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Enzymatic Depolymerization of Polysaccharides

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SUMMARY

A comprehensive review of polysaccharide enzymatic depolymerization studies is presented. Three structural classes of polyglucose materials are considered; i.e. crystalline cellulose and granular starch, amorphous soluble dextrans, and gel-forming starch pastes. In addition, various groups of depolymerizing enzymes are discussed: exo-acting and endo-acting polysaccharide depolymerases. The interrelationships of the enzyme action patterns and the substrate's structure and its related susceptibility toward hydrolysis are considered in this treatise. Mass transfer and adsorptive phenomena of solid substrates are treated. A resumé of current experimental methodologies is included. State of the art biochemical kinetic models for simulating these processes are collected here. Evidence is presented in support of the thesis for these natural polymeric substrates, that it is both the mode of action of the catalysts and the conformational states of the polysaccharides which must be accounted for if realistic physical and mathematical models of the dynamic events are to be understood.

INTRODUCTION

Over the last decade or two, considerable interest has developed in the academic and industrial communities dealing with the utilization of renewable polysaccharide materials for the production of foods, fuels and specialty chemicals. Of particular interest is the potential these materials possess in supplying inexpensive non-fossil energy resources (e.g. ethanol) or non-cane sugars (glucose/fructose) to regions of the world where traditional materials are either not available or in short

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supply. The principal polysaccharides which have been considered for these purposes have been 'waste' cellulosic materials for ethanol production and over produced starch containing materials for sugar production. Estimates as to the potential values of these commodities and their relative impacts on the food and petrochemical industries have stimulated considerable debate which will undoubtedly continue into the near future. In spite of this, fundamental scientific and engineering research has continued and significant progress has been made in understanding the basic problems inherent with these materials. Irrespective of their commercial value, the knowledge gained from these studies will be of greater utility ultimately as biotechnology and biochemical engineering become more established and begin work in new areas with other biopolymers. It is certainly not intended to imply that all problems have been either solved or realized at this time, but certain common themes have emerged from these investigations that may well relate to the study of other biopolymeric systems or polymer systems in general. It is for this reason that these studies are reviewed here.

Recently, the National Research Council of the United States *ad hoc* Panel on Polymer Science and Engineering (National Research Council, 1981) devoted considerable discussion to state-of-the-art research opportunities and national needs for polymers of biological and medical importance as well as for agricultural and food production. This document highlighted much of the important scientific and engineering studies which will be required in order to fully understand and exploit biological polymers such as polynucleic acids, proteins and polysaccharides. It may be somewhat fortuitous that as the biotechnology field became established, so much attention was focused onto polysaccharides. Both proteins and polynucleic acids are ion-containing polymers. The presence of these charged moieties, although most likely quite necessary for their specific biological roles, clearly will complicate their understanding (Eisenberg & King, 1977) and thereby hinder research efforts. The biopolymers which have received the greatest attention by biotechnologists and biochemical engineers, namely polysaccharides, are for the most part uncharged polymers and therefore, by chance, have avoided these problems. However, even for neutral polysaccharides, researchers have learned that it is the inherent nature of these polymers in contact with the required processing fluids which to a large extent dictates the manner in which they behave and thus can be used and manipulated.

Specifically, for these most thoroughly studied polysaccharides (starch and cellulose), primary attention has been given to depolymerization reactions. In order to convert the polymers to more useful materials, they first must be degraded to low molecular weight entities, ideally monomeric sugar units. In theory, this conversion could be accomplished by either chemical (e.g. acids) or enzymatic means, but the latter methods are preferred owing to their highly selective mode of action and thus elimination of unwanted by-product formation. In consequence, this review is limited to polysaccharide depolymerization reactions by biological catalysts in aqueous solution.

It is widely believed (Plowman, 1972; Metzler, 1977) that enzymatically catalyzed reactions are controlled (in the kinetic sense) by properties that are intrinsic solely to the catalysts. Particularly in the case of polymeric substrates (polysaccharides, proteins and polynucleic acids), three enzymatic 'attack patterns' are recognized (Roybt & French, 1967); these being single chain attack, multichain attack and multiple chain attack. The first attack pattern is a single polymer chain degradation, the latter is a random attack of all chains, and the other attack pattern is intermediate between these two extremes. Provided that enzyme molecules have unlimited access to the polymeric substrate and bond linkages are common, the kinetics of degradation can be properly described by one of these forms. For these cases, the manner of degradation is controlled specifically by the mode of action of the enzymes.

Exceptions to this have been observed in the case of highly crystalline substrates. This situation involves reactions on a solid substrate surface such as cellulose or lignin. Clearly, if the substrate forms a non-continuous phase by participating in a molecular association such as a crystal, then it is easily recognized; the state of the substrate must affect the degradation. As it is observed that such extreme cases exist for polymeric substrates, it is clear that substrate structure (or more generally, macromolecular conformation) can determine the manner in which degradation reactions will proceed. Therefore, the physical chemical state of the substrate must be considered when modeling the kinetic degradation. Moreover, it is conceivable that a wide variety of intermediate states between amorphous soluble polymers and highly crystalline solid polymers can exist. The role that such substrate conformational states play in directing the course of enzymatic degradation reactions of biopolymers has not been reviewed.

Polysaccharide depolymerization by biological catalysts is complexed by both inter- and intramolecular associations as well as by branching

points, all of which modify the manner in which the enzyme can act on the substrate. The degree to which each of these physicochemical effects dominate the course of degradation can be understood only by thorough understanding of systems which exhibit widely varying structural properties. These effects are strongly influenced by the type of covalent polymer bond linkages. The following sections review current knowledge in these areas.

STRUCTURAL GLUCAN CLASSES

Ever since the polymeric nature of certain biologically derived materials with specific functionality was recognized, considerable interest has been devoted to elucidating the relationships between biopolymer structure and function (Dickerson & Geis, 1969). Until recently, polysaccharides have not received the same level of attention that proteins and polynucleic acids have. Renewed interest has been stimulated by both fundamental and commercial concerns.

Native polysaccharides that are composed exclusively of glucose monomers can be classified by their relative degree of structural order. To a large extent, the degree of structural order is directly related to the type of hydrolytic linkage which joins the monomer units along the polymer backbone (see Fig. 1).

Crystalline glucans

Native cellulose is considered a highly crystalline material although the fine structure is still in debate (Colvin, 1980). At least four polymorphic crystalline forms (designated as cellulose I, II, III, IV) are known to exist. Of these, cellulose I (native cellulose) and cellulose II (regenerated cellulose) have been studied most extensively. For native cellulose, the fringed micelle concept is the most widely accepted view of cellulose structure. Excellent reviews are available elsewhere (Hearle, 1967; Bikales & Segal, 1971; Shafizadeh & McGinnis, 1971). In this model, the individual polymer molecules composed of β-1,4-bonded glucose monomer units (see Fig. 1) pass repeatedly through crystalline regions and then through disordered regions of the microfibrils (see Fig. 2(a)). The ordered regions are of sufficient size and ordered enough to result in defined X-ray diffraction patterns. In order for the cellulose mole-

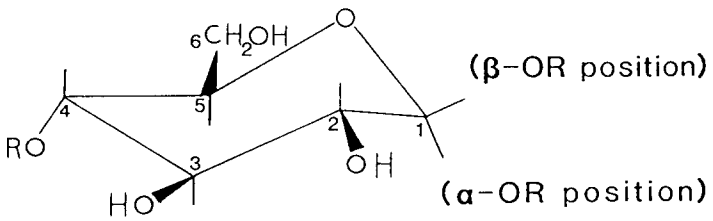

Fig. 1.

cules to pass between regions of high order to regions of disorder, there must be a transitional zone or fringes around the crystallite; hence the name 'fringed' micelle. An alternative model proposed for cellulose crystallite structure is that of a folded chain (Chang, 1971). In this model, a single cellulose molecule folds back and forth on itself within the 101 plane of the crystallite with a fold length of one 'leveling off degree of polymerization' (LODP) unit forming a platelet (see Fig. 2(b)). Several platelets are packed in the crystallographic registry in the 101 direction making up the cellulose crystallite. This model was stimulated

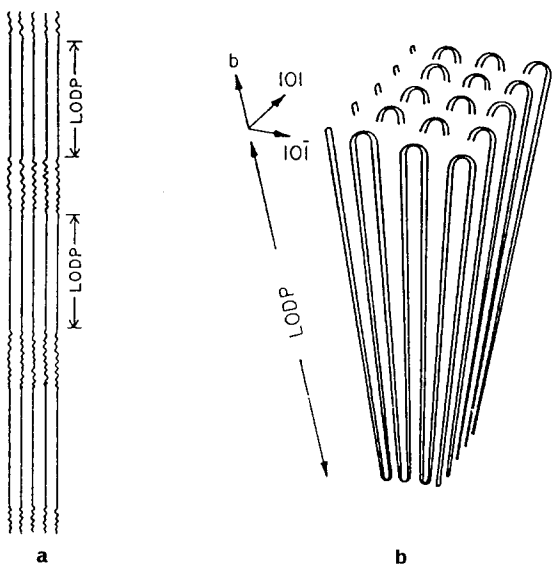

Fig. 2.

by the work of Keller and co-workers (Blundel *et al.*, 1966; Williams *et al.*, 1968*a*) in the case of polyethylene. Estimates of the size of cellulose crystallites range from a few hundred to approximately 2000 monomeric units, depending on the source of cellulose. Although experimental evidence for chain folding was presented (Chang, 1974), 'Keller transformations' in native cellulose were not demonstrated. Earlier experimental evidence (Sakurada *et al.*, 1962) and theoretical studies (Jaswon *et al.*, 1968) on the elasticity of cellulose crystalline regions appear to exclude this model. Moreover, more recent X-ray diffraction (Gardner & Blackwell, 1974), electron diffraction (Claffey & Blackwell, 1976), and stereochemical structural (Sarko & Muggli, 1974) data aimed at elucidating whether the cellulose chains run parallel or antiparallel in the crystallites show that at least in one course of cellulose, *Valonia*, the chains run parallel, thereby refuting the universality of the folded chain model. In other forms of cellulose, the situation is not as clear (French, 1978; Stipanovic & Sarko, 1978; Bose, 1980). Chain folding may exist in cellulose II and not in cellulose I. In addition, it has also been suggested that pretreatment effects may change the crystalline structure (Kolpak & Blackwell, 1976), but the manner in which these effects alter the depolymerization process is unknown. The various structural models for cellulose are still in dispute and it is unlikely that the debate will be resolved in the near future.

