Simulation of Starch Degrading and Branching Reactions

Shane Latham, School of Mathematical Sciences, Australian Natuional University, ACT 0200 and Matthew .K. Morell, CRC for Plant Science, PO Box 475, Canberra, ACT 2601 and CSIRO, Division of Plant Industry, PO Box 1600, Canberra, ACT 2601

Abstract. The degradation and modification of polymers by enzymes is an important process in biological systems and in industrial transformations of materials. However, the determination of the key parameters which drive the modification of polydisperse polymer populations towards specific end-points is difficult experimentally. One method of resolving complex biological proceses is to construct forward simulations, varying the paramters until a good match is obtained with experimental data. In this paper, we have used forward simulations to model the modification of starch, a plant polymer, by enzymes which either cleave or branch the polymer. While these simulations do not provide unique solutions to the problems addressed, they have been highly valuable in suggesting how further experimentation can resolve key issues.

1. INTRODUCTION

Starch is an important plant storage compound which provides the bulk of the carbohydrate and energy reserves found in cereals and tubers. At the level of its primary chemical structure, starch is a polydisperse macromolecule in which a single monomer unit, glucose, is linked via 2 types of chemical bonds, the α-1,4 bond (linking linear arrays of glucan), and the α-1,6 bond, (introducing branch points into the molecule) (Ball *et al* 1996). We are trying to define the deterministic and non-deterministic rules which govern the way particular enzymes cleave and join starch polymers.

The analysis of the kinetic properties of enzymes is very well established for those enzymes which interconvert discrete molecules into discrete products. However, enzymes which modify the structure of polymeric substrates present some unique problems.

- the substrate is usually a population of polydisperse molecules, not a discrete entity.
- the product of one catalytic event is usually a potential substrate for the next round of catalysis.
- some enzymes can cleave at any point along the polymeric substrate chain
- both bond forming and bond cleavage probabilities need to be defined for branch-forming enzymes.

One approach to resolving complex biological questions is to construct forward simulations of the processes involved and then vary the parameters in the forward simulation until a good match is obtained with the experimental data. This is the basic simulation methodology which has been exploited in the current research. The difficulty with this approach is the possibility that more than one set of parameters in the forward simulation will yield an equally good fit to the experimental data. When such non-uniqueness occurs, there is a need to seek independent information about the processes involved in order to resolve the issue. Such inherent non-uniqueness is a direct mathematical consequence of the parameter identification situation being solved. However, the model serves as a valuable tool in defining precise questions which can be asked through further rounds of experimentation.

2. MODELLING STARCH DEGRADING AND MODIFYING REACTIONS

We are interested in α-amylases, which degrade starch polymers by cutting internal bonds within the starch polymer, and branching enzymes, which rearrange the polymer in a two step "cleave then join" transfer reaction. As a first step in discovering these rules, a program written in the C++ language was coded to simulate various cutting and joining strategies.

3. THE MODEL

The "Reaction" program is coded to simulate the action of a pool of "enzymes" which act on individual "bonds" selected at random along the length of "polymers" drawn at random from a "polymer pool". Polymers have directionality such that their ends (defined from their chemical properties as being reducing "R" or non-reducing "NR" ends) differ in properties. During each clock tick of the simulation, each enzyme in the enzyme pool is presented with an individual polymer drawn and the enzyme interacts with that polymer at a bond position selected at random. Probability functions based on the bond position with respect to either end of the selected chain, or a pre-exisitng branch point, are invoked to activate either bond cleavage or polymer release.

4. EXAMPLES OF SIMULATIONS

Simulations have been designed to model either α–amylase or branching enzyme reaction. Our aim in these simulations was to explore the parameters which are likely to be important in defining the properties of these enzymes which direct them to synthesise the types of molecular structures found in plant starches.

