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A Computer Study of the Changes in Composition-Distribution Occurring during Random Depolymerisation of a Binary Linear Heteropolysaccharide

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The random depolymerisation is considered of a binary linear heteropolysaccharide of high molecular weight, in which the monosaccharide residues are arranged according to a statistical model. With the help of the digital computer, the changes occurring in the distribution of the monosaccharide units among the various fragments are studied. In general, an initially unimodal distribution becomes successively bimodal, trimodal, polymodal, and finally bimodal again as the reaction mixture is simplified into a mixture of two monomers. The bi- and trimodal phases are due to the appearance of bi- or trimodal distributions in chains of the same length, whereas the polymodal phase is a result of the presence of chains of different lengths. The separate degrees of scission at which these changes occur are determined by the composition of the heteropolysaccharide, and the way in which the monosaccharide units are arranged along its chains. The changes resemble those occurring, in the reverse order, during the biosynthesis of the heteropolymer. The significance of experimental data obtained with alginic acid is discussed in the light of these findings.

Upon mild acid-hydrolysis, alginic acid is rapidly cleaved into electrophoretically distinct polymeric fragments of markedly different composition.[1–3] It is therefore important to ascertain whether this behaviour is due to the existence of weak linkages between differently composed segments of the chains, or whether it can be accounted for simply in terms of random cleavage. To this end, a simple theoretical model for a binary linear heteropolysaccharide is now considered, and the changes in composition-distribution occurring upon random cleavage of the chains are studied with the help of the digital computer. The theoretical model is not regarded as an adequate description of the alginate molecule, but it is hoped that it may serve as a first step towards arrival at such a description. In the discussion, the terms "heterogeneous" and "polydisperse" are used in the sense defined by Gibbons.[4]

It is self-evident that, when any copolymer is randomly degraded, even when originally of a unimodal distribution, the reaction mixture must ultimately become heterogeneous with respect to composition, since a simple mixture of different monomers is finally obtained. However, the degree of scission at which compositional heterogeneity first appears, and the way in which this is related to the distribution of monomer units along the chains of the parent copolymer, require some clarification. For synthetic copolymers, the problem of composition-distribution has been treated by Simha and Branson,[5] and by Stockmayer,[6] who derived expressions approximately valid for long chains, and more recently by Frensdorff and Pariser,[7] who employed a matrix development to arrive at a formula for chains of any length. These workers, however, were concerned primarily with compositional polydispersity in copolymer preparations, and did not consider either the transient heterogeneity occurring during the copolymerisation reaction, or its reappearance upon depolymerisation.

THEORY

The hypothetical heteropolysaccharide, composed of linear chains of monosaccharide units A and B, is considered to be synthesised *in vivo* by stepwise enzymic transfer of A and B from suitable donors, on to one end of a growing chain. The assumptions are detailed elsewhere,[8] together with the formulation of the copolymer's overall composition and monomer sequence distribution in terms of enzymic substrate specificities a and b, and a priming specificity, P_1^A. The priming specificity is the probability, that in a particular chain, the first unit added to the primer is an A. The substrate specificities a and b are defined as $K_{A\to A}/K_{B\to A}$ and $K_{B\to B}/K_{A\to B}$, respectively, where $K_{A\to B}$, for example, is the rate of addition of a unit of A to a chain whose terminal unit is a B. The probability (P_i^A) of a unit of A occupying the i-th position in the chain, is also a function [8] in a, b, and P_1^A. The question of chain termination, and hence of the molecular-weight distribution in the heteropolysaccharide, is again avoided by assuming that the chains are very long.

It is convenient to begin by considering all the chains obtained after addition of n units to the primer. These will consist of chains containing n units of A only, chains containing n units of B only, and mixed chains, ranging in composition from 1 unit of A and $(n-1)$ units of B, to $(n-1)$ units of A and 1 unit of B.