Irrespective of the specific structural model for cellulose it is agreed that cellulose is a highly crystalline material and very insoluble in aqueous media (Kolpak & Blackwell, 1976; Fan *et al.*, 1980). This property has been exploited in studies of acid cellulose hydrolysis, employing measurements of weight loss of the cellulose substrate with various reaction conditions (Millet *et al.*, 1954; Sharples, 1971). These latter experiments seem to confirm the general idea that cellulose is composed of at least two distinguishable regions; a rapidly hydrolysable amorphous region and a more chemically resistant region of cellulose crystallites. More will be said about this below.

Starch is another naturally occurring polysaccharide which exhibits crystalline characteristics. After cellulose, it is the next most abundant compound synthesized by plants. Starches are naturally produced polyglycans linked α-1,4 along the polymer backbone, but also contain significant amounts of α-1,6 branch points (see Fig. 1). Traditionally, it is said that starch is composed of two chemical constituents; amylose and amylopectin. The linear fraction, amylose (composed exclusively of

α-1,4 bonds), typically constitutes 15–25% of the total starch of a particular plant genotype; the remaining component is amylopectin. Certain plant varieties are known to differ from this 'normal' range of amylose and amylopectin. Waxy varieties contain little or no amylose and so-called high amylose varieties may have up to 80% by weight of the linear fraction.

The reported degree of polymerization (DP) (i.e. the number of glucose monomer units covalently linked within the polymer) of amylose is of the order of several hundred units (Sterling, 1978). Amylopectin is of higher molecular weight than amylose with a published DP ranging from approximately 1000 to nearly 10^6 (Greenwood, 1956; Banks *et al.*, 1972). Approximately 97% of the bond linkages in amylopectin are the same α-1,4 type as in the linear molecule, but in the classical 'tree-like' structure shown in Fig. 3, every 20–30 monomer units along the polymer backbone, at any given part, an α-1,6 branch point exists (see insert of Fig. 3). The structure of glycogen, the equivalent animal storage polysaccharide, is similar to that of amylopectin with the exception that approximately 20% of the bond linkages are

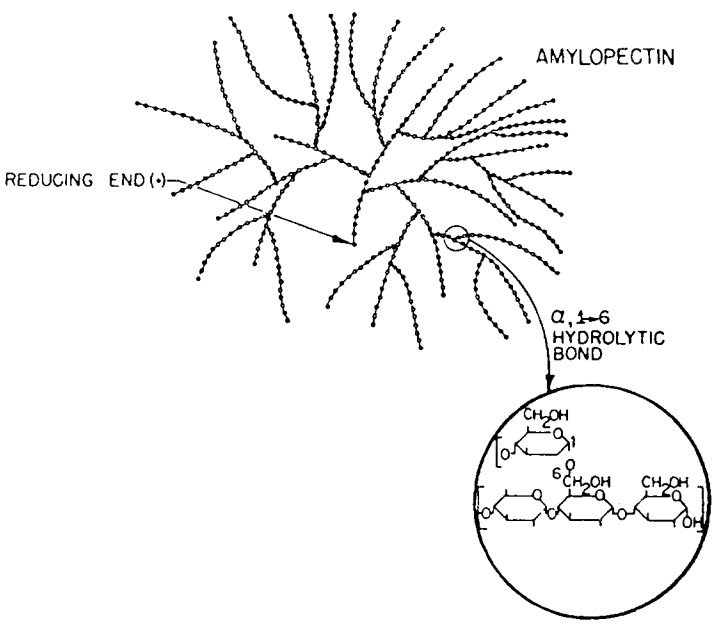

Fig. 3.

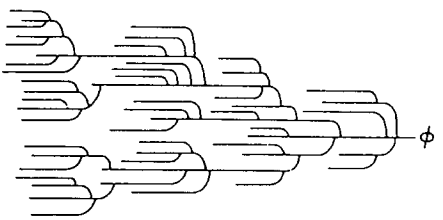

Fig. 4.

α-1,6 branch points (i.e. occurring every 3–6 units along the main chain) and the molecular weight is generally not as high as that of amylopectin (Meyer, 1943; Metzler, 1977). French (1972) has suggested that the structure of amylopectin is not that of the classical, tree-like structure of Fig. 3, but rather like that of a racemose (see Fig. 4), or grape-like clustering of branch points with longer straight chain portions. This concept has been gaining acceptance recently (Banks & Greenwood, 1975; Robin *et al.*, 1975; Hood & Mercier, 1978; Nikuni, 1978; Banks & Muir, 1980) as its structure fits well into the fibrillar model of starch granule construction (see below). It is interesting to note that in the so-called unitarian theory, amylose and amylopectin do not exist *per se*, but are artifacts of fractionation process by which they are isolated. In this concept, the starch granule contains only a single complex polysaccharide. Banks & Muir (1980) state that there is little evidence to support this concept at the present time, but note that it is not possible to prove it erroneous. Nikuni (1978) takes an opposing view.

In the natural product, starch is not present as a random assortment of macromolecules (disregarding the unitarian concept), but is organized into a larger body called the starch granule. The size (usually in the range 5–30 μm) and shape of the granules vary with the source and are characteristic for specific plant varieties (Sterling, 1978). All starch granules have certain common features in their supermolecular structure. A tangential lamellar construction of the granule is evident with both light and electron microscopy (Buttrose, 1962, 1963). These concentric shells appear as successive darker and lighter bands. Each of the lamellae are approximately the same size (0·4–0·5 μm). The darker bands are known to be more compact and are highly crystalline (Sterling, 1978) and therefore more resistant to chemical and enzymatic

attack (Kerr, 1952; Cowie & Greenwood, 1957; Musselman & Wagoner, 1968; Gallant & Guilbot, 1971; Hood *et al.*, 1971; Gallant *et al.*, 1973; Robin *et al.*, 1974; Hollinger & Marchessault, 1975; Allen *et al.*, 1976). The starch molecules are arranged within the starch granule essentially parallel to each other along radial lines (French, 1978; Sterling, 1978). For this reason, the starch grain is birefringent and polarizes light. This general structure has been confirmed by a variety of experimental techniques.

The fine ultrastructure of the granule is still unknown, although two postulates have been advanced: the blocklet concept and the fibrillar concept. The blocklet concept (Muhlerthaler, 1965) assumes the existence of microcrystalline bodies called micelles. These micelles are somewhat similar to crystallites in cellulose although the geometry is not as well defined. Little experimental evidence has been presented in support of this concept. The more accepted model is that of the fibrillar concept (Meyer, 1952). In this model, the highly crystalline regions consist of parallel chains of amylose and the straight chain portions of amylopectin molecules, but discrete crystallites are not present (see Fig. 5). The occurrence of the observed lamellar structure in the granule is due either to the specific growth pattern of the granule (possibly related to the light–dark cycle) or due to some intrinsic properties of the macromolecules. French's racemose concept (1972) fits the fibrillar granule model well (Nikuni, 1978). An individual amylopectin molecule could traverse several lamellae with the majority of α-1,6 branching points being in the less dense amorphous regions. The amylose molecules could then either associate (via hydrogen bonding) with the crystalline regions of the amylopectin molecules or be present in the amorphous regions in a more random form.

Amorphous glucans

At the opposite end of the spectrum are polysaccharides which do not form strong inter- or intramolecular associations in aqueous solvents. These materials include a large group of naturally produced or modified polysaccharides that can either be linear or branched polymers and may or may not contain ionic moieties. Strictly speaking, the use of the word amorphous would only imply a lack of structure or form without distinct crystalline characteristics. Clearly, many polymeric materials may be amorphous but yet form distinct phases when in contact with

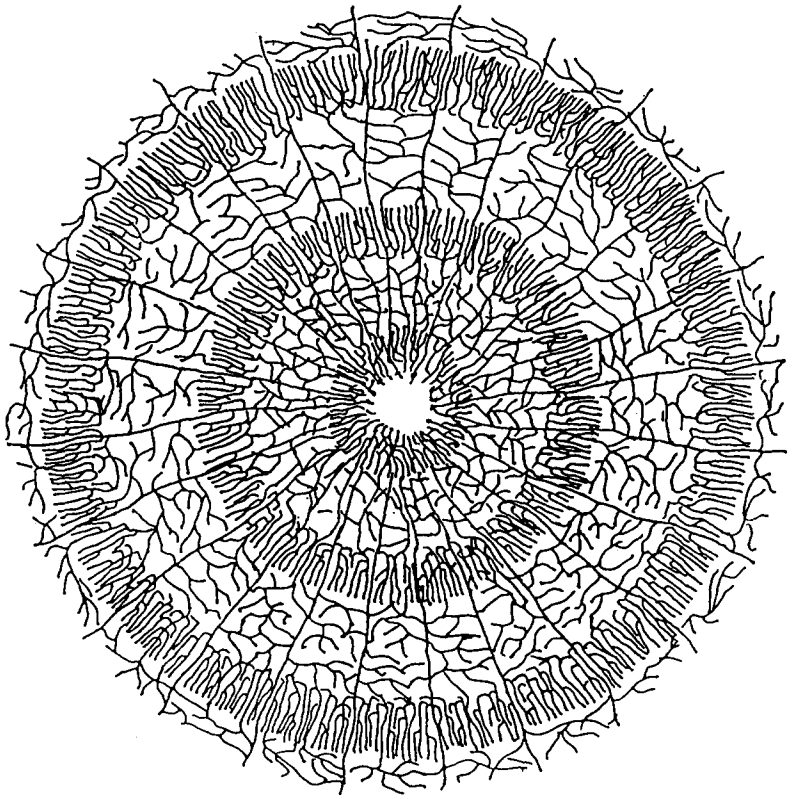

Fig. 5.

aqueous solution owing to their insolubility as in a suspension or gel. Cellulose and starch (rendered amorphous by mechanical means, e.g. ball-milling) could then be considered to be members of this class. However, for materials prepared in this manner, there would also exist significant mass transfer considerations between the amorphous insoluble polymeric substrate phase and the soluble biological catalyst in the aqueous phase. Such phenomena, though clearly present in many systems (see below) necessarily complicate the process of enzymic depolymerization. Therefore, for this discussion, the meaning of amorphous glucans will be restricted to soluble polysaccharides. Clearly such materials must be hydrophylic substances. These would include water soluble derivatives of starches and cellulose (e.g. carboxymethyl-

cellulose, etc.), ion-containing polysaccharides (pectate, alginate, sulfonic esters of carrageenan and deacylated chitin) and dextrans. Some of these materials may form true solutions at low concentrations of the polymeric substrate, but exhibit gel-like and/or colloidal-like characteristics at higher concentrations or only exist in solution over specific ranges of pH and/or ionic strength. Most neutral, linear polysaccharides do not solvate easily under any conditions and are therefore not suitably defined under the above stated restrictions. Whistler (1973) defines the final state of dissolution as when the polysaccharide chain is completely surrounded by an atmosphere of partially immobilized water molecules. Polymer chemists (Flory, 1953; Yamakawa, 1971; Vollmert, 1973) would describe solvation in a different fashion, referring to the dilute solution conformation of these materials as being random coils or statistical coils, that is to say, materials which do not form inter- or intramolecularly associated complexes in solution.