4.1 α-amylase

In the simplest form of the model, enzyme is presented with a specific bond in a linear polymer and either cleaves the bond or releases the polymer, depending on the outcome of a probability based test at that site. Figure 2 shows outcomes from 2 simulations. The probability factors used are given in Table 1. Figure 2A shows the chain length distribution produced by an enzyme which has a probability of cleavage of 1 at all bond positions. The polymer is rapidly broken down, quickly yielding oligomers and eventually monomers. Figure 2B shows the result of a typical simulation in which the enzyme was restricted by being unable to cleave polymers within 8 monomers of either end. This value was chosen because it produces a chain length distribution with a similar average chain length to the amylopectin chain length distribution, although the shape of the distribution clearly differs (Figure 4). Exploration of a wide range of probabilities lead to the conclusion that simple combinations of probabilities of cleavage with respect to the R and NR ends could not produce chain length distributions which resemble the chain length distribution of the linear chains which collectively constitute a branched starch molecule (see Figure 4).

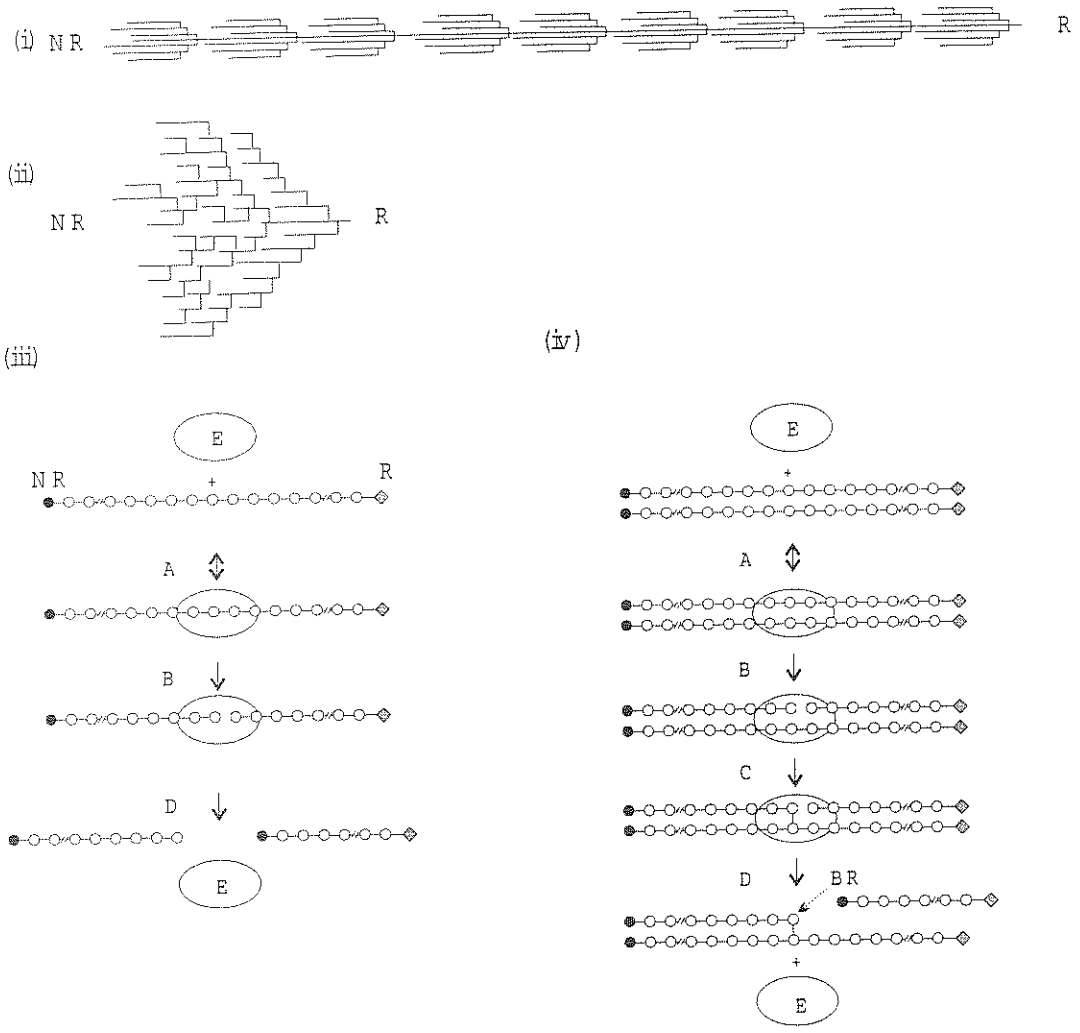

Figure 1. Chemical Structures and transformations. (i) schematic structure of amylopectin (ii) schematic structure of glycogen (iii) reaction catalysed by α-amylase (iv) reaction catalysed by branching enzxyme. A, substrte binding reaction; B, cleavage reaction; C, joining reaction; D, product release; E, enzyme; R, "reducing" end; NR, "non-reducing" end;BR, branch point

Table 1

Probabilities of cleavage or joining at positions with polymeric substrates.