The fraction ($F_{A=n,B=0}$) of all the chains that contain n A's only is

$$F_{A=n,B=0} = P_1^A \left(\frac{a}{1+a}\right)^{n-1} \tag{1}$$

Similarly, the fraction ($F_{A=0,B=n}$) containing n B's only is

$$F_{A=0,B=n} = P_1^B \left(\frac{b}{1+b}\right)^{n-1} \tag{2}$$

where $P_1^A + P_1^B = 1$, and n is not less than 1.

Now, the fraction of all chains of length n that contain $(n-x)$ units of A and x units of B is given by

$$F_{A=(n-x),B=x} = P_1^A \varphi(A) + P_1^B \varphi(B) \quad (3)$$

where

$$\varphi(A) = \left(\frac{a}{1+a}\right)^{n-x-1}\left(\frac{b}{1+b}\right)^{x-1}\left[\left(\frac{1}{1+a}\right)\sum_{c=1}^{x}\left(\frac{1}{ab}\right)^{c-1}\binom{x-1}{c-1}\binom{n-x-1}{c-1} + \left(\frac{1}{1+b}\right)\left(\frac{1}{a}\right)\sum_{c=1}^{x}\left(\frac{1}{ab}\right)^{c-1}\binom{x-1}{c-1}\binom{n-x-1}{c}\right] \quad (4)^*$$

and

$$\varphi(B) = \left(\frac{a}{1+a}\right)^{n-x-1}\left(\frac{b}{1+b}\right)^{x-1}\left[\left(\frac{1}{1+a}\right)\left(\frac{1}{b}\right)\sum_{c=1}^{x}\left(\frac{1}{ab}\right)^{c-1}\binom{x-1}{c}\binom{n-x-1}{c-1} + \left(\frac{1}{1+b}\right)\sum_{c=1}^{x}\left(\frac{1}{ab}\right)^{c-1}\binom{x-1}{c-1}\binom{n-x-1}{c-1}\right] \quad (5)$$

$$(n>1;\ x\geqslant 1;\ n>x)$$

It may be noted that

$$F_{A=n,B=0} + F_{A=0,B=n} + \sum_{x=1}^{n-1} F_{A=n-x,B=x} = 1 \quad (6)$$

Eqns. (4) and (5) are of the same form as those arrived at earlier by Simha and Branson,[5] and an explanation of the physical meaning of the terms has been given by Stockmayer.[6] By way of further explanation in the present case, it is sufficient to point out that $F_{A=(n-x),B=x}$ is simply a sum of C_x^n expressions, each giving the fraction of the total number of chains in which $(n-x)$ units of A and x units of B are arranged according to a particular sequence. The expression for any one of these individual sequences can be written down just by inspection. That for AAABAAA, for example is

$$P_1^A\left(\frac{a}{1+a}\right)^2\left(\frac{1}{1+a}\right)\left(\frac{1}{1+b}\right)\left(\frac{a}{1+a}\right)^2 \quad (7)$$

However, the expression is not different for every sequence. For example, expression (7) is also obtained for ABAAAAA, AABAAAA, AAAABAA, and AAAAABA. This makes it possible to divide the C_x^n expressions into groups, and thus arrive at a reasonably compact formula.

For the present development, it is necessary to recognise that eqns. (1), (2), and (3) describe not only whole molecules, but also parts of molecules. For example, a group of M-mers may be considered, such that $M>n$. In this case, eqn. (3) gives the fraction of these molecules whose first n units consist of $(n-x)$ units of A and x units of B. Similarly, the fraction containing segments of the same composition, lying between the $(i-1)$th and the $(i+n)$th units, is

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* $\binom{n}{x}$ signifies C_x^n, the usual notation for the combination $n!/x!\,(n-x)!$

$$P_i^A\varphi(A)+P_i^B\varphi(B) \tag{8}$$

It must also be noted that the mole fraction (F^A) of units of A in all chains of length M is given by