Probably the best studied system of amorphous, water soluble polysaccharides are dextrans. Dextrans are bacterially produced polyglycans linked α-1,6 along the polymer backbone (see Fig. 1). Some species of the polymer contain short side chains (one to three glucose units long) that are bonded to the main chain α-1,4-, α-1,3- or α-1,2- (Bourne *et al.*, 1962; Metzler, 1977). Although the degree of branching varies somewhat with the source (Jeanes *et al.*, 1954), commercial dextran (from Pharmacia Fine Chemicals, Uppsala, Sweden) derived from a particular strain of *Leuconostoc mesenteroides* contains approximately 4–5% of such branch points and thus can be described as a comb-branched polymer. For these biopolymeric substrates of number-average molecular weight greater than approximately 2000 a random coil configuration has been confirmed by intrinsic viscosity studies (Gekko, 1971; Gekko & Noguchi, 1971), thermodynamic data (Basedow *et al.*, 1980*a*), and various spectroscopic data (Seymour & Julian, 1979; Seymour *et al.*, 1979). For low molecular weight dextran polymers (less than 2000 daltons or approximately 12 glucose unit long), the solution configuration is no longer randomly coiled, but rather that of an uncoiled, stretched or rod-like form. Presumably this effect is caused by steric hindrances along the polymer backbone or the polymers' associated solvent layers. Basedow *et al.* (1980*a*) did not find that the coil configuration of dextrans above 2000 daltons was affected by aqueous salt solutions. The author concurs with this finding from his studies of the dilute solution viscosity measurements over widely

varying ionic strengths (Rollings, 1981; Bose *et al.*, 1982). However, the possibility of aggregated materials being formed in minute quantities cannot be eliminated entirely in view of the results of Brant and co-workers (Jordan & Brant, 1978) who studied the dilute aqueous solution behavior of pectin and pectic acids with light scattering methods. In any case, the limited degree to which such conformations exist and participate in kinetic degradation will be small and hence precludes their discussion at this time.

Gel-forming glucans

The natural crystalline granule structure of starch, unlike cellulose, can be disrupted rather easily. The overall process of granule macromolecular order disruption is referred to as gelatinization (Sterling, 1978). Gelatinization occurs when starch is heated in excess water, which causes a series of physicochemical changes to occur. The process can be summarized as follows (Blanshard, 1979): first water diffuses into the unswollen granule, followed by a hydration-facilitated melting process which disrupts the crystalline character of the granule. This can be observed by a loss of birefringence on heating. Finally, the granule swells and imbibes large amounts of water. If the crystalline structure of the granule is homogeneous, then the entire structure will disrupt (or melt) when subjected to the same treatment (Flory, 1953). However, this is not the case (Zobel, 1984). For an individual granule, the melting process, as measured by birefringence loss, occurs over a temperature range 1·5–2·0°C (Gough & Pybus, 1971), but variations among granules also exist. Generally, within a given starch variety, the entire population of granules gelatinize over a range 10–15°C (Banks & Muir, 1980; Zobel, 1984). Although it has been reported (Schoch and Maywood, 1956; Collison, 1968) that the gelatinization range is related to granule size (smaller granules being more resistant to gelatinization), it has been demonstrated using birefringent measurements and enzyme digestibility (Rollings, 1981; Rollings, in press) that this is not universally true. Moreover, in light of the fact that the gelatinization range is strongly dependent upon starch variety it is more likely that the physical chemistry of starch gelatinization is related to some intrinsic properties of the constituent macromolecules (Lelievre, 1973). Related physical phenomena are discussed below.

Numerous experimental techniques have been employed for studying the phenomena of starch gelatinization. An excellent review of and resumé to these methods is presented by Zobel (1983). These methods include: light microscopy, electron microscopy, light transmission, viscometric measurements, swelling and solubility data, enzymic digestibility, nuclear magnetic resonance (NMR), laser light scattering and differential scanning calorimetry (DSC). Depending upon particular objectives, any or various combinations of these methods are employed for gelatinization studies, and for the most part (with the possible exception of some viscometric information (Rollings, 1981; Rollings, in press)) are complementary, at least for the major transition. The most direct evidence for treating starch gelatinization as a melting process has come from DSC measurements (Lelievre, 1973, 1976; Zobel, 1984) which are most amenable to thermodynamic analysis. Flory (1953) demonstrated that under appropriate conditions, the conversion of a polymer from a partially crystalline to an amorphous state may be treated as a first order transition. In such cases, the equilibrium temperature, T_m, at which the last traces of polymer crystallinity disappear is related to the concentration of solvent.

Application of this technique has been to demonstrate many gelatinization phenomena (Wootton & Bamunuarachchi, 1979*a*, *b*; Donovan & Mapes, 1980; Kugimiya *et al.*, 1980). Donovan & Mapes (1980) observed not only the primary melting transition of starches, but also order–disorder transitions at temperatures above those required for gelatinization as seen by other techniques. This has been interpreted as being evidence of macromolecular structural change occurring beyond the birefringence end point (Donovan & Mapes, 1980; Zobel, 1984).

It is well known that starch–water complexes are not stable (Watson, 1964; Collison, 1968; Sterling, 1978). Water is a poor solvent for starch (Jordan & Brant, 1978; Zobel *et al.*, 1965; Donovan, 1979) and as a consequence a clear solution of starch in water will gradually become turbid and eventually some of the carbohydrate will precipitate. In the literature, this phenomenon is called retrogradation (Watson, 1964). It will be shown below that it is related to the polymer conformation states of this material.

As stated above, many physical properties of starches are related to their botanical source. It is not intended to review these in great detail here as excellent reviews are available elsewhere (Banks & Greenwood,

1975; Banks & Muir, 1980). Some pertinent information collected from these and other sources (Leach & Schoch, 1961; Nara, 1979; Rutenberg, 1980; Zobel, 1984) is summarized in Table 1. This information can be related in part to similar data on cellulose (Kast, 1953). It is clear that a much broader range of variation in the physical properties of starch is available for study than is the case for cellulose. In addition, starch can be pretreated to alter its properties in a predictable fashion (Sair & Fetzer, 1944; Blackwell *et al.*, 1969; Wu & Sarko, 1977; Sarko & Wu, 1978; Banks & Muir, 1980) which is related to macromolecular morphology. Similar transformations in retrograded starch have not been demonstrated (Charbonnière *et al.*, 1968). All of the physicochemical states shown by starch from various botanical sources can be treated in a systematic fashion in much the same way as synthetic polymers. This concept has laid the foundation for much of the important calorimetric data presented above. In addition, other important relationships between starch structure and function should be expected as it is the macromolecular nature and breadth of the molecular weight distribution (MWD) of polymers which results in variations in their gelatinization (melting) behavior (Flory, 1953) as well as the observed depolymerization behavior of these materials with enzymes. Why is it then that such a wide variation in physical characteristics exists in materials that are so similar chemically? The answer must lie in the physical chemistry. For example, if the nature of the starch crystallites are similar for various species (in the physicochemical sense) then one would expect to observe phase transitions in similar thermal environments (Flory, 1953; Kaufman & Falcetta, 1977) even though the amount of material undergoing the transition (order–disorder) varies. On the other hand, if variations in the gelatinization (melting) behavior are observed, then they must be attributed to structural defects present in the polymer caused by chain folding, branching or atacticity. This will clearly affect the manner in which the polymer will be degraded.

DEPOLYMERIZATION ENZYMES

Depolymerization, by definition, involves the cleavage of covalent polymeric substrate backbone linkages. Nature supplies a myriad of catalysts which can accomplish this objective for polysaccharides and, conse-

TABLE 1

Starch variety	*Amylose (%)*	*X-ray pattern type*	*Gelatinization range (°C)*	*Granule size (μm)*	*Fraction of starch crystallized*	*Enthalpy of gelatinization (cal g^{-1} dry starch)*	*% Iodine affinity*	*% Digestibility with α-amylase (24 h, 50°C)*
Cereals								
Corn	22-28	A	62-72	5-26	0·42-0·45	4·1-4·9	4·83	49·5
Waxy corn	<1	A	63-72	5-26	0·30-0·31	4·8		57·8
Sorghum	23-28	A	68-78	6-30			4·90	50·2
Wheat	17-27	A	56-64	2-35	0·44-0·45	2·9	5·30	48·5
Rice	16-17	A	68-78	3-8		3·4-3·9	4·70	32·6
Amylomaize	50-80	B	63-[a]	3-24		6·7	11·90	14·0
Roots								
Potato	23	B	56-64	15-100	0·32-0·34	4·5	4·42	18·4
Casava	17-22	B	62-73	5-25	0·45-0·47	4·0	3·15	55·7

[a] No well-defined end point.

quently, there is a wide variety of pathways leading to a particular distribution of product molecular weights.