		Cleave			Join			
Set	NR	R	BR	NR	R	BR	RP	Data
1	all 1	all 1	NA	NA	NA	NA	NA	Fig. 2A
2	8 x 0	8 x 0	NA	NA	NA	NA	NA	Fig. 2B
3	8 x 0	8 x 0	8 x 0	all 1	all 1	1 x 0	0	Fig 3A
4	8 x 0	8 x 0	8 x 0	7 x 0	7 x 0	7 x 0	0	Fig 3B
5	(a)	(b)	(c)	5 x0	5 x 0	2 x0	0	Fig 4
6	(a)	(b)	(c)	5 x0	5 x 0	2 x0	0	Fig 5
7	(a)	(b)	(c)	5 x0	5 x 0	2 x0	0.1	Fig 5

NA = not applicable

all 1 = all positions in the polymer have a probability of 1

8 x 0 = the eight positions closest to the end have a probability of zero, the remainder have a probability of 1.

1 x 0 = the position adjacent to the branch point has a probability of 0

(a) Reducing end probabilities 0.0 0.0 0.0 0.0 0.0 0.005 0.01 0.05 0.25 0.5 1.0 1.0 1.0 1.0 1.0

(b) Non-Reducing end probabilities 0.0 0.0 0.0 0.0 0.0 0.005 0.01 0.05 0.25 1.0 1.0 1.0 1.0 1.0 1.0

(c) Probabilities adjacent to a Branch 0.0 0.0 0.0 0.0.0.0 0.005 0.01 0.05 0.25 0.5 1.0 1.0 1.0 1.0 1.0

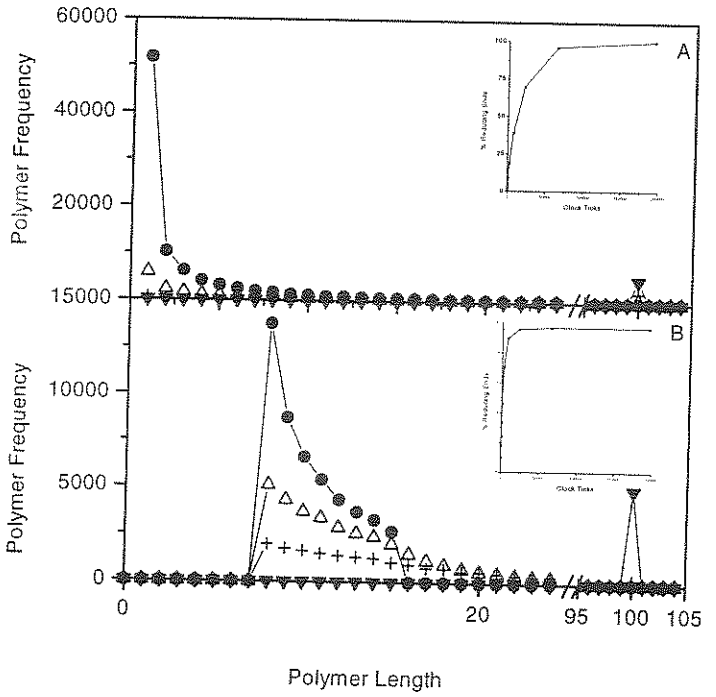

Figure 2. Simulations of an endo-cleavage reaction for a linear polymer containing 100 monomeric units. Data shown are the polymer frequency as a function of polymer length at clock ticks 800, 2400 and 194400. The probability of cleavage for each bond positions relative to the reducing and non-reducing ends are given in Table 1. (A) simulation of an enzyme which has an equal probability of cleaving at any bond position (see Table 1, set 1), (B) simulation of an enzyme which is unable to cleave within 8 units of either end of a polymer (Table 1, set 2). The overall progress of the simulation is indicated in the insets in each panel which give the percentage of momomers which are reducing ends relative to the number of monomers in the polymer pool as a function of clock ticks.