$$F^A = \frac{1}{M}\sum_{i=1}^{M} P_i^A \tag{9}$$

The random depolymerisation of all chains of length M can now be considered. In the sense used here, random depolymerisation implies that the different types of linkages present between the monomers all are hydrolysed at the same rate. For a degree of scission α, the yield of n-mer obtained by splitting the linkage between the $(i-1)$th and the ith units, and that between the $(i+n-1)$th and the $(i+n)$th units is $(n/M)\alpha^2(1-\alpha)^{n-1}$, where this is expressed as a weight-fraction of the total number of units in all chains of size M. The fraction of the n-mer so obtained that consists of $(n-x)$ units of A and x units of B is given by expression (8). Now i can have any value from 1 to $(M-n)$, so therefore the total yield, $Y_{A=(n-x),B=x}$, of n-mer having the stated composition is obtained by summing over all these values, and adding an extra term for the yield obtained by splitting the linkage between the $(M-n)$th unit and the $(M-n+1)$th unit:

$$\begin{aligned} Y_{A=(n-x),B=x} = {} & \frac{n}{M}\left\{\sum_{i=1}^{M-n}\left[P_i^A\varphi(A)+P_i^B\varphi(B)\right]\right\}\alpha^2(1-\alpha)^{n-1} \\ & +\frac{n}{M}\left\{P^A{}_{M-n+1}\varphi(A)+P^B{}_{M-n+1}\varphi(B)\right\}\alpha(1-\alpha)^{n-1} \end{aligned} \tag{10}$$

Having ignored chain-termination effects, due to the present lack of knowledge of these mechanisms in enzymic systems, it is now necessary to allow M to tend to infinity. If this is done, and eqns. (9) and (10) are combined, the unknown quantities P_i^A and P_i^B disappear, and a very simple relationship is obtained:

$$Y_{A=(n-x),B=x} = n[F^A\varphi(A)+F^B\varphi(B)]\ \alpha^2(1-\alpha)^{n-1} \tag{11}$$

In this equation, the factor $n\alpha^2(1-\alpha)^{n-1}$ is immediately recognised as the well-known Kuhn formula [9] for the yield of n-mer obtained upon random depolymerisation of a long-chain homopolymer. The factor $[F^A\varphi(A)+F^B\varphi(B)]$ is then the fraction of all fragments of length n having a particular quantitative composition.

By a similar reasoning, the corresponding equations for n-mer composed of units of A only or B only are readily obtained:

$$Y_{A=n,B=0} = nF^A\left(\frac{a}{1+a}\right)^{n-1}\alpha^2(1-\alpha)^{n-1} \tag{12}$$

$$Y_{A=0,B=n} = nF^B\left(\frac{b}{1+b}\right)^{n-1}\alpha^2(1-\alpha)^{n-1} \tag{13}$$

It is clear that

$$Y_{A=n,B=0} + Y_{A=0,B=n} + \sum_{x=1}^{n-1} Y_{A=(n-x),B=x} = n\alpha^2(1-\alpha)^{n-1}$$

RESULTS AND DISCUSSION

With the advent of the digital computer, expressions such as (4) and (5) can now be readily evaluated without the methods of approximation used by the early workers,[5,6] and exact distribution curves obtained. However, to facilitate graphical comparison of distributions for chains of different lengths, it was necessary in much of the present work to introduce a simplification. This consisted in dividing the possible range of compositions (0—100 % of B) into "boxes", and instructing the computer to print out only the sums of the weight-fractions of all molecules in each box, which were then plotted on the graphs.

Fig. 1 compares the composition-distributions for chains of various specified lengths, produced either by biosynthesis (eqns. 1—3; $P_1^A=0.7$) or random

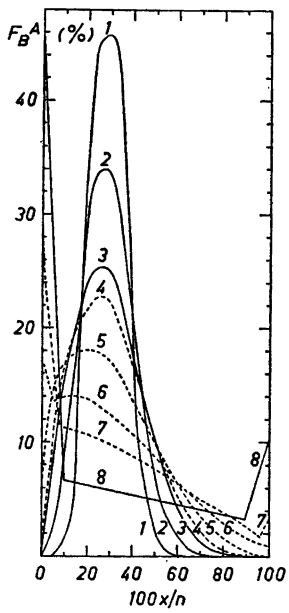

Fig. 1. Dependence of composition-distribution upon chain length. The polymer is characterized by the substrate specificities $a=20$ and $b=8$, the priming specificity $P_1^A=0.7$, and the mole fraction of A, $F^A=0.7$. 1, $n=500$; 2, $n=200$; 3, $n=100$; 4, $n=80$; 5, $n=50$; 6, $n=30$; 7, $n=20$; 8, $n=10$.

