INTERPRETATION OF DEPENDENCY OF RATE PARAMETERS ON THE DEGREE OF POLYMERIZATION OF SUBSTRATE IN ENZYME-CATALYZED REACTIONS. EVALUATION OF SUBSITE AFFINITIES OF EXO-ENZYME

Keitaro Hiromi

Department of Chemistry, Faculty of Science,
Kyoto University, Kyoto, Japan

Received May 7, 1970

SUMMARY: The dependency of maximal velocity and Michaelis constant of polymer-degrading enzymes on the degree of polymerization (DP) was interpreted in terms of the subsite affinities by assuming that the intrinsic rate of hydrolysis in the productive complex is independent of DP. The apparent dependency of maximal velocity on DP arises merely from the probability of productive complex formation, which is determined by the arrangement of subsite affinities in the active site of the enzyme. The theory was applied to exo-enzyme, and the method for determining the subsite affinities from the rate parameters was proposed.

In hydrolase-catalyzed degradation of polymer substrates, it has often been observed that the rate parameters, the Michaelis constant (K_m) and the maximal velocity (V), are intimately related to the degree of polymerization (DP) of substrates. Thus in amylase- and lysozyme-catalyzed hydrolyses of oligosaccharides (1-4), the increase in DP of substrate results in the increase in V as well as the affinity for binding, which is reflected in the decrease in K_m. The increase in affinity with DP may well be expected in terms of increased number of interactions between the enzyme active site and the substrate residues. However, the increase in V with DP is not easy to understand, since it seems unlikely that the actual rate of hydrolysis of a particular linkage in ES complex is affected by the substrate residues considerably remote from the linkage to be hydrolyzed. In this paper, an interpretation of the effect of DP on V will be made in terms of the probability of formation of productive complex, assuming a priori that the intrinsic rate of hydrolysis in the productive complex is actually independent of DP of substrate. Based on this hypothesis, K_m and

and V are simply correlated with the subsite affinities of the enzyme active site for substrate residues. Hydrolysis of homopolymer substrates catalyzed by exo-enzyme (e.g., glucoamylase-catalyzed hydrolysis of linear oligosaccharides (1)) will be the first subject of application of the theory, because of the simplicity that there is only one productive complex for a given substrate.

THEORETICAL

Basic assumptions employed for the treatment of exo-enzyme catalyzed hydrolysis of homopolymer substrates are as follows:

1) The active site (including the binding and catalytic sites) of an exo-enzyme involves m subsites each of which has its own subsite affinity A_i for a residue of substrate molecule. The subscript i refers to the number of subsite counting from the terminal at which the catalytic site is situated. The model is schematically illustrated in Fig. 1.

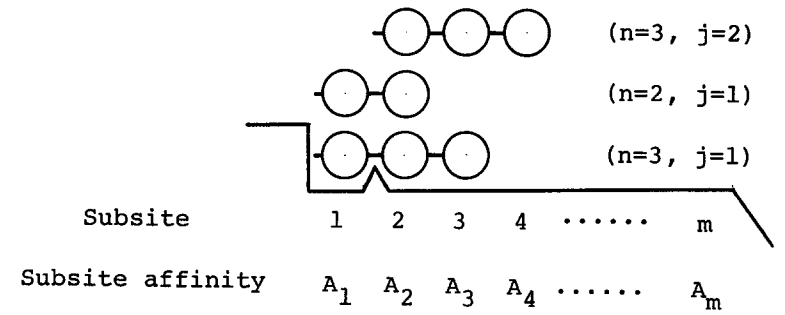

Fig. 1. Schematic model for the active site of an exo-enzyme and for various modes of binding of linear substrates. The circle designates a residue (monomer unit) of substrate, and the wedge represents the catalytic site. The subsites are numbered as indicated (i=1,2,...,m). A_i refers to the subsite affinity of the i-th subsite. The mode of binding is specified by j, which is equal to the number of subsite at which the particular terminal residue is situated. n represents the degree of polymerization (DP) of substrate.

---

2) A substrate molecule can be bound in a variety of mode of binding (designated by j) to form one productive and several nonproductive complexes. The number of binding mode, j, is taken to be equal to the number of subsite at which the particular terminal residue from which the exo-enzyme attacks the substrate

is situated. Thus the binding mode j=1 yields a productive complex, and j=2,3,...,m the nonproductive ones.

3) The molecular binding affinity of n-mer substrate (DP = n) in the j-th binding mode, designated by $B_{n,j}$, is assumed to be equal to the sum of the subsite affinities of the subsites which are covered by the substrate in this mode of binding. For the productive complex (j=1), which covers the catalytic site, we assume that there is strain free energy (5), designated by D (positive in sign), which is to be subtracted from the sum of the subsite affinities. $B_{n,j}$ is directly related to the standard free energy change for this binding process, $\Delta G^{\circ}_{n,j}$, corrected for the change in free energy of mixing, ΔG_{mix}, which is common for a bimolecular association process (6). Therefore, we have:

$$B_{n,j} = -\Delta G^{\circ}_{n,j} + \Delta G_{mix} = \sum_{i}^{cov.} A_i - D\delta(j-1) \qquad (1)$$

where $\sum_{i}^{cov.}$ indicates that the sum is taken only for the covered subsites in the j-th binding mode, and $\delta(j-1)$ is equal to unity for j=1 and equal to zero for $j \neq 1$.

