis [9] suggested, and it must be noted that both aldehydic groups form inter-residue hemiacetals in preference to intra-residue hemiacetals.

Kinetic studies of the present kind may be useful in applications of periodate oxidation to the structural analysis of polysaccharides. For example, if it were not already well established that amylose is a uniformly α-1,4-linked

glucan, the constancy in the reactivities of the four sites named above would have provided strong evidence for the virtual absence of anomalous linkages in the material. This would apply not only to the positions of the linkages, but also to their anomeric form, because the anomeric centres of both oxidised and unoxidised glucose residues are present in the hemiacetal rings, and it is very unlikely that the positions of the equilibria would be unaffected by their configuration.

The same information would not, of course, be provided by the Malapradian oxidation-limit alone, since 1,2- or 1,5-linked glucose residues would also consume one mole of periodate without liberating formic acid, in either the α- or the β-form. In this connection, it is noteworthy that oxidised units in 1,5-linked sequences would be unable to inhibit the oxidation of neighbouring units by 6-membered hemiacetal-ring formation, while in 1,2-linked units, an oxidised unit could form such a hemiacetal with only one unoxidised neighbour. Periodate-oxidation kinetics might therefore be expected to show high sensitivity to every structural detail in a polysaccharide, and indeed it is already clear that the positions of the equilibria are substantially different even in the three 1,4-linked polysaccharides, alginate,[6,7] xylan,[8] and amylose.[9]

The present study has also shown that the reactivity coefficient (α_{LR}) for a doubly inhibited unit is simply the product of the separate contributions, α_L and α_R, of the two oxidised neighbours. This implies that hemiacetal formation with one of the secondary hydroxyl groups of an unoxidised unit neither favours nor disfavours formation of a hemiacetal with the other. In general, this condition would be expected to apply only if the formation of either hemiacetal was unaccompanied by any substantial change in the conformation of the unoxidised unit. Since it is sterically impossible for a glucose residue in the 1C conformation to form a hemiacetal at position 3 in a partly oxidised amylose chain (cf. a cognate discussion on alginate [7]), it is very probable that most of the glucose residues in amylose exist, in solution, in the C1 conformation at 20°. This is in agreement with physical evidence.[20,21]

Within the possible range of reactions of polymers that exhibit auto-inhibitory effects, the periodate oxidation of amylose is probably a model of simplicity rather than a typical example.

It is apparent, however, that the Monte-Carlo procedure possesses the versatility that is needed to treat more complex cases. A minor modification of the programme described here would permit the simulation of reactions in which the reactivities of the next-nearest and still more remote neighbours of reacted units are modified, and, by filling the array initially with more than one kind of arbitrary integer, the behaviour of copolymers could also be studied.

Copolymers that are polydisperse with respect to composition and structure could be represented by filling the initial array with sequences of integers generated, according to the kinetics of copolymerisation, by Monte-Carlo techniques [22] or by exact enumeration with statistical weighting.[23] Populations of simple branched-chain polymer molecules, whose structures can be formally accommodated in two dimensions, could be represented in the computer by three-dimensional arrays, while four-dimensional arrays could be used to represent populations of three-dimensional polymeric networks.

REFERENCES

1. Gordon, A. H., Martin, A. J. P. and Synge, R. L. M. *Biochem. J.* **35** (1941) 1369.
2. Stein, W. H., Moore, S. and Bergmann, M. *J. Biol. Chem.* **154** (1944) 191.
3. Synge, R. L. M. *Biochem. J.* **39** (1945) 351.
4. Meyer, K. H. and Gonon, W. F. *Helv. Chim. Acta* **34** (1951) 294.
5. Whelan, W. J. and Roberts, P. J. *J. Chem. Soc.* **1953** 1298.
6. Larsen, B. and Painter, T. J. *Carbohydrate Res.* **10** (1969) 186.
7. Painter, T. J. and Larsen, B. *Acta Chem. Scand.* **24** (1970) 813.
8. Painter, T. J. and Larsen, B. *Acta Chem. Scand.* **24** (1970) 2366.
9. Painter, T. J. and Larsen, B. *Acta Chem. Scand.* **24** (1970) 2724.
10. Banks, W. and Greenwood, C. T. *Carbohydrate Res.* **7** (1968) 349.
11. Banks, W. and Greenwood, C. T. *Carbohydrate Res.* **7** (1968) 414.
12. Rao, V. S. R., Yathindra, N. and Sundararajan, P. R. *Biopolymers* **8** (1969) 325.
13. Simha, R., Wall, L. A. and Blatz, P. J. *J. Polymer Sci.* **5** (1950) 615.
14. Simha, R., Wall, L. A. and Bram, J. *J. Chem. Phys.* **29** (1958) 894.
15. Boyd, R. H. *J. Chem. Phys.* **31** (1959) 321.
16. Schaad, L. J. *Am. Inst. Chem. Engrs. J.* **85** (1963) 3588.
17. Cohen, G. D. *Ind. Eng. Chem. Fundamentals* **4** (1965) 471.
18. International Business Machines Corp., *Random Number Generation and Testing,* Ref. Manual C20-8011, IBM Corp., New York 1959.
19. Van Gelder, A. *J. Assoc. Computing Machinery* **14** (1967) 785.
20. Rao, V. S. R. and Foster, J. F. *J. Phys. Chem.* **69** (1965) 636.
21. Casu, B., Reggiani, M., Gallo, G. G. and Vigevain, A. *Tetrahedron* **22** (1966) 3061.
22. Smidsrød, O. and Whittington, S. G. *Macromolecules* **2** (1969) 42.
23. Larsen, B., Painter, T. J., Haug, A. and Smidsrød, O. *Acta Chem. Scand.* **23** (1969) 355.