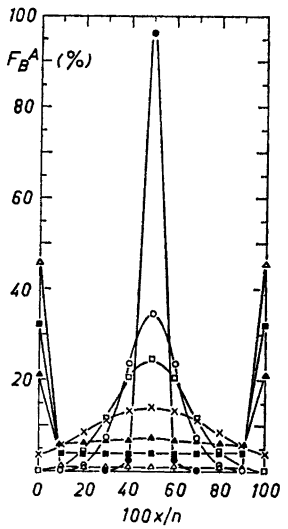

Fig. 2. Dependence of composition-distribution upon substrate specificities a and b: $n=10$; $F^A=0.5$; $P_1^A=0.5$. ● $a=b=0.01$; ○ $a=b=0.5$; □ $a=b=1.0$; × $a=b=3.0$; ▲ $a=b=10$; ■ $a=b=20$; △ $a=b=100$.

degradation (eqns. 11—13), with a set at 20 and b at 8. In this figure, $F_{A=n,B=0}$ and $F_{A=0,B=n}$ are plotted separately, and the range of intermediate compositions is divided into 10 boxes. The first of these includes all molecules containing from 1 to $n/10$ units of B inclusive, and the last is for mixed chains containing more than $9n/10$ units of B. The last box is therefore a little smaller than the others, but the amount of material falling into it is very small in the present case, and the resultant error in the curves is negligible.

Fig. 1 illustrates the broadening in the composition-distribution with decreasing chain length, culminating in the appearance of compositional heterogeneity. When the chain-length is sufficiently large, there is a single, unimodal distribution, corresponding, in Gibbons' terminology,[4] to a preparation which is homogeneous, but compositionally polydisperse. As the chain-length decreases, there comes a point at which $F_{A=n,B=0}>F_{A=(n-1),B=1}$, and the distribution is then bimodal. Later, another point is reached such that $F_{A=0,B=n}>F_{A=1,B=(n-1)}$, and the distribution is then trimodal. For symmetrical distributions, these two events occur simultaneously. It should be noted that, although the exact values of n at which these two events occur are readily calculated from eqns. (1), (2), and (3), these are not shown in Fig. 1, because of the box summation. The values of n indicated by Fig. 1 (approximately 40 and 25, respectively) are of much greater practical interest than the absolute ones, since they indicate results that could be expected by application of a physical method capable of separating molecules differing in composition by 10 %.

Fig. 2 shows symmetrical distributions (occurring when $a=b$) for chains of 10 units, and illustrates the effect of varying a and b. The box method was not necessary here, and the curves are exact representations of the distributions. In considering this effect, it is useful to recall [8] that the average length of the groups of contiguous units of A is $(1+a)$, and that of the B-groups is $(1+b)$. An interesting consequence of this fact is shown in Fig. 3, which illustrates, for large values of a and b, the effect of varying n proportionately with a and b. The near-identity of the curves illustrates a simple means of obtaining distribution curves for very large chains. This is very useful in saving computer time; even with the most economical programme that it was pos-

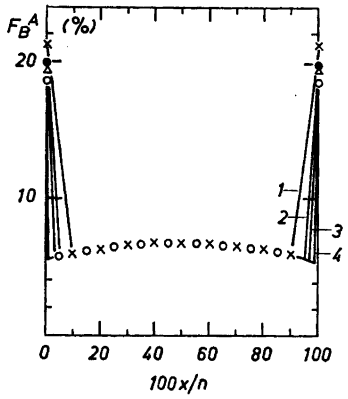

Fig. 3. Effect of simultaneous variation of a, b, and n for large values of a and b. $F^A=P_1{}^A=0.5$. 1 ×, $a=b=n=10$; 2 ●, $a=b=n=20$; 3 △; $a=b=n=30$; 4 ○, $a=b=n=100$.

sible to write, the running time increased as the square of n, and with $n=1000$, it amounted to about 20 min. Fig. 3 shows only the result when $a=b=n$, but the relationship still holds good when $a\neq b\neq n$.