Certain enzymes cleave internal polymeric substrate bonds and are referred to as endo-acting enzymes or endo-depolymerases. If it is assumed that all such linkages are identical (i.e. possess the same bond energies), all bonds will have equal reactivity, and there will be no substrate molecular weight dependency on the reaction rate. Such a situation is said to exist for unbranched dextran molecules acted on by the extracellular endodextranase (E.C. 3.2.1.11) of *Penicillium lilacinum* and *P. funiculosum* (Bourne *et al.*, 1962; Wheatley & Moo-Young, 1977; Lim & Rollings, 1983) with the possible exception of terminal glucosidic bonds (Hutson & Weigel, 1963). As stated above, many dextrans contain branch points along the polymer backbone and these endoenzymes are also unable to hydrolyze main chain linkages in the vicinity of these branch points. With these exceptions, however, these dextranases appear to react in a random fashion. Product distributions of truly random hydrolysis are themselves randomly distributed but are also dependent upon initial substrate molecular weight distributions (Basedow *et al.*, 1980*b*). A parallel situation does not exist for starches and celluloses (Freudenburg *et al.*, 1930; Kuhn, 1930). It may be conjectured that this non-randomness is due to the action pattern of the enzyme (Roybt & French, 1967), but it is equally likely that this result is due to intrinsic properties of the substrate (see below). α-Amylases (E.C. 3.2.1.1) of various sources (thermally stable bacterial sources are the most commonly used industrially) cleave the α-1,4 main chain polysaccharide linkages of starch. Here, however, both the hydrolysis pattern and hydrolysis rate are dependent upon the substrate size (Roybt & French, 1963; Okada *et al.*, 1968). Any attack pattern other than a true random, multichain attack will necessarily result in, at least, a bimodal molecular weight product distribution. Cellulose degradation appears to be a much more involved process from the enzymatic standpoint involving a complex enzyme system of more than one type of catalyst, each with separate functions, and therefore is not directly related to this type of degradation pattern (Blanch & Wilke, 1982).

Exo-acting depolymerases preferentially cleave off monomers or dimers from the end of a polymeric substrate. Most often, if one end of the substrate is characteristically distinguishable from the other (or others in the case of tree-branched type polymers (Burchard, 1983), such as with polysaccharides, these endoenzymes will act on only one

end of the molecule. This situation exists for β-amylase, a starch degrading enzyme. Enzymatic action patterns of this type are believed to result because of the specific means in which the substrate molecule is associated with the enzyme and its active site. Possibly there exists certain functional groups of the protein which are responsible for 'holding' the polymer molecule and others which catalyze the depolymerization. Enzymes of this type could react with the substrate in either a single chain, multiple chain or multi-chain attack pattern. In any case, product distributions from exo-acting enzymes must be bimodally distributed. Given the physical sizes of the catalyst and its substrate and the high turnover numbers of the enzymes, it may be possible that such attack pattern variations are due to simple diffusional processes (Granath, 1958; Burchard, 1983) as well as the specific mode of action of the enzyme. This same argument could also be made for endo-acting enzymes as well.

Certain enzymic systems which specifically depolymerize polysaccharides appear to be designed so that more than one enzyme is assigned to the overall process and these appear to act in a synergistic manner. Wheatley & Moo-Young (1977) studied the synergistic effect of combined exo- and endo-acting enzymes in the dextran–dextranase system with both soluble and insoluble forms of the substrate. As expected, the exoenzyme acting alone resulted in a bimodal product distribution and a slow increase in the degradation index, DI, defined as:

$$\mathrm{DI} = \frac{M_n(0)}{M_n(t)} - 1$$

where $M_n(0)$ and $M_n(t)$ are the number-average molecular weights initially and at time t after reaction, respectively, whereas the endoenzyme rapidly increased this parameter. Surprisingly, however, a combination of these enzymes resulted in a more rapid increase in DI than would be expected by simple summation principles for the soluble substrate. The exact physical reasons for such synergism could not be explained with the data presented although it did follow trends predicted by a theoretical treatment (Suga *et al.*, 1975*a*). Such synergistic effects have been demonstrated for a more physically realistic picture in the case of cellulose. The literature on this subject is reviewed elsewhere (Ghose & Gas, 1971; Humphrey, 1979; Blanch & Wilke, 1982). The cellulose enzyme system is currently believed to consist of three different enzymes each with a different function. The isolated components

are identified as: (1) an endo-β-1,4-glucanase or β-1,4-glucan glucanohydrolase (E.C. 3.2.1.4) which hydrolyses the polymeric substrate randomly with glucose and cellobiose as end products; (2) an exo-β-1,4-glucanase or β-1,4-glucan cellobiohydrolase (E.C. 3.2.1.91) which cleaves cellobiose units from the non-reducing end of this molecule; and (3) cellobiase or β-glucosidase (E.C. 3.2.1.21) which splits cellobiose into two glucose units (Reese, 1959; Whitaker, 1959; Li *et al.*, 1965; Selby & Maitrand, 1967; Nisisawa *et al.*, 1972; Eriksson & Pettersson, 1975*a*,*b*; Wood, 1975). The original postulate of the effect is due to Eriksson (1969, 1974) and has been supported by recent experiments (Okasaki & Moo-Young, 1978; Ghose & Bisaria, 1979). Again, the combined action of these different enzymes, each having specific attack patterns with the polymeric substrate, results in a degradation that is more rapid than the summation of the contributions from the individual catalysts. The situation for cellulose is, however, more easily envisaged than that of soluble dextrans, as a large fraction of the substrate exists as a crystalline solid; therefore, one enzyme may assist in liberating smaller substrate molecules which are then degraded by another enzyme. This latter enzyme (or group of enzymes) may have little or no activity on the original solid substrate. Hence, the synergism is realized. However, this process does involve substrates in more than one phase and consequently the possibility of mass transfer effects and other physicochemical phenomena exists.

SUBSTRATE CONFORMATION DURING DEPOLYMERIZATION

For polymeric substrates that form true solutions and thereby maintain intimate contact with the same continuous phase containing the biological catalyst, the macromolecular conformation still may be important in terms of the form of the degradation profiles. As stated above, the conformation of unbranched or low degree of branching dextran molecules seems to be dependent upon molecular weight (Gekko, 1971; Gekko *et al.*, 1971; Seymour & Julian, 1979; Seymour *et al.*, 1979*a*,*b*; Basedow *et al.*, 1980*a*), molecules with less than a molecular weight of 2000 being rod-like in character, while larger molecules are more randomly coiled. Presumably, if the substrate molecule is randomly coiled and if the kinetics of depolymerization are controlled or dictated in any significant fashion by the translational

diffusion of the enzyme molecule, then there may exist a greater opportunity for that same enzyme molecule to attack the same substrate molecule at another available and suceptible bond linkage in the near vicinity of the previous encounter even though this may be a considerable distance along the primary structure of the substrate. On the other hand, if the molecules are more fully extended, then this possibility may be decreased (i.e. in the absence of intrinsic enzymic action patterns). Clearly, this phenomenon, if it exists, may assist in explaining more fundamental questions involving the exact mode of action of various enzymes, specifically the concept of multiplicity (Roybt & French, 1963). This possibility may not exist, however, if for the large randomly coiled substrate molecules the mass action is governed by the relative diffusion of the smaller enzyme molecule and if for the small rod-like substrate molecule the mass transfer is governed by its own rate of diffusion with respect to the now larger enzyme molecule. Although such effects have not been investigated at this time, it may be possible to examine this phenomenon with well chosen model systems. For example, in the case of polyelectrolytic substrates (e.g. pectins and pectates (Fuoss, 1948; Pals & Hermans, 1952; Jordan, 1976)), substrate conformation can be altered by variation of bulk solution ionic strength (Eisenberg & King, 1977). Such questions cannot be answered until better understanding of the dilute solution conformation statistics of biopolymers are available. These conformational states can be observed by a variety of experimental methods, including their solution properties, spectroscopic behavior and simulated conformational mapping. The allowable states will be directly related to their intra- and intermolecular bonding patterns.

Solution properties are of fundamental and paramount interest here as they provide the necessary relationships between the biopolymers' structure, shape and function devoid of unwanted intermolecular interactions (Flory, 1953). Applicable measurements include osmotic pressure, light scattering, sedimentation equilibria, chromatographic and various hydrodynamic methods which provide information on the material's molecular weight and molecular weight distribution (MWD). Probably the most extensive information on polysaccharides has been collected from starch fractions (particularly amylose) in various solvents. Considerable debate concerning the conformation of amylose in solution exists in the literature. Banks & Greenwood (1971*a*,*b*; 1972*a*,*b*) have considered amylose to be a random coil configuration in solution,

Szejtli and co-workers (Hollo & Szejtli, 1957; Hollo *et al.*, 1968; Szejtli, 1971) have proposed a tightly coiled helix, and Senior & Hamorie (1973) have taken an intermediate view; a loosely coiled or 'worm-like' chain (Yamakawa, 1971). This seemingly wide disparity has recently been addressed by Kodama *et al.* (1978) who found the conformational states of amylose in aqueous solution to be strongly dependent upon molecular weight; corresponding to the so-called 'dissolving gap' reported much earlier by Burchard (1963). Burchard, who was interested in amylose–water stability (retrogradation), determined that if the molecular weight of amylose is between 6500 and 160000 daltons it is indissoluble in water and soluble only if its molecular weight is outside of this region, referred to as the 'dissolving gap' by him. This molecular weight corresponds to approximately 35–900 DP, a range easily envisioned for the straight chain portions of amylopectin in light of the currently accepted fibrillar concept of starch granule construction. Kodama *et al.* (1978) further determined by light scattering techniques that this undissoluble material is asymmetric in conformation and could be that of a rod. Amylose of a size in this range readily forms aggregates of presumably rigid form as evidenced by the high slope of these fractions in plots of radius of gyration versus molecular weight. The structure inferred from their data is that of a double helix of two intertwined amylose molecules consistent with the proposed structure of Kainuma & French (1972) for B-starch. This material was further shown to be *resistant* to enzymic attack that could not be attributed to α-1,6 branching bonds. This information is consistent with experimental data and the hypothesis (Dintzis & Tobin, 1969; Tanner & Berry, 1974) advanced earlier for polyglycans systems as well as data reported by Brant and co-workers (Goebel & Brant, 1970; Jordan & Brant, 1980) in mixed solvent systems. It is interesting that amylose even in so-called 'good solvent' systems may form into aggregates which cannot be removed (Jordan, 1976), indicating strong intermolecular interactions (Brant, 1980). Although the molecular size of these aggregates is larger (Jordan & Brant, 1980) than that reported by Kodama *et al.* (1978) or Burchard (1963) it is likely that this effect is strongly solvent dependent. More generally, we can presume that aggregate formation of the polymer will be a function of solvent–polymer interaction as well as an intrinsic property itself predicted by intramolecular bond angles. This generalization is clear in the light of the findings for the pectin and pectic acids (polyelectrolytes) in dilute aqueous solutions (Jordan & Brant, 1978).