4) The intrinsic rate constant, k_{int}, for the decomposition of the productive ES complex into products is assumed to be independent of DP (= n) of substrate.

It is readily shown (7) that K_m and V for an enzyme-catalyzed reaction in which multiple binding modes are involved with only one productive complex are represented by:

$$1/K_m = \sum_{j} K_{n,j} \qquad (2)$$

$$V = V_{int} \times K_{n,1} / \sum_{j} K_{n,j} \qquad (3)$$

where $K_{n,j}$ represents the association constant of n-mer substrate to form an ES complex in the j-th binding mode, ES_j;

$$K_{n,j} = (ES_j)/(E)(S)$$

hence $K_{n,1}$ is the association constant of the productive complex, and $V_{int} = k_{int}(E)_o$.

From Eqs. 2 and 3, we have a useful relationship;

$$V/K_m = V_{int} \times K_{n,1} \qquad (4)$$

which involves the parameters for productive complex only.

Since $K_{n,j} = \exp(-\Delta G^{\circ}_{n,j}/RT)$, from Eq. 1 we have:

$$K_{n,j} = \exp(-\Delta G_{mix}/RT) \times \exp\left[\sum_{i}^{cov.} A_i - D\delta(j-1)/RT\right] \qquad (5)$$

where R is the gas constant, T the absolute temperature, and ΔG_{mix} is equal to 2.4 kcal/mole at 25°C for a bimolecular association process in aqueous solution (6).

By the use of Eqs. 2-5, we can calculate the experimentally obtainable rate parameters, K_m and V, if the individual values of A_i and D are known. Conversely, the subsite affinities, A_i's may be evaluated from the rate parameters for substrates of various DP. For this purpose, it is convenient to use Eq. 4. Since we have assumed that V_{int} is independent of DP, V/K_m is directly proportional to the association constant of productive complex, $K_{n,l}$, which is in turn proportional to $\exp(B_{n,l}/RT)$, as

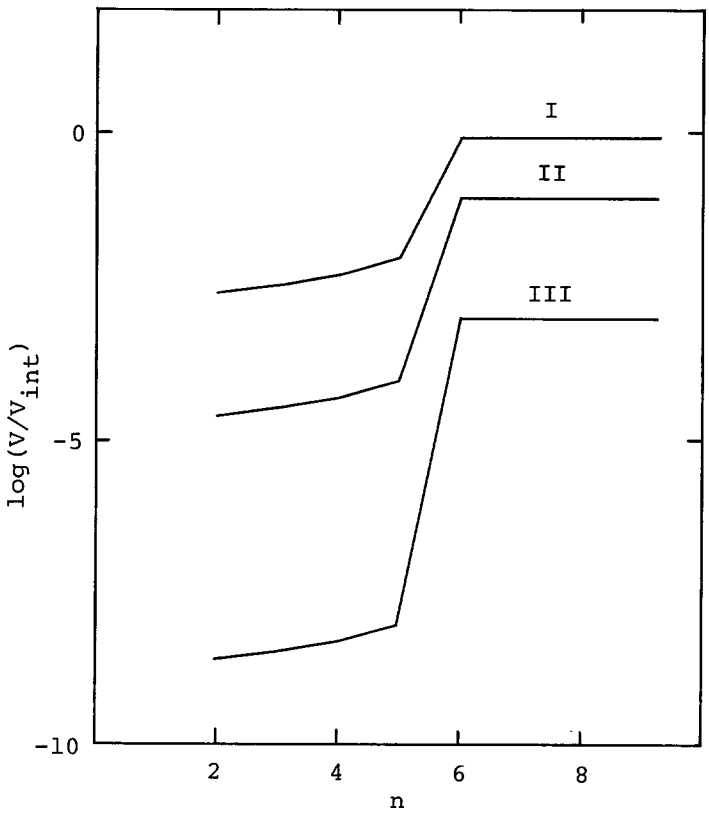

Fig. 2. Plots of log V versus n calculated for an exo-enzyme catalyzed hydrolysis of n-mer substrates. The subsite affinity A_i was assumed to be equal for all the subsites. The number of subsites m was taken to be 6.

Curve I : A_i = 4.1 kcal/mole, D = -2.7 kcal/mole.
Curve II : A_i = 4.1 kcal/mole, D = -5.4 kcal/mole.
Curve III: A_i = 6.8 kcal/mole, D =-10.9 kcal/mole.

seen from Eq. 4. Therefore, from the ratio of V/K_m values for n-mer and (n-1)-mer substrates ($n \leq m$), we can evaluate the n-th subsite affinity, A_n, as would be understood from Fig. 1.

$$(V/K_m)_n/(V/K_m)_{n-1} = \exp\left[(B_{n,1} - B_{n-1,1})/RT\right] = \exp(A_n/RT) \quad (6)$$

In this way, we can determine A_i's for i=3,4,...,m with the substrates of DP=2,3,...,m. The evaluation of the individual values of A_1, A_2 and D, however, is difficult in general, although it may be possible by further assumptions. A detailed treatment of this problem will be made elsewhere.