Construction of the composition-distribution curves for the entire population of chains of different lengths obtained upon random cleavage requires summation over all values of n for various specified compositions (eqns. 11—13). For given values of α, the computer was instructed to sum the weight-fractions of all chains having a particular x/n ratio, starting with $n=1$, and working upwards. Since there is no maximum possible value for n in the present instance, the computation was carried out until a minimum of 95 % of the total weight of the material was accounted for. Owing to the fact that computation time increases with n^2, lower recoveries of material had to be accepted for $\alpha=0.02$ and 0.01 (93.3 and 93.0 %, respectively). The possible range of compositions (0—100 % of B) was divided in this case into 20 boxes, the first for chains containing 0—5 % of B inclusive, and the 20th for chains containing more than 95 % of B. Typical results are shown in Figs. 4—7 inclusive.

As a result of the arbitrarily chosen box size and arrangement, and in some cases the impossibility of accounting for the total weight of the chains, there are some artificial effects in the distribution curves which must be distinguished from the meaningful ones. Owing to the fact that the first box is larger than the others and that the box arrangement therefore is not symmetrical about 50% (*e.g.* box 45 exclusive — 50 inclusive is not symmetrical with box 50 exclusive — 55 inclusive), a certain degree of artificial asymmetry is imparted to the curves; this can be seen in Figs. 5—7, where the curves should clearly

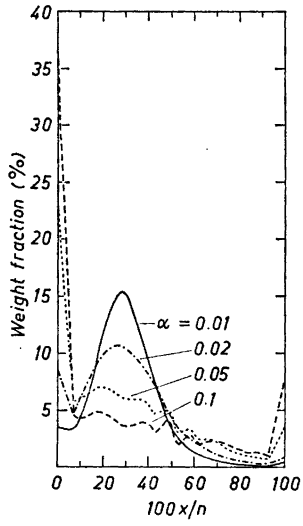

Fig. 4. Composition-distribution in random depolymerisation of a polysaccharide of infinite chain length characterized by the substrate specificities $a=20$ and $b=8$. α is the degree of scission.

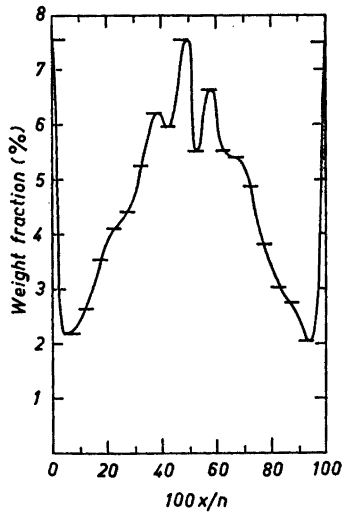

Fig. 5. Composition-distribution in random depolymerisation. $\alpha=0.05$; $a=b=10$.

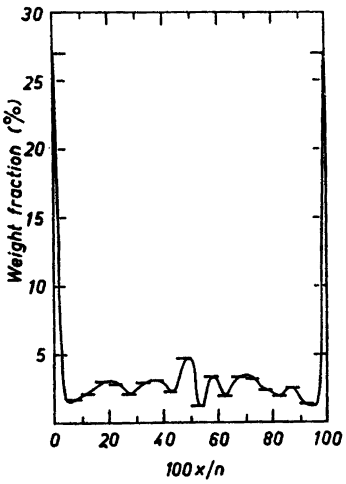

Fig. 6. Composition-distribution in random depolymerisation. $\alpha=0.2$; $a=b=10$.

