BIOCHEMICAL LETTERS

Enzyme kinetics of cellulose hydrolysis

The recent paper by Woodward *et al.* (1988) about synergism in cellulose hydrolysis highlights the fact that there is usually no suitable framework in which to discuss the enzyme kinetics of cellulase. Their data imply that maximum hydrolysis rates occur at enzyme concentrations ($\text{mg} \cdot \text{ml}^{-1}$) which are close to the conventional. Cellulases turn over so slowly that workers do not always choose to digest cellulose at infinitely small enzyme concentrations; in many experiments it cannot be assumed that Michaelis–Menten kinetics are applicable. An alternative approach is proposed below.

The measurement of reaction velocity as a function of substrate concentration is a very artificial convention when reaction takes place on a hydrated solid within which it is impossible to change the concentration of substrate sites. It seems more suitable to establish the rate of cellulose hydrolysis explicitly as a function of the other variable, enzyme concentration. In principle the rate of hydrolysis will increase with E_0 , the total enzyme concentration, and it must saturate. Accordingly it is convenient to define a maximal velocity, V'_{max} , and a corresponding, arbitrary, half-saturation constant, K . Proceeding by analogy:

$$v = V'_{\text{max}} E_0 / (K + E_0) \quad (1)$$

This equation is an exact steady-state solution if E_0 is in excess over substrate. As velocity decreases below V'_{max} , it becomes more and more approximate. The point may be tested by examining $1/v$ versus $1/E_0$ plots for deviations (concave upwards) from linearity. Cellulose is a heterogeneous substrate and it may be that the approximation involved in the use of eqn. (1) is especially acceptable because of the special imprecision of initial rate measurements of this substrate. In some cases, a more complicated equation may be needed to derive V'_{max} and K .

The preferable solution is to use the equation:

$$v = V'_{\text{max}} E / (K_m + E) \quad (2)$$

where E is the concentration of free enzyme. The equation can also be expressed in the alternative form:

$$v = k_{\text{cat}} S_0 E / (K_m + E) \quad (3)$$

The two equations are exact steady-state solutions and do not rely on assumptions about the relative molar quantities of enzyme and substrate. They are not usually used because of the difficulty of measuring the concentration of free enzyme. However, cellulose hydrolysis is an ideal application because it is extremely simple to separate bound and free enzyme (by centrifugation or even by sedimentation under gravity) and because it can be arranged that the time required for separation is extremely small in comparison to the lifetime of the enzyme–substrate complexes and to the usual sampling times of cellulase assays.

Enzyme kinetics studied by this approach would use double-reciprocal plots of $1/v$ versus $1/E$ (or $1/E_0$) and this data presentation is of the type required in studies of synergism now that Woodward *et al.* (1988) have demonstrated the importance of enzyme concentration. They have demonstrated a synergistic effect which disappears at high enzyme concentration, i.e. a ‘competitive’ synergism. The analogy, of effects between enzymes on $1/v$ versus $1/E$ plots and effects between substrates/inhibitors on $1/v$ versus $1/S$ plots, may be a fruitful source of hypotheses.

The parameters K_m and V'_{max} from eqns. (1)–(3) have some intrinsic interest. They are not interchangeable with K_m and V_{max} from the usual Michaelis–Menten equation only because cellulose concentrations cannot be given in molecular units. K_m from eqn. (2) is in enzyme concentration units, molecular or specific. V'_{max} is the rate of substrate turnover at saturation with enzyme. The specific activity of substrate turnover, V'_{max}/S_0 , is the maximum rate of substrate depletion, readily presentable in units of percentage weight $\cdot$ time $^{-1}$. For cellulases, it is not interchangeable with the specific activity of enzyme turnover. V'_{max}/S_0 is of crucial concern if the aim is to find rapid processes to convert cellulose to sugars.

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Specificity of ribosome-inactivating proteins with RNA *N*-glycosidase activity

A number of widely distributed plant proteins, provisionally identified as ribosome-inactivating proteins (RIPs), exist either as single-chain proteins (RIPs type 1) or, less frequently, as A-chains linked to a B-chain with lectin properties (RIPs type 2). The latter are potent toxins, the best known of which is ricin [1]. All RIPs tested, of either type, possess a unique RNA *N*-glycosidase activity and cleave the glycosidic bond of an adenine residue of 28 S rRNA [2–5], specifically adenine-4324 of rat liver 28 S rRNA. This base is in a highly conserved sequence in rRNA from a wide range of organisms, from bacteria to mammals [6]. However, RIPs do not act on bacterial ribosomes, and ricin A-chain (and presumably other RIPs as well) is much less active on purified rat liver 28 S rRNA than on whole rat liver ribosomes, and is not active at all on denatured 28 S rRNA. This led Endo *et al.* [2] to suggest that integrity of the secondary structure of rRNA is important for the action of RIPs and that ribosomal proteins must have an important role in maintaining the secondary structure of rRNA.