The instability of starch–water systems (retrogradation phenomenon) mentioned above is related to starch–water solution properties and aggregate formation. Although the general phenomenon has been known for some time, it is only recently that the physics of retrogradation is being understood (Kodama *et al.*, 1978). Rollings *et al.* (1983*b*) were able to independently conclude that starch substrate structure and susceptibility are intimately involved in the degradation pattern observed during depolymerization of starch, but the physical model of the process presented is probably overly simplistic. Infrared and Raman spectroscopy (Cael *et al.*, 1973) and proton nuclear magnetic resonance (NMR) studies (Casa *et al.*, 1966; Jaska, 1971; Cael *et al.*, 1973; St Jacques, 1976; Hennig & Lechert, 1977; Bradbury & Collins, 1979) of starches and starch degradation products, clearly indicate that strong intramolecular hydrogen bonding, from OH(3′) to OH(2), exists in all compounds. Intermolecular H-bonding has also been suggested to exist (Brant, 1976, 1980; Banks & Muir, 1980). This type of bonding along the polymer chain induces specific steric conformation to the chain. This information has been used together with conformational theory (Rao *et al.*, 1969; Brant & Dimpfl, 1970; Goebel *et al.*, 1970; Brant, 1976; St Jacques *et al.*, 1976; Jordan *et al.*, 1978) to construct the structure of the amylosic chain in solution. The most recent picture that emerges from these investigations is that of a loosely coiled helical chain consistent with earlier proposed structures (Senior & Hamorie, 1973; Kodama *et al.*, 1978). The models presented are still incomplete, however, as there still exists a debate concerning left or right chirality (St Jacques *et al.*, 1976; Jordan & Brant, 1980) to the helix. Irrespective of this problem it is clear that starch and starch components in solution or aggregated from solution will be present in a narrow range of conformational states. These states are caused by both the intrinsic properties of the biopolymer as well as polymer–solvent interactions. The specific molecular size of these aggregates will be directly related to the source of starch. Depolymerization of the polysaccharide by a random acting catalyst will not occur in a random fashion due to the fact that not all of the polymer is freely accessible or equally accessible to the catalyst. For these reasons, the conformational states of polysaccharides during depolymerization must be considered if applicable kinetic models are to be developed.

The situation for cellulosic materials is somewhat similar to that of granular starch. Both materials are believed to consist of crystalline and amorphous regions (see above), although the specific nature of

these regions is a property of the material and the source of the material. Various effects of cellulose substrate structure on the depolymerization kinetics have recently been reported (Fan *et al.*, 1980*a*, *b*; Fan *et al.*, 1981; Ryu *et al.*, 1982; Saddler *et al.*, 1982; Tassinari *et al.*, 1982; Lee *et al.*, 1983). Earlier studies were instrumental in directing these developments. Preferential hydrolysis of amorphous regions on regenerated cellulose was demonstrated by Norkrans (1950) and Walseth (1952*b*). This action results in an increase in substrate crystallinity during degradation (Caulfield & Moore, 1974) and a greater resistivity toward enzymic attack (Reese *et al.*, 1957; Nisisawa, 1973). Fan *et al.* (1980*b*) list several major structural features that may contribute to substrate susceptibility which include (1) crystallinity, (2) surface area, (3) macromolecular packing arrangements and (4) cellulose fiber structure. Of the first two contributions, crystallinity effects seem to be of greater importance in guiding the depolymerization reactions (Fan *et al.*, 1980*b*, 1981; Lee *et al.*, 1983). If the cellulosic substrate used in the study has associated with it material such as lignin (another biopolymer possessing definite structural characteristics (Goring *et al.*, 1979; Lindstrom, 1979; Connors *et al.*, 1980; Sarkanen *et al.*, 1981, 1982) then surface area effects may be more important (Saddler *et al.*, 1982). Lee *et al.* (1983) have incorporated the salient features of these results into a physical description of the influence of cellulose structure on the cellulase reaction mechanism. Although no universally accepted view of the process has yet been presented, it is clear that these studies are beginning to unravel the complexities associated with cellulose depolymerization. Rollings *et al.* (1983*b*) used aqueous size exclusion chromatographic analysis to examine the MWD profiles of corn starch at various thermal states. For the reactions of intact granular starch (below the granule melting point), the high molecular weight fraction (i.e. greater than 10^7 daltons) appears to exhibit a similar two phase depolymerization process (i.e. crystalline and amorphous regions) as that of cellulose (work in progress by H. Hong and J. E. Rollings), but additional non-random degradative hydrolysis is also present. This may be attributed to other associational complexes inherent to starch substrates or to its bonding patterns.

Under appropriate conditions, amorphous polysaccharides may reassociate with themselves and in some cases crystallize. Water addition is known to result in an increased crystallinity caused by recrystallization of highly amorphous cellulose (Howsmon & Marchessault, 1959; Caulfield & Steffes, 1969). Similarly, starch will reassociate (or

retrograde) although the crystallinity of this material is not clear (Nara, 1979; Rutenberg, 1980). The rate and extent of retrogradation depends upon the concentration and type of starch (amylose retrogrades more quickly than branched starch), the molecular weight of starch, temperature and other components in solution (Ciacco & Fernandes, 1979). Retrogradation is undesirable in depolymerization reactions as the reassociated (or recrystallized) material is more resistant to enzymic hydrolysis than are amorphous substrates and this will decrease overall yield (Boruch & Pierzgalski, 1979; Rutenberg, 1980). Retrogradation is difficult to measure, but most techniques involve determining the amount of soluble material present in solution by some chemical or physical means. The causes for such phenomena are as yet not clear. However, certain recent observations on various polysaccharides are uncovering some common themes. Undoubtedly, the physical chemistry of these macromolecules and the fluids they are in contact with result in the types of allowable associational states which exist. That is to say, the macromolecular conformational state and its overall configuration determines to a large extent, the manner in which the biopolymeric substrate will ultimately be degraded. Therefore, in order to understand the larger question, detailed information must be available about the substrate's structure. It may well be that cellulose molecules will preferentially form into crystalline arrays of various polymorphic forms, each of which must be considered a separate phase. Starches and their degradative products may also have certain preferred conformations and those conformations may change depending upon the intrinsic properties of both the polysaccharide and the continuous liquid medium. This is evident in the light of the findings of Burchard (1963) and Kodama *et al.* (1978). Moreover, the depolymerization process is by nature a dynamic process as is the formation and disruption of these allowable polysaccharide configurations. Consequently, the global process is quite complicated yet is dictated by a finite set of physical and chemical events.

DETERMINATION OF POLYSACCHARIDE MOLECULAR WEIGHT DURING DEPOLYMERIZATION

In order to obtain sufficient information for modeling kinetic depolymerization of large molecular weight natural polymers like polysaccharides

ides, it is necessary to determine the MWD of the resulting products. Size exclusion chromatography (SEC) is the most powerful analytical tool currently being used for this purpose (Tung & Moore, 1972; Yau *et al.*, 1979) and has provided valuable information on depolymerizations (Blundel *et al.*, 1966; Williams *et al.*, 1968*a*,*b*; Willmouth *et al.*, 1968; Bendetskii & Yarovenko, 1975; Linko *et al.*, 1975; Henriksnas & Bruun, 1978; Henriksnas & Lovgren, 1978). In order to use SEC as a quantitative measure for kinetic studies, a number of factors must be examined in parallel with the main study. General reviews of this topic have been published by Rollings *et al.* (1983*a*) and others (Stendlund, 1976; Dubin, 1981; Hagnauer, 1982) which will now allow for appropriate kinetic measurements to be obtained.

The principal separation mechanism in SEC is the partitioning of macromolecules between the flowing solvent and the solvent within the porous matrix of the SEC column packing. Separation occurs because the effective volume of the column depends upon the size of the polymer molecules in solution. Smaller molecules will, on average, spend a longer period of time in the relatively stationary solvent inside the porous matrix as compared to the larger macromolecules which are unable to enter the small pores. Thus, the larger macromolecules have a smaller pore volume available to them and elute from the column earlier than the smaller molecules. Since the principal separation mechanism is the size of the macromolecule, the dilute solution conformation statistics of water soluble polymers are of paramount importance.

The main objective in using SEC is to determine the molecular weight and/or MWD of the macromolecules. However, the separation process depends primarily on the size of the polymer in solution. In order to effectively interpret SEC elution data, the relationship between the size of the macromolecule in solution and the molecular weight must be available. Flory (1953) has proposed that the molecular weight, M, is related to the hydrodynamic volume $\langle r^2 \rangle^{3/2}$ as follows:

$$[\eta] = \Phi_0 \langle r^2 \rangle^{3/2} / M \tag{1}$$

where $[\eta]$ is the intrinsic viscosity, $\langle r^2 \rangle$ is the mean square end-to-end distance of the molecule, and Φ_0 is a constant equal to $3{\cdot}6 \times 10^{21}$ dl cm^{-3}. Grubisic and co-workers used the relationship in eqn (1) as the basis of calibration in SEC for synthetic polymers in non-polar solvents (Grubisic *et al.*, 1967). The product $[\eta]M$ is proportional to the hydrodynamic volume; therefore, a plot of $\log[\eta]M$ versus the SEC elution volume

yields a common curve for a given chromatographic column irrespective of the chemical structure of the polymer. Similar or slightly modified calibration procedures (incorporating excluded volume concepts, present in many water soluble polymer systems, not accounted for in Flory's original development (Ptitsyn & Eizner, 1960; Coll & Prusinowski, 1967) have also been shown to be applicable to aqueous SEC systems (Spatorio & Beyer, 1975; Bose *et al.*, 1982; Rollings *et al.*, 1982). If the intrinsic viscosity can be independently measured for an unknown polymer, then its molecular weight can be determined from the elution volume and the SEC calibration curve, thereby alllowing for the development of appropriate kinetic models of depolymerization reactions.