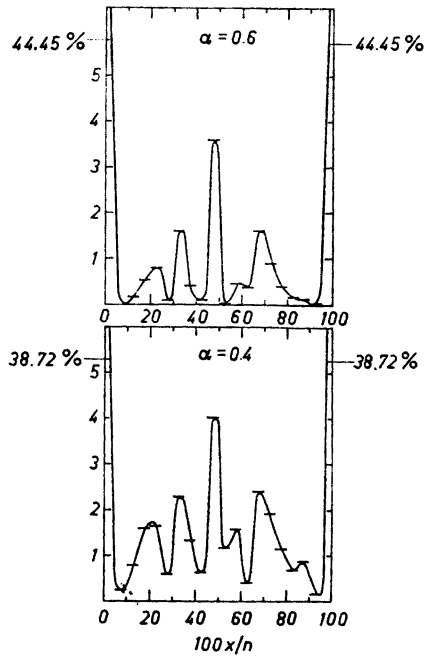

Fig. 7. Composition-distribution in random depolymerisation. $a=b=10$.

be symmetrical in theory. Another effect, not so clearly seen, arises from the fact that smaller chains contain a higher proportion of homopolymeric fragments than the larger ones, so that any chains not accounted for in the summation represent an error occurring mainly in the part of the curve where $x/n \approx F^{B}$.

The division of the distribution curve for mixed chains into numerous small peaks and shoulders is a real effect, becoming more pronounced as α increases. This is clearly to be expected when it is remembered that, when $\alpha=0.5$ for example, about 90 % of the reaction mixture consists of monomer to pentamer inclusive. These oligomers can only contain an integral number of units of B, and so peaks are to be expected mainly at values for x/n of 20, 25, 33, 40, 50, 60, 66, 75, and 80 %.

It is necessary here to recognise that distribution curves for mixed chains of the same length (eqn. 3) can also be represented as a series of different peaks, and for short chains it might be experimentally possible to observe them. The smooth curve drawn through the apices of these peaks would, however, for mixed chains still be unimodal (Fig. 2), and with this qualification, Gibbons' nomenclature is consistent and meaningful. For mixtures of chains of different lengths of the type just considered, it is, however, impossible to draw a unimodal curve through the apices of the peaks.

In summary, two different types of compositional heterogeneity can be said to arise during random depolymerisation of a heteropolysaccharide obeying the present theoretical model. The first type is associated with the appearance of a di- or a tri-modal distribution within chains of the same length; the maximum possible number of peaks is three, and these would consist of two different homopolymers, and one compositionally polydisperse heteropolymer. The degree of scission at which this type of heterogeneity becomes experimentally detectable depends upon the magnitude of a and b and the resolving power of the physical method used for fractionation. The choice of a box size of 5 % seems not to be unrealistic in relation to the capacity of existing methods of fractionation, and Fig. 4 gives some idea of the magnitude of the effect at different degrees of scission, for the case when $a=20$ and $b=8$. It is seen that it can be quite possible to detect heterogeneity in such a copolymer at degrees of scission in the range 0.01—0.02.

The second type of compositional heterogeneity arises within the heteropolymeric chains themselves, and is due only to the fact that these are not all of the same length. The distribution for mixed chains of a given length is in every case unimodal, with the qualification given above. This effect is illustrated in Fig. 8 with the help of a very simple example. The random depolymerisation is considered of a simple pentasaccharide fraction, such as might be obtained by partial acid-hydrolysis of a long-chain heteropolysaccharide obeying the present model. The yields of the various n-mers for a degree of scission α are given by an equation originally due to Montroll and Simha,[10] and subsequently derived in a simpler treatment by Painter.[11] If this is combined with a suitable distribution factor (*cf.* eqn. 11), the following relationship is obtained:

$$Y_{A=(n-x),B=x} = (n\alpha/N)[F^{A}\varphi(A)+F^{B}\varphi(B)]\ (1-\alpha)^{n-1}[2+(N-n-1)\alpha] \qquad (14)$$

where N is the number of units in the parent chain.