4.2 Branching Enzymes.

A more complex configuration of the model presents enzyme with a polymer pool which contains both linear

and branched polymers. The enzyme carries out an operation which involves two distinct steps, firstly a bond cleavage operation analogous to the action of α-amylase, and secondly, a bond forming reaction joins one of the two new polymers produced by the cleavage reaction (that portion of the polymer containing the original NR end) to a second polymer via a branch. The second polymer may be the other half of the cleaved polymer or it may be a second polymer drawn at random from the polymer pool. Discrimination between these options is based on a release probability factor "RP". The probability parameters used to generate the data shown in Figures 3 and 4 are given in Table 1 and include probabilities for cleaving and joining with respect to R and NR ends, and pre-existing branch points. Probabilities were systematically tested for first the cleavage and then the joining reactions. In Figure 3A, a simulation is presented in which cleavage probabilities were as for Figure 2B, however with the inclusion of a non-specific joining reaction (Table 1). The output shown is the chain length distribution of the individual linear chains which are linked to form the branched polymer. This simulates the chain length distribution obtained after debranching a starch using a starch debranching enzyme. The chain length distribution obtained is a better fit to a debranched starch distribution than the α-amylase reaction as it generates a tailing region above a chain length of 10 monomers. The inclusion of selective joining probabilities, as in Figure 3B, further improves the fit to the typical shape of a starch distribution. In Figure 4, a simulation has been conducted in which the joining probabilities are the same as those of Figure 3B, however the cleavage probabilities have been optimised using an iterative process to obtain probabilities which yield a distribution which is highly similar to that of starch.

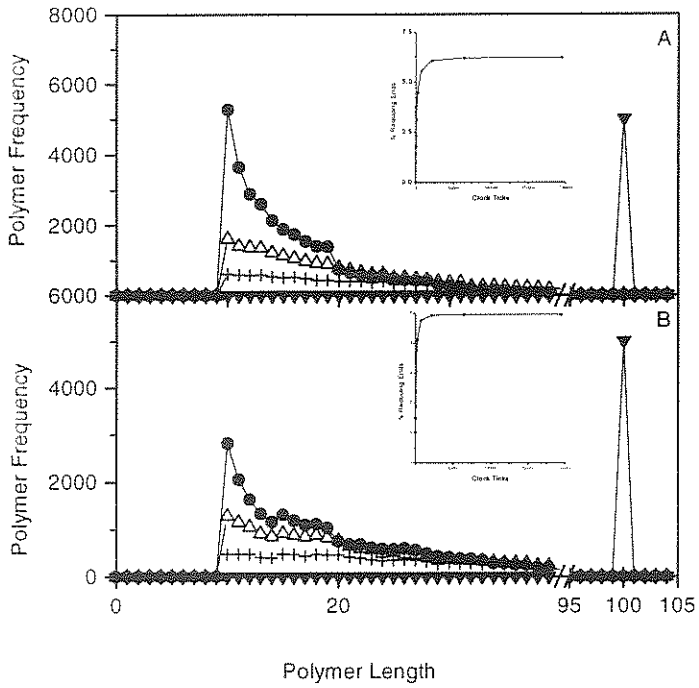

Figure 3.Simulations of a transferase reaction. Data shown are the distribution of linear chains after debranching the polymer pool at clock ticks 800, 24000 and 194400. The probability of cleavage and joining reactions for each bond position relative to the reducing and non-reducing ends, and existing branch points, are given in Table 1. (A) simulation of transferase reaction which is restricted in cleavage specificity but not in joining (Table 1 Set 3) (B) simulation of transferase reaction which is restricted in both cleavage and joining specificity (Table 1, Set 4). The progress of the reactions are shown in the insets, which give the percentage of momomers which are reducing ends or branch points relative to the number of monomers in the polymer pool, as a function of clock ticks.