A key problem is encountered when trying to determine the value of the intrinsic viscosity for many water-soluble polymer systems. Most polymers of interest are not obtainable commercially as narrow MWD, well characterized samples, nor is it practical to prepare these samples for routine kinetic studies. This situation is compounded for polysaccharide studies given the association effects mentioned above. Therefore, for many systems it is not practical to independently determine the Mark–Houwink parameters, K and a, by classical methods.

$$[\eta] = KM^a \tag{2}$$

Ouano and coworkers (Ouano, 1973; Ouano *et al.*, 1974) have described the use of a continuous capillary viscometer capable of determining the viscosity of a polymer solution during its SEC elution. If the sample concentration, c_i, is determined from differential refractive index (DRI) measurements, intrinsic viscosity can be calculated from the relationship given in eqns (3) and (4):

$$[\eta] = \lim \frac{1}{c_i} \ln \frac{\eta}{\eta_0} \tag{3}$$

$$[\eta] = \lim_{c_i \to 0} \frac{1}{c_i} \left(\frac{\eta}{\eta_0} - 1 \right) \tag{4}$$

where η and η_0 are the viscosities of the polymer solutions and solvent, respectively. Letot *et al.* (1980) have described the performance of a similar unit. Both of these groups have worked only on synthetic polymer systems in neutral organic solvents. The extension of these techniques to aqueous solvent systems should be straightforward given

that the appropriate incorporation for the excluded volume concept (Yamakawa, 1971) has been determined (Coll & Prusinowski, 1967; Kolpak & Blackwell, 1976; Rollings *et al.*, 1983*a*) and shown to be applicable to polysaccharide kinetic studies (Rollings *et al.*, 1982, 1983*b*).

Coupled concentration (DRI) and viscometric detectors would be generally suited for kinetic degradation studies provided the polymer is linearly bonded. For studies of starch and dextran degradations, the presence of branching points present an additional complication in analysis. These polysaccharide hydrolysates must be independently assayed for branching or the molecular weight of the material (branched and linear) must be independently measured. The latter approach is more generally convenient and appropriate as: (i) chemical assays for branching require large sample sizes, thereby necessitating parallel development of preparative SEC techniques; (ii) the chemical techniques are relatively inaccurate and provide only a bulk measurement that is insensitive to small changes in branching degree (Whistler, 1964); and (iii) the use of direct molecular weight measurements would provide an internal check on the viscometric studies mentioned above and hence be of more general utility in supplying accurate intrinsic parameters necessary for kinetic model development. Recently, low angle laser light scattering (LALLS) techniques (Kaye, 1972; Kay & Havlik, 1973) have been combined with conventional SEC (Quano & Kaye, 1975; Quano, 1976; MacRury, 1979) to obtain more accurate MWD than can be obtained with standard SEC alone. This combined system is ideally suited for polysaccharide studies as the light scattering technique is extremely sensitive to the high molecular weight fraction which will be present, can be used to effectively eliminate the need for band broadening corrections (MacRury, 1979), and is applicable to both linear and branched polymers and mixtures of linear and branched polymers (McConnell, 1976). The technique has been demonstrated to be suitable for the study of branched and linear aqueous soluble polymers (Fukutomi *et al.*, 1980). Light scattering techniques are quite standard in polymer studies and theories are reviewed elsewhere (Flory, 1953). The use of low angle measurements ($\sim 2^\circ$ in commercial units) eliminates complex angular extrapolations (Zimm plots) used in conventional light scattering and therefore provides rapid molecular weight and MWD determinations.

Sufficient kinetic parameter determination can be obtained for the study of soluble polysaccharide hydrolysis through a combination of

SEC, intrinsic viscometric and LALLS methods, but it may be necessary to employ all these experimental techniques to ensure that accurate measurements are obtained and self-consistent. Theoretically then there is little problem in attaining the required data provided the polysaccharide under study is soluble and that the solvent is itself compatible with appropriate chromatographic resins, etc. Dextran studies pose no significant problems as they are by nature water soluble. The retrogradation phenomenon clearly indicates that water is a poor solvent for starch; however, starch is readily soluble in 0·1–1·0 M alkali (Foster, 1965) – this is due to ionization of mildly acidic hydroxyl groups in the polymer ($pK \cong 12$). Although certain organic solvents (e.g. DMSO) could also be used, aqueous base solutions are very convenient as they also act as a 'stop' for the enzyme activity (i.e. at pH 12 and above the enzymes are denatured and exhibit no activity).

Solubilization of cellulosic materials is more difficult due to their strong inter- and intramolecular bonding. Various cellulose solvents are known (Dale, 1979) which include: (1) strong mineral acids; (2) transition metal complexes; (3) quaternary ammonium bases; and (4) aprotic reagents such as DMSO, paraformaldehyde, etc. The latter two systems are not often used as they are either poor solvents or they cause cellulose to degrade. Strong mineral acids (H_2SO_4, HCl, H_3PO_4) will dissolve cellulose only in specific ranges of acid strengths (Jayme & Lang, 1963; Bose, 1980). Because of their ability to dissolve cellulose with little or no degradation, the transition metal complex solvents have been used most often for cellulose solution studies (Henley, 1961; Jayme, 1971). One of these transition metal complex solvents, cadoxen, is particularly attractive as it is colorless, odorless, and readily dissolves large amounts of cellulose at room temperature. In addition, the capacity of cadoxen to dissolve cellulose can be increased with the addition of sodium hydroxide to the solution (Donetzhuber, 1960). Bose (1980) and Bao *et al.* (1980) have reported the use of such solvent systems for size-exclusion chromatography of cellulose.

PROPOSED MODELS OF POLYSACCHARIDE DEPOLYMERIZATION

It is evident from the information presented above, that a number of kinetically atraditional phenomena must be considered if appropriate

models of polysaccharide depolymerization are to be realized. Even for the 'simplest' system presented (dextran–dextranase) the polymeric nature of the substrate significantly complicates model development. Suga *et al.* (1975*a*) considered the random fission of glycosidic bonds to follow Michaelis–Menton kinetics and expressed the local rate of enzymatic action, $\mathcal{R}$, as:

$$\mathcal{R}(C_i, C_E) = -\frac{k_3 C_E (i-1) C_i}{K_M + \sum_{j=2}^{\infty} (j-1) C_j} + \frac{2k_3 C_E \sum_{j=i+1}^{\infty} C_j}{K_M + \sum_{j=2}^{\infty} (j-1) C_j} \quad (5)$$

where C_E and C_i are the local concentrations of the endodextranase and of a polymeric substrate i monomer units long, respectively, and k_3 and K_M are model parameters. The first term represents the rate of depolymerization of C_i and the second term represents the formation of C_i from all polymeric substrates larger than i monomer units. Rate data were determined using bulk solution chemical assays via established procedures (Hoffman, 1937). The basic model parameters K_M and V_M (V_M is the product of k_3 and C_E), were determined from Lineweaver–Burk plots and found to be reasonably constant irrespective of the substrate molecular weight. This result was interpreted as evidence of the random endo-acting characteristics of the chosen enzyme. Reasonable agreement between experimental observation and theory was obtained in spite of the fact that constant substrate diffusivity was assumed. This same dextran–dextranase system was used in conjunction with an exo-acting dextranase by Wheatley & Moo-Yung (1977) to study the depolymerization reaction with multiple enzyme systems experimentally. Their results did demonstrate that combined enzyme systems enhance substrate depolymerization in a synergistic manner, but further model refinement was not presented.

Studies of the dextran–dextranase system were, to a large degree, stimulated by a need to understand more complicated biopolymer degradation reactions, specifically cellulose hydrolysis. Clearly, this latter system is complexed by both the heterogeneity of the substrate and the multiplicity of the employed enzyme system, as explained above. Early progress and thoughts on the phenomenological factors controlling cellulose depolymerization are reviewed elsewhere (Ghose & Das, 1971). Since 1975 considerable progress has been made in

understanding this system's dynamics. Howell & Struck (1975) viewed cellulose hydrolysis as being primarily controlled by cellobiose inhibition of the *Trichoderma viride* enzyme system. Product inhibition by the dimer was found to be non-competitive, and glucose inhibition as well as inhibition by intermediate products and cellulose itself (less than 15% by weight) were not found to be significant. Although substrate multiplicity was not included within their model development, they concluded that a multisubstrate model with both substrate and product inhibitory functions would be the most reasonable description of cellulose kinetics. Huang (1975) studied the hydrolysis of amorphous cellulose (Walseth, 1952*a*) with the same enzyme system as Howell and Struck but incorporated an adsorption step in the kinetic development, modeled after Langmuir (1916). The rate expression derived by Huang (eqn (6)) required evaluation of four model parameters; k_2, a rate constant, k_1, the ratio of forward and reverse kinetic constants for enzyme and substrate complex formation, X_{1m}, the saturation amount of adsorbed enzyme per unit mass of substrate and k_3, the ratio of forward and reverse kinetic constants for inactive product and enzyme inhibitory complex formation.

$$v = \frac{k_2 X_{1m} k_1 (E)_0 (S)}{1 + k_3(P) + k_1(E)_0 + k_1(S)[X_{1m} - X_1]} \tag{6}$$

where v is the reaction velocity, $(E)_0$ is the initial enzyme concentration, (S) is the substrate concentration, (P) is the product concentration and X_1 is the amount of enzyme absorbed per unit mass of substrate. This equation could be linearized by reciprocation provided that $X_{1m} \gg X_1$ and the model parameters determined by Lineweaver–Burk plots (Huang, 1975). Substrate concentrations were maintained low enough in this experimentation to prevent substrate inhibitory effects and good agreement between theory and experimental observations was obtained for conversions up to approximately 70%. Beyond this Huang believed the assumption of $X_{1m} \gg X_1$ to be no longer valid. Again, although the heterogeneous character of the polymeric substrate was recognized, for real systems, such model incorporation was not performed. Howell & Mangat (1978) developed a kinetic model to account for product inhibition and enzymatic deactivation. Their experimental observations also led them to conclude that substrate heterogeneity should be incorporated into model development. The

non-intrinsic characteristics of the model parameters were explicitly recognized by Huang (1975) but no attempt was made to separate the individual contributions of the various hydrolysis enzymes. This latter deficiency of previous models for cellulose hydrolysis was recognized by Lee *et al.* (1978) who extended the concepts of Suga *et al.* (1975*b*) to incorporate the various action patterns of the cellulase enzyme system. Their model required determination of eight parameters and could only be solved numerically. Experimental tests were performed on soluble cellulose (CMC) substrates (Lee, 1977) again to avoid the heterogeneous characteristics of native cellulose. The model which they presented is shown in eqns (7)–(9).