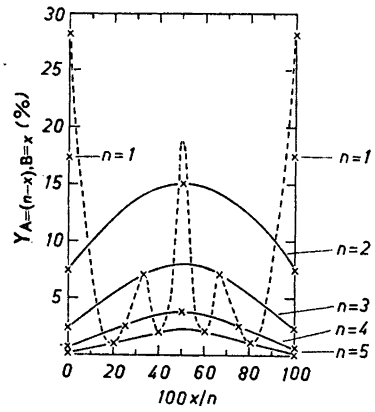

Fig. 8. Composition-distribution obtained by further random depolymerisation of a total pentamer fraction whose composition is governed by formula (11). $\alpha=0.5$; $a=b=1$.

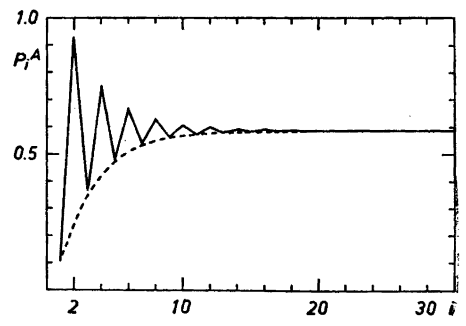

Fig. 9. The probability (P_i^A) of finding an A in the i-th position as a function of i; $P_1^A=0.1$: ——— $a=0.43$, $b=0.001$ ($x=-0.7$, see Ref. 8). - - - - $a=7.15$, $b=4.66$ ($x=+0.7$).

Fig. 8 was obtained by putting $N=5$, $\alpha=0.5$, $a=b=1$, and $F^A=F^B=0.5$. It depicts the four different possible unimodal composition-distributions, one being that of the residual undegraded pentamers (6.25 % of the total), and the other three for the tetramers (10 %), the trimers (18.75 %), and the dimers (30 %), respectively. It will be appreciated here that the unimodal curves drawn through the apices of the peaks are intended only to show which peaks belong to the distribution for a particular n-value, and are not intended to imply continuity. Another curve is then drawn through the apices of all the peaks, duly summed where coincident (*i.e.* at 0, 50, and 100 % of B), and it is seen to be trimodal with respect to the chains of mixed composition.

Returning to the long-chain heteropolysaccharide, Fig. 4 shows that this second type of heterogeneity is of practical significance only at high degrees of scission. It is present at low degrees of scission, and indeed even in the parent heteropolysaccharide when this is polydisperse with respect to molecular weight, but it is then experimentally indistinguishable from compositional polydispersity in the strict Gibbons sense.

Despite the difference in mechanism between stepwise biosynthesis and random degradation, the distribution functions for chains of a given length are closely similar: $[P_1^A\varphi(A)+P_1^B\varphi(B)]$ and $[F^A\varphi(A)+F^B\varphi(B)]$, respectively. It is therefore of interest to examine to what extent the changes in composition-distribution taking place upon random depolymerisation can be regarded as a reversal of those occurring during biosynthesis. This is governed by the relationship between P_1^A and F^A, and the similar one between P_1^B and F^B. Now the relationship between P_1^A and P_i^A is given elsewhere,[8] and Fig. 9 illustrates the variation of P_i^A with increasing chain length for arbitrarily chosen values of a, b, and P_1^A. The figure shows that, regardless of the value of P_1^A, P_i^A tends to a constant value, identical with F^A, as i increases. This period of approach to a stationary state has previously been discussed by Fueno and Furukawa[12] within the context of free-radical polymerisation. The speed of approach depends upon the magnitudes of a and b, and also upon P_1^A. It follows that, as the chain length increases, the composition-distribution should approach more and more closely that obtained upon random depolymerisation. This effect is illustrated in Fig. 10, in which F^A is given the value 0.7, and P_1^A a very different value, 0.1.

The effect of decreasing P_1^A from 0.7 to 0.1 for any value of n is clearly to decrease the fraction of chains containing A only to one-seventh of its original value, with a corresponding 3-fold increase in the fraction of chains containing B only, and an overall increase in the amount of B in the copolymer. Subject to the influence of the box summation, this effect is seen in Fig. 11, which is a replica of Fig. 1, except that P_1^A is in this case 0.1. With the box size used, the chains of pure A are not now visible as a separate component, even when $n=10$. It is of course also possible for P_1^A to be zero, in which case there would be no chains composed of A only at all.