Table 1. Effect of ribosome-inactivating proteins on ribosomes from various plant and protozoan species

IC₅₀ (nM) values are given, representing the concentration of the various RIPs causing 50% inhibition of poly(U)-directed phenylalanine polymerization. The IC₅₀ values for rat liver ribosomes range from 6 nM (for abrin) to 23 nM (for dianthin). See [7] and [9]. The origin of the RIPs is as follows: dianthin, *Dianthus caryophyllus* (family Caryophyllaceae); saporin, *Saponaria officinalis* (family Caryophyllaceae); PAP-S, *Phytolacca americana* (family Phytolaccaceae, closely related to Caryophyllaceae); momordin, *Momordia charantia* (family Cucurbitaceae); gelonin, *Gelonium multiflorus* (family Euphorbiaceae); ricin, *Ricinus communis* (family Euphorbiaceae); abrin, *Abrus precatorius* (family Leguminosae).

Source of ribosomes	RIPs type 1 (single chain)					RIPs type 2 (two-chain, toxins)	
	Dianthin 32	Saporin 6	PAP-S	Gelonin	Momordin	Ricin	Abrin
Plants							
<i>Dianthus caryophyllus</i> leaves	Inactive	—	3330	Inactive	1365	Inactive	Inactive
<i>Phytolacca americana</i> leaves	3330	—	Inactive	Inactive	3330	Inactive	Inactive
<i>Nicotiana tabacum</i> leaves	133	—	83	Inactive	3330	Inactive	448
<i>Triticum aestivum</i> germ	33	53	18	3330	1265	Inactive	2108
<i>Daucus carota</i>							
Leaves	450	—	2131	Inactive	3330	Inactive	1660
Cells	10	—	50	3330	782	—	1245
<i>Ricinus communis</i> leaves	260	—	1232	Inactive	Inactive	Inactive	2656
<i>Bryonia dioica</i> leaves	—	8	12	820	3330	—	—
Protozoa							
<i>Tetrahymena pyriformis</i>	30	2630	1570	3330	300	1700	1700
<i>Acanthamoeba castellanii</i>	7	17	1230	3330	1130	1030	100
<i>Leishmania infantum</i>	27	33	21	2590	3330	1700	5
<i>Trypanosoma brucei</i>	66	116	17	3330	3300	1700	49

In Table 1 we point out further evidence for the remarkable specificities of RIPs. Not surprisingly, RIPs are inactive on ribosomes from their own plant species but it is very striking that ribosomes from a particular plant show a large range of sensitivities to different RIPs [7], e.g. at least 400-fold for *Bryonia dioica* (Table 1). Ribosomes from four protozoan species also respond in a different manner to several RIPs examined [8,9] and it appears that ribosomes from *Leishmania* and *Trypanosoma*, which belong to the same order (Kinetoplastida), are more similar to each other in their sensitivity to RIPs than to ribosomes from *Tetrahymena* and *Acanthamoeba*, which belong to other orders (Table 1). By the same token, RIPs obtained from taxonomically related plants are more similar to each other than to RIPs from distantly-related species (Table 1).

These data indicate that a particular spectrum of specificities to various RIPs could be a species-related property of eukaryotic ribosomes and that amongst rRNAs from different organisms there are conformational differences ('epitopes'), maintained by ribosomal proteins, that can be recognized by some but not all RIPs. Recently, direct evidence for toxin-induced conformation changes, involving proteins either at the A-site or P-site of the ribosome, has been obtained and a role in the action of ricin and α -sarcin on 28 S rRNA proposed [10].

The observations summarized above could have clinical implications: a specificity of RIP action according to the origin of ribosomes could offer a chance of therapy by specific action of a selected RIP on an invading organism. Moreover, if differences in the sensitivity to

RIPs also exist among ribosomes from mammalian cells, including for example malignant cells, then in the search for new RIPs some toxins of potential therapeutic value could have been missed if the material tested contained a RIP inactive on the ribosome used in the assay.

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