$$\frac{dC_1}{dt} = z\frac{k_3 C_{Et}\sum_{j=2}^{\infty} C_j}{K_M + \sum_{i=2}^{\infty}(i-1)C_i} + 2\frac{k_3' C_{Et} C_2}{K_M' + \sum_{i=2}^{\infty} C_i} + \frac{k_3' C_{Et}\sum_{i=3}^{\infty} C_i}{K_M' + \sum_{i=2}^{\infty} C_i}$$

$$+ \frac{k_3'' C_{Et} C_3}{K_M'' + \sum_{i=3}^{\infty} C_i} + 2\frac{k_3''' C_{Et}''' C_2}{K_M''' + C_2} \tag{7}$$

$$\frac{dC_2}{dt} = -\frac{k_3 C_{Et} C_2}{K_M + \sum_{i=2}^{\infty}(i-1)C_i} + 2\frac{k_3 C_{Et}\sum_{j=3} C_j}{K_M + \sum_{j=2}^{\infty}(i-1)C_i} - \frac{k_3' C_{Et}' C_2}{K_m' + \sum_{i=2}^{\infty} C_i}$$

$$+ \frac{k_3' C_{Et}' C_3}{K_M' + \sum_{i=2}^{\infty} C_i} + \frac{k_3'' C_{Et}'' C_3}{K_M'' + \sum_{i=3}^{\infty} C_i} + 2\frac{k_3'' C_{Et}'' C_4}{K_M'' + \sum_{i=3}^{\infty} C_i}$$

$$+ \frac{k_3'' C_{Et}'' \sum_{i=5}^{\infty} C_i}{K_M'' + \sum_{i=3}^{\infty} C_i} - \frac{k_3''' C_{Et}''' C_2}{K_M''' + C_2} \tag{8}$$

and for $i = 3, 4, \ldots, \infty$

$$\frac{dC_i}{dt} = -\frac{k_3 C_{Et}(i-1)\,C_i}{K_M + \sum_{i=2}^{\infty}(i-1)\,C_i} + 2\frac{k_3 C_{Et}\sum_{j=i+1}^{\infty} C_j}{K_M + \sum_{i=2}^{\infty}(i-1)\,C_i} - \frac{k_3' C_{Et}' C_i}{K_M' + \sum_{i=2}^{\infty} C_i}$$
$$+ \frac{k_3' C_{Et}' C_{i+1}}{K_M' + \sum_{i=2}^{\infty} C_i} - \frac{k_3'' C_{Et}'' C_i}{K_M'' + \sum_{i=3}^{\infty} C_i} + \frac{k_3'' C_{Et}'' C_{i+1}}{K_M'' + \sum_{i=3}^{\infty} C_i} \qquad (9)$$

In eqn (7), which represents rate of glucose production, the first term accounts for the random action of the endoglucanase, the second and third terms account for exoglucanase activity and the remaining terms account for cellobiase reaction. Rate of cellobiase production is represented by eqn (8), where, terms one and two are the result of endoglucanase activity, terms three and four are for exoglucanase reaction, the next three terms are for cellobiosylhydrolase activity and the last term is for cellobiase action. Equation (9) is a recursive formula for oligomeric and polymeric species larger than the dimer. This last equation includes the contributions of endoglucanase, exoglucanase and cellobiosylhydrolase. For a complete derivation and list of the various terms in the model, the reader is referred to the original work (Lee, 1978). The combined action of such multiple enzyme systems has also led to the observed synergistic effects mentioned above (Eriksson, 1969; Eriksson *et al.*, 1974; Eriksson & Pettersson, 1975*a*, *b*; Okasaki & Moo-Young, 1978; Ghose & Bisaria, 1979). Clearly, kinetic modeling of this type is a formidable task and one that leads to various interpretations for experimental data.

Peitersen & Ross (1979) used a state variable model containing 38 adjustable parameters coupled to an optimization routine to simulate published data. The basic assumptions of their model are that (1) two forms of cellulose exist (amorphous and crystalline) and that the crystalline cellulose transforms to the amorphous form before reaction ($C_1 - C_x$ theory of Reese *et al.* (1950)); (2) the enzyme system is composed of both an endo-acting and an exo-acting species; (3) end products are glucose and cellobiose; and (4) an inductorepressor messenger RNA mechanism will represent enzyme formation by *T.*

reesei. The authors used Michaelis–Menton kinetics for the enzymatic reactions, all subject to product inhibition while the organism which grows on the monomeric product follows Monod growth kinetics. (For complete model development and methodology, see Peitersen & Ross (1979).) Significant discrepancies between model prediction and published experimental observations were noted for end products formed during hydrolysis, although the model was able to simulate reasonable agreement at the end of the fermentation. The authors conclude, however, that a more exact model would require better understanding of the process, particularly relating to cellulose substrate structure and regulation of the enzyme system. This model does clearly demonstrate the complexities of the hydrolysis reaction and the associated fermentation process.

In addition to the specific role in cellulose hydrolysis of the substrate's structure, other phenomena are related to the fact that the substrate is not intimately in contact with the continuous aqueous medium, specifically adsorption processes and mass transfer effects. The capacity for cellulose to preferentially adsorb or position the cellulase enzymes has been recognized for some time (Mandels *et al.*, 1971). Initially this was believed to assist the overall process in an economic sense as the hydrolytic agent would naturally associate with the substrate and therefore lower enzyme requirements. No attempt was made to determine which of the cellulase enzymes were preferentially adsorbed. Clearly, for such systems, the enzymes must first come in contact with the substrate before hydrolysis can proceed.

Peitersen *et al.* (1977) studied the adsorption process for various cellulose systems and determined that the amount of adsorbed enzyme was more related to the surface area of the substrate than its crystallinity. For fermentation of cellulose substrates with *T. viride*, Binder & Ghose (1978) determined that physical adsorption of the cellulose is required in order to induce cellulase production by the living microorganism. Such processes were not included in the Peitersen & Ross (1979) model of coupled fermentation and hydrolysis reactions although they recognized such contributions. Castanon & Wilke (1980) examined the adsorption process in more detail and concluded that under the conditions studied, all the cellulase components were essentially adsorbed onto the cellulosic matrix within the first hour of incubation. However, the adsorption rates of the individual enzyme components were not equal, C_x enzymes being adsorbed preferentially during the

early stages of hydrolysis when compared to C_1 enzymes. During later stages, the reverse was observed. During the entire process of degradation, a continuous decrease in soluble protein was noted in contrast to earlier reports (Mandels *et al.*, 1971; Brant *et al.*, 1973; Wilke & Yang, 1975*a*, *b*). This indicates that once the enzymes become adsorbed they remain immobilized (Castanon & Wilke, 1980). Beltrame *et al.* (1982) later found that the total amount of adsorbed enzyme was related to the substrate's susceptibility to hydrolysis by the catalyst. It should be recognized however that, potentially, all these phenomena (adsorption and mass transfer effects, macrostructure of the cellulose substrate, ability of the enzyme and substrate to form an active complex, as well as the intrinsic mechanistic kinetic steps) are interrelated to the entire process.

Lee *et al.* (1982) concluded that adsorption of the enzymes onto the substrate governs the rate of cellulose hydrolysis, but the affinity of the enzyme to hydrolyze the substrate (or the substrate's susceptibility) is related to the structural properties of the cellulose. Adsorption will therefore depend on both the crystallinity and surface area of the substrate. The rate of hydrolysis, although clearly dependent on the amount of adsorbed enzyme, is more directly related to the crystallinity of the substrate; the rate of reaction slows down more markedly for crystalline material than for amorphous material. These same authors explain that previously reported discrepancies are due to the availability or accessibility of adsorption sites. Lee & Fan (1982) take a different view of the process. They concluded that the various mass transfer steps do not control the hydrolysis rate, but are, rather, controlled by a surface reaction step promoted by the adsorbed enzyme. Since the transport of the enzyme from the bulk to the surface is very rapid (occurring in the first minute of contact) compared to reaction, they concluded that the surface reaction is rate limiting. In their study, the amount of adsorbed enzyme did not exactly parallel the hydrolysis rate. Two possible explanations were given to explain this observation: (1) multiple layers of adsorbed enzyme are formed, but only the monolayer adjacent to the cellulose surface is capable of participating in reaction; or (2) the surface of the cellulose substrate is composed of two fractions, one containing active sites and the other containing inactive sites – only the active sites are involved in reaction. These authors also concluded that the major structural features of cellulose (i.e. surface area and crystallinity) greatly influence hydrolysis rate, and

the susceptibility of the substrate to be hydrolyzed is most strongly dictated by its crystallinity. The heterogeneous characteristics of the cellulose–cellulase system may preclude the applicability of Michaelis–Menton type kinetics. In a later paper, these same authors (Lee & Fan, 1983) refer to the structural transformation of cellulose into a less digestible form during hydrolysis which was suggested earlier by others (Brant *et al.*, 1973). It is unlikely that this transformation process which they refer to, can be likened to the change of cellulose from one crystalline polymorph to another, and they doubt that crystallinity is the only major factor contributing to the structural transformation. Specific surface area variations observed during the initial period of hydrolysis in this report are large and the authors suggest that this may be a probable cause of the rate variation. This claim was supported by visual observation of small cellulose particle formation during hydrolysis. Formation of such particles may in part be due to a preferential attack pattern of the cellulase enzymes on the cellulose crystallites (Betrabet & Paralikas, 1977), a phenomenon supported in part by recent observations of Lee *et al.* (1983). It is as yet unclear, however, if a true transformation of the cellulose structure from one form to another exists or if this is a manifestation of the two-phase model for cellulose hydrolysis.