In general, therefore, the changes occurring in composition-distribution during stepwise biosynthesis can be regarded as a reversal of those occurring during random depolymerisation, inasmuch as the composition-distribution for chains of a given length passes successively through trimodal and bimodal

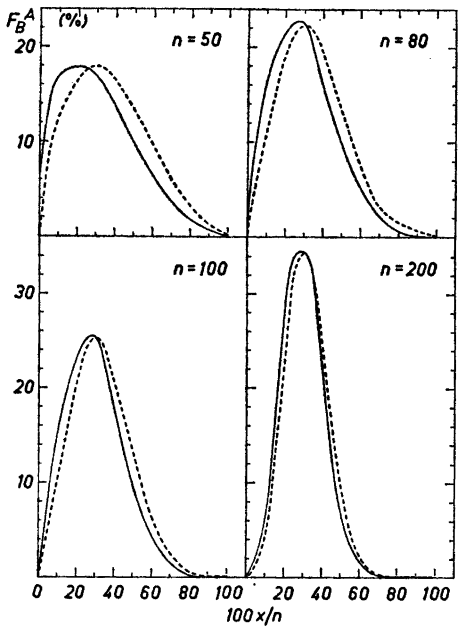

Fig. 10. Dependence of composition-distribution upon P_1^A and F^A. $a=20$; $b=8$; ——— $P_1^A=F^A=0.7$; - - - - $P_1^A=0.1$.

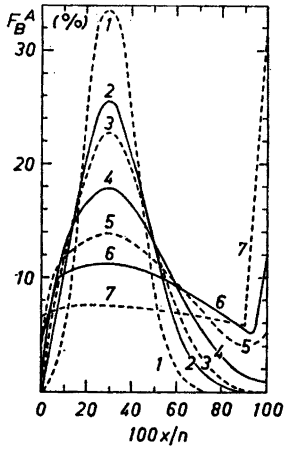

Fig. 11. Dependence of composition-distribution upon chain length. $a=20$; $b=8$; $P_1^A=0.1$.

phases before becoming unimodal, but it is possible for the trimodal phase not to appear at all (*i.e.*, when $P_1^A=0$ or 1).

When alginic acid is subjected to limited depolymerisation by acid, it becomes heterogeneous, and can be fractionated into a component consisting largely of mannuronic acid residues, a component containing mainly guluronic acid residues, and a third component having about the same composition as the original alginate.[1-3] These components are formed at degrees of scission of the same order as those which are here shown to be consistent with the appearance of heterogeneity of the first type. Studies of the molecular weights and compositions of these components show that alginate must be regarded as a block copolymer, in which long groups of contiguous mannuronic acid residues and similar groups of guluronic acid residues are linked together through chains of mixed composition, containing a surprisingly high proportion of alternating mannuronic and guluronic acid units.

In the present theoretical model, a sort of block copolymer is obtained when a or b (or both) is large, and it is this type of arrangement that leads to the early appearance of the kind of heterogeneity actually found in the case of alginate (*i.e.* two homopolymers and one heteropolymer). Although the assumption of random hydrolysis is not strictly valid,* this measure of agree-

* It is valid only at a particular pH. This question will be discussed in a forthcoming publication.

ment between the actual behaviour of alginate and that predicted theoretically on the assumption of random cleavage is sufficiently good to discourage "weak link" hypotheses, and to encourage the belief that a first step has been taken towards a description of alginate structure in terms of the substrate specificities of the alginate polymerase system in the living alga.

The agreement, however, is by no means perfect. For example, the present theoretical model cannot account for the high proportion of alternating sequences in the "mixed" segments of the chains. It is therefore proposed to set up more complex theoretical models, taking account of penultimate unit effects [8] during biosynthesis and of non-random degradation patterns,[13] in an attempt to describe the structure and degradation of alginate more exactly. Some progress has already been made in this respect, and will be reported later.

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