Figure 5 shows the results of simulations carried out using the cleavage and joining probabilities used in Figure 4, however the "RP" factor was either 0 or 0.1. The data shown are the number of monomers per

polymer. Allowing the enzyme to transfer the cleaved chain to a second molecule drawn form the polymer pool has a dramatic effect on the overall molecular size distribution obtained. The simulation has lead to the development of a simple hypothesis about an important aspect of the action of the enzyme which can be readily experimentally tested.

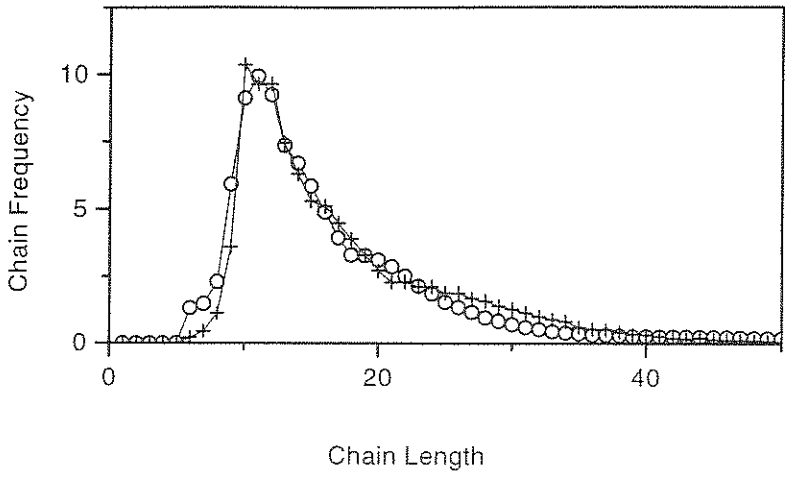

Figure 4. Comparison of the chain length distribution of the the branched fraction of wheat starch (O'Shea and Morell 1996) (-o-), with a simulation (-+-) carried out using the probabilities given in Table 1(Set 5). An iterative approach was used to select probabilities which produce a chain length distribution which approached that of wheat starch.

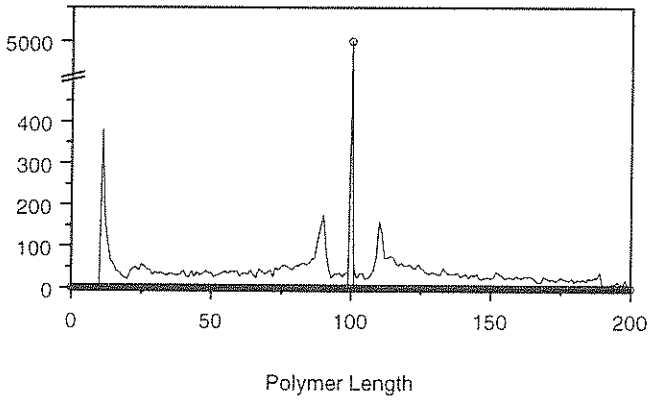

Figure 5. Comparison of the polymer size distribution of polymers produced by the transferase enzyme from a product pool containing polymers of 100 monomeric units when the release probability factor, "RP"was either zero (Table 1, Set 6, -o-), or set at 0.1 (Table 1 Set 7, unbroken line).

5. DISCUSSION

This paper illustrates ways in which forward simulations of polymer degrading or branching

enzymes can be used to resolve questions about the processes involved. The simulations do not in themselves necessarily offer unique solutions to these problems but can suggest often simple experiments to test the predictions of the model and the nature of the processes. For example, Figure 5 demonstrates that a definitive experiment can be conducted to test whether branching enzymes are capable of catalysing the transfer of chains from one polymer to another by determining whether the molecular weight distribution of the polyme pool changes over the course of the enzyme reaction. If such a change is observed, the rate of change in molecular weight distribution relative to the overall rate of branching will allow calculation of this probability.

Branching enzymes with differing kinetic properties have been isolated from a range of sources, however, their specific differences in their patterns of action have yet to be accurately defined. The approach used here to conduct forward simulations of branching enzyme reactions is allowing us to design experimental approaches which will allow us to dissect out the key differences between these enzymes. This information is necessary if we are to understand the roles of individual forms of these enzymes in moulding of the final structure of plant starches.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

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