The kinetics of starch depolymerization, as stated above, can be realized only with thorough knowledge of both the physical chemistry of the substrate as well as the action pattern of the enzyme employed. Here we must be concerned with both the bonding patterns of the starch and any associational complexes in which it is present. These phenomena may be dependent upon the source of substrate and/or its molecular size. To understand the kinetics of degradation with the biological catalyst we must know the type of enzyme and if it is in soluble or immobilized form. All things considered, modeling hydrolysis reactions is clearly more complicated for starch than for cellulose.

Wheetall & Havewala (1972) used corn starch substrate and hydrolyzed it with immobilized glucoamylase for the production of glucose. The authors modeled the reaction with Michaelis–Menton kinetics and their reactor system was a packed bed. They determined that immobilization did not appear to stabilize the enzyme thermally and only resulted in a slight shift in the pH optimum of the reaction. The Michaelis constant was lowered by immobilization, but they also stated that Michaelis–Menton kinetics was inadequate for modeling the observed changes even if the inhibitory effects of the product were

included in the model. They pointed out that little information was available at the time on the kinetics of macromolecular substrates and suggested the possibility that simple first order enzymatic decay might not be adequate for such systems.

Rosendal & Nielsen (1979) further studied the problem of enzymatic stability in such systems. Specifically, they examined the stability of bacterial α-amylase in starch liquefaction using conventional (Somogyi, 1955) reducing sugar measurements (DE). Their model employed a lumped differential equation and first order enzymatic decay as shown in eqns (10) and (11).

$$\frac{\mathrm{d}}{\mathrm{d}t}(\mathrm{DE}) = aC_t f(\mathrm{DE}) \tag{10}$$

$$C_t = C_0 \exp\{-bt\} \tag{11}$$

where a = a factor dependent on pH, temperature and enzyme dosage; C_0 = initial enzyme dosage; b = decay constant ($= \ln 2/t_{1/2}$); $f(\mathrm{DE})$ = a correction for non-linearity ≈ 1 for $\mathrm{DE} < 12$ which decreases to $\approx 0{\cdot}5$ at $\mathrm{DE} = 22$. The half-life, $t_{1/2}$, of the enzyme was shown to follow Arrhenius dependency and it was concluded that first order thermal enzymatic decay was appropriate for such systems.

The question of the effects due to variation in starch bonding type (α-1,6- and α-1,4-glucosidic) linkages was addressed by Roels & van Tilbury (1979). These authors used a system of moderately low DE (= 15) starch substrates hydrolyzed by the amyloglucosidase of *A. niger*. This enzyme is an exo-acting enzyme which splits glucose off from the non-reducing end of the polymer. In their model, all reactions were developed from simple mass action laws; the reactions were also permitted to be reversible. Due to the mode of action of the enzyme, they assumed that kinetic rate constants and equilibrium constants were independent of the substrate's degree of polymerization. Their differential kinetic and equilibrium model for the interconversion of glucose, maltose and isomaltose explicitly neglected formation of oligosaccharides larger than the dimers. For the higher molecular weight substrate studied, the basic results of the model developed for the case of the monomer and dimers were incorporated, here though lumping all carbohydrate substrates as either α-1,6 or α-1,4 bonds. In this manner, differential equations were written for the rate of change of time for each of these bond types. The basic assumptions used in the develop-

ment and analysis are: (1) any α-1,4 or α-1,6 bonds at the non-reducing end of the molecule have the same probability of being attacked by the enzyme, but the rate of reaction differs for each bond type; and (2) estimates as to the total number of non-reducing ends within the reactor at a given time could be estimated from material balance and knowledge of the average DE at that time. The principal contribution of this model was its ability to simulate the reversibility of the reaction at high levels (DE approximately 90) of conversion. An appropriate model for low levels of conversion was not attempted.

A model for exo-acting glucoamylase on soluble starches was published by Kusunoki *et al.* (1982). These authors employed modified Michaelis–Menton kinetics with product inhibitory effects. They established that both V_{max} and K_M vary linearly with substrate molecular weight. Under the conditions used, only two coupled non-linear differential equations were required to be solved simultaneously: one for glucose production and a second for the starch substrate. These are shown in eqns (12) and (13).

$$\frac{d[G]}{d\theta} = \frac{V'_M [S_0]}{[S_0] + K'_M (1 + [G]/K'_i)} \tag{12}$$

where $[S_0]$ = initial molar starch concentration, $[G]$ = molar glucose concentration, V'_M = maximum velocity of reaction based on mole concentration, K'_M = Michaelis constant based on mole concentration, K'_i = inhibitor constant based on mole concentration and θ = time.

$$-\frac{dS}{d\theta} = \frac{V_M S/1{\cdot}1}{S + K_M (1 + G/K_i)} \tag{13}$$

where S = mass concentration of starch, V_M = maximum velocity of reaction based on mass concentration, K_i = inhibitor constant based on mass concentration, G = mass glucose concentration. They determined that both K_M and K_i decrease with molecular weight where as the forward rate constant for the depolymerization reaction increase with molecular weight. Good agreement between their simulation and experimental results were obtained.

Subramainan (1980) proposed a power law rate expression of the form

$$\text{Rate} = \frac{K_1 E^n S^n}{K_2 + S^n} \tag{14}$$

where K_1, K_2 = constants at a given temperature, S = substrate molar concentration, E = enzyme molar concentration. They used the data of Mudhgankar *et al.* (1977) to determine the proposed exponential factors. Their analysis found a power dependency of approximately 1·5 for enzyme concentration and approximately 0·2 for the substrate. No significance was given to these values.

The kinetics of mixed endo- and exo-acting enzyme systems has been presented by Fujii *et al.* (1981). Their system consisted of soluble starch substrate hydrolyzed with endo-acting α-amylase from *B. subtilis* and commercial glucoamylase. They assumed that the rate of reaction of both enzymes toward each substrate molecule are the same when the substrate molecules are sufficiently large and that the endo-acting enzyme produces substrate molecules primarily for the exo-acting enzyme. In this manner, a synergistic effect (similar to that of cellulose–cellulase) will be exhibited under appropriate conditions. In both cases, Michaelis–Menton kinetics were assumed to represent the action of the enzymes. In the case of α-amylase kinetics, linear Linewever–Burke plots resulted when the substrate concentration (here both linear and branched substrates of various molecular weights) was represented on a mass scale. This indicates that the bond dependency of the reaction rate is more important than the number of polymeric substrate molecules for the action of the endo-enzyme. The data presented for the exo-acting enzyme show that the opposite is the case: that is to say, that here the molecular population and not the bond population is the guiding parameter as no molecular weight dependency of the substrate is observed. An interesting experiment for a similar two enzyme system (fungal α-amylase and glucoamylase), was reported on by Hausser *et al.* (1983). These authors employed separate immobilized membrane reactors for each enzyme fed initially with starch syrup with DE of 42 in series and demonstrated that this reactor geometry could be employed to alter the levels of the monosaccharide and disaccharide produced by altering the operational parameters (primarily temperature and flow rate). These data were not used to advance a kinetic model however.

The most complete model of starch hydrolysis published to date has been presented by Marc *et al.* (1983). These authors proposed a model which incorporated the main physical and chemical phenomena that occur during mashing operations. Physical factors included the dissolution of both substrates and enzymes as well as the gelatinization rate for the starch substrate. The kinetic equations employed assumed first

order rate of reaction for both soluble and insoluble starch with α-amylase, but different kinetic constants for the production of dextrins and maltotriose. Similarly, first order kinetics was found to be applicable for β-amylase hydrolysis of dextrins to glucose, maltotriose and limit dextrins as well as first order enzymatic thermal deactivation. The Michaelis–Menton kinetics of β-amylase hydrolysis with dextrins to produce maltose was required. All kinetic constants were assumed to follow Arrhenius expressions. A solution of 18 simultaneous differential equations was required with various parametric values. The reader is referred to the original work for a complete description. Good agreement was found between the simulations and experimentation even for studies involving thermal programming operations.

Rollings *et al.* (1983*b*) examined the MWD of corn starch hydrolyzed with commercial α-amylase and determined that the product distributions are not random but, rather, collected into four distinct molecular weight groups. At least in one case, the material corresponded to insoluble starch that is of the same size as the material observed during amylose retrogradation (Burchard, 1963; Kodama *et al.*, 1978). Based on this observation, Rollings & Thompson (1984) proposed a simple five parameter model using a first order reaction rate expression for each of the four molecular size groups and the thermal enzymatic deactivation. Reasonable agreement was found here. The observation of these resistant intermediate products is not accounted for explicitly in any of the other models presented above. Parallel to the cellulosic studies (Lee & Fan, 1983) these authors also reported polarized light microscopic observation of small starch particles which exhibited birefringence. The earlier MWD models (Henrikshas & Bruun, 1978; Henrikshas & Lovgren, 1978) do not account for such possibilities.

CONCLUSION

It is evident that considerable progress has been made recently in understanding the fundamental processes which govern enzymatic polysaccharide depolymerization reactions, although our knowledge is as yet not complete. Clearly, we must possess detailed information on the enzymatic action patterns specifically in terms of its multiplicity of attack, as it is the nature of these catalysts to perform highly specific kinetic functions. We should not, however, attribute all experimental

observation to properties solely associated with the enzyme. This latter statement results as numerous physicochemical properties of the biopolymeric substrate also direct the dynamics of degradation. Specifically, when examining crystalline polysaccharides, we must be cognizant of contributions pertaining to the substrate's crystallinity (e.g. size, type, amount, etc.) as well as physical mass transfer and surface area effects. In describing depolymerization of easily solubilized materials, such as dextrans and ion-containing polysaccharides, knowledge of the substrate's molecular size, branching patterns, solution conformations and configurations, along with complications associated with the presence of charged moieties and solubility limitations must be considered. Finally, gel-forming substrates, such as starches, may pose the greatest challenge. Here concentration effects, the mechanism and kinetics of gel formation, multimerization processes as well as the manner and degree of branching within the substrate must be thoroughly understood in order that fundamental descriptions (in either the physical or mathematical sense) be advanced. In part, these phenomena have been incorporated into various published kinetic model descriptions. No doubt, further studies will be presented in the near future as this area is still being actively explored. As can be anticipated, these studies will lead to more useful models which will find applications both academically and commercially and assist our understanding and utility of many other biopolymeric systems.

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