RESULTS AND DISCUSSION

An example of calculation of V for an exo-enzyme catalyzed

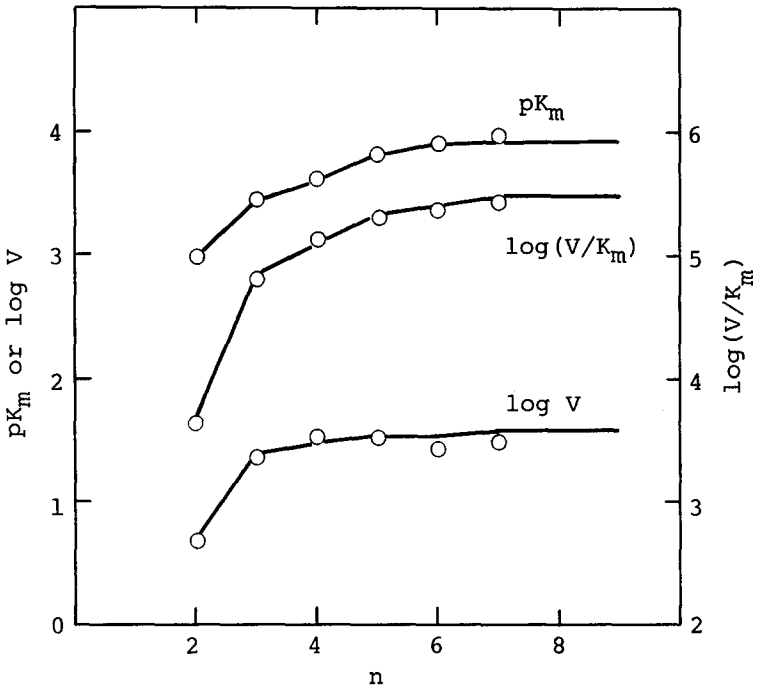

Fig. 3. Dependency of rate parameters on DP of substrate for glucoamylase catalyzed hydrolysis of linear oligosaccharides. The open circles indicate the experimental values obtained at 25°C and pH 4.5 (8). The solid lines are the theoretical curves calculated from Eqs. 2-5 by using the following values for subsite affinities:

(A_1-D)	A_2	A_3	A_4	A_5	A_6	A_7	
0	4.85	1.59	0.43	0.22	0.11	0.10	(kcal/mole)

V_{int} = 77 sec^{-1} for unit molar concentration of enzyme.

hydrolysis of n-mer substrates by using the assumed values of A_i, D and V_{int} is shown in Fig. 2, in which all A_i values are taken to be equal irrespective of i. The experimental values of rate parameters obtained for glucoamylase-catalyzed hydrolysis of linear substrates (malto-oligosaccharides) are shown by the open circles in Fig. 3 (8). From the comparison between Fig. 2 and log V curve in Fig. 3, it is obvious that A_i should not be equal but should be greatest near the catalytic site and decrease with the distance from the catalytic site, to be consistent with the experimental results for glucoamylase. The solid lines in Fig. 3 are the theoretical curves calculated by using the A_i values shown in the legend, which were chosen to fit the experimental rate parameters. A_i's for $i > 2$ were obtained by using Eq. 6. The successful agreement between the theoretical curves and the experimental points suggests the validity of the basic assumptions employed. Thus the apparent dependency of V on DP can be accounted for solely in terms of the probability of productive complex formation, even though the intrinsic rate of hydrolysis, k_{int}, is independent of DP.

The treatment along this line may be extended to endo-amylases (2,3), for which more than one productive complexes are involved.

ACKNOWLEDGMENTS

The author wishes to acknowledge with gratitude Professor S. Ono of the University of Osaka Prefecture and Professor H. Hatano of Kyoto University for their active interest and continuing encouragement throughout this work.

REFERENCES

1. S. Ono, K. Hiromi and M. Zinbo, J. Biochem. (Tokyo), 55, 315 (1964).
2. Y. Nitta, M. Mizushima, K. Hiromi and S. Ono, submitted to J. Biochem. (Tokyo).
3. S. Iwasa, Thesis for Master Degree at Kyoto University (1970).
4. J. A. Rupley and V. Gates, Proc. Natl. Acad. Sci., 57, 496 (1967).
5. C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips and V. R. Sarma, Proc. Roy. Soc., Series B, 167, 378 (1967).
6. D. M. Chipman and N. Sharon, Science, 165, 454 (1969).
7. G. E. Hein and C. Niemann, J. Am. Chem. Soc., 84, 4495 (1962).
8. K. Hiromi, C. Numata, Y. Nitta and S. Ono, in preparation.