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Reaction kinetics of some important site-specific endonucleases

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ABSTRACT

Reaction kinetics of the site-specific endonucleases BamHI, BglII, ClaI, EcoRI, HpaII, PstI, SalI, SmaI, and XorII were investigated employing some frequently used substrates. Six of these enzymes could be analyzed under steady-state conditions. Kinetic data were obtained from progress curves applying an integrated Michaelis-Menten equation. K_M ranged from $4 \cdot 10^{-9}$ M to $4 \cdot 10^{-11}$ M. Activities also spanned two orders of magnitude. In the case of ClaI the analysis of the pre-steady-state kinetics ("burst reaction") allowed the assessment of several rate constants. The rate-limiting step is the very slow dissociation of the enzyme-product complex (0.22 min^{-1}). This complex is formed from the enzyme-bound nicked intermediate at a rate of 1.7 min^{-1}. The introduction of the first cut is again faster by a factor of about 6. SmaI and XorII resembled ClaI in their kinetics. The burst reaction can be used for the easy and unambiguous determination of molar concentrations of site-specific endonucleases in any preparation, which is free of non-specific DNases.

INTRODUCTION

The importance of site-specific endonucleases for recombinant DNA technology has stimulated a great effort in the search for such activities. Now more than 200 such enzymes have been described and many of them show identical specificities. The list compiled by R. Roberts [1] may be used as a reference to bacterial sources, nomenclature, and basic properties of these enzymes. In principle one can now choose the protein with optimal properties for any particular application. It is therefore highly desirable not only to characterize growth conditions and purification procedure, but also stability and activity of such enzymes. We are here concerned about the activity, i.e. kinetics of restriction endonucleases. In two preceding papers [2,3] we investigated the reaction kinetics and substrate recognition of BamHI in detail. These experiments were now extended to a number of similar enzymes, which are frequently used, in order to show the validity of the evaluation procedure (agarose gel technique) and the applicability of Michaelis-Menten-kinetics [4].

Aside from practical considerations site-specific endonucleases are interesting objects for the study of protein-DNA interactions. The specific cleavage of DNA is a process which is composed of a large number of different steps including i) non-specific association and dissociation in search for the specific site by some kind of enhanced diffusion, ii) two cleavage reactions, and iii) dissociation of the enzyme from the intermediate and from the final products. While these reactions proceed, there may occur independent alterations in the structure of the substrate like supercoil relaxation, which in turn influence the interaction with the enzyme.

Some restriction endonucleases like BamHI, EcoRI, and several enzymes from Haemophilus strains have already been characterized with respect to the features which are required for specific recognition [3,5,6].

However our knowledge of quantitative relations between these steps in the complete cleavage reaction is based on few experiments only. The known data for BamHI and EcoRI e.g. are not necessarily representative of 200 other endonucleases with recognition sites which vary in length, base composition etc. So rather different types of kinetics are to be expected. One such case is XorII, which has recently been described qualitatively in much detail [7]. We wish to present here a quantitative analysis of another such example, namely ClaI, along with data for some frequently used enzymes which follow simple BamHI-type kinetics.

MATERIALS AND METHODS

Enzymes: BamHI was prepared as described earlier [2]. BglII was isolated by P11-phosphocellulose-, DEAE-cellulose-. and hydroxyapatite-chromatography and showed no contamination by BglI. ClaI was purified by DEAE-cellulose-chromatography or obtained from Boehringer (Mannheim). EcoRI was purified similarly as in [8] using phosphocellulose-, hydroxyapatite-, and hexylsepharose-chromatography. HpaII was obtained from Biolabs (Beverly, Ma.). PstI was highly purified as reported previously [9]. SalI was isolated using P11-phosphocellulose-and DEAE-cellulose-chromatography. For the preparation of SmaI phosphocellulose-, DEAE-cellulose-, and again phosphocellulose-chromatography were employed. XorII was obtained from BRL (Neu-Isenburg). All of the enzymes were free of contaminating non-specific nucleases.

Substrates: pBR322, pBR325, pJC80, ColEI, and NTP14 DNA were prepared by the method of Goebel and Bonewald [10], λ-DNA by the method of Bøvre and Szybalski [11] and generously provided by H. Mayer. SV40 DNA was a gift from W. Fiers. DNA concentrations were determined photometrically assuming that

an absorbance of 1.0 at 260 nm and 1 cm lightpath corresponds to a concentration of 50 mg/l. Molecular weights were taken as $2.8 \cdot 10^6$ for pBR322 [12], $4.0 \cdot 10^6$ for pBR325 (Johannsen, W. and Mayer, F., unpublished electronmicroscopic data), $3.2 \cdot 10^6$ for pJC80 (Hohn, B. and Collins, J., unpublished data), $4.2 \cdot 10^6$ for ColEI [13], $10.7 \cdot 10^6$ for NTP14 [14], and $32 \cdot 10^6$ for λ [15]. Poly(dG)• poly (dC) was purchased from Boehringer (Mannheim). Nuclease-free bovine serum albumin was supplied by BRL (Neu-Isenburg) . All other reagents were of highest commercially available purity.

Assay: The enzymes were assayed by a continuous sampling procedure as published previously [3]. In brief, aliquots were withdrawn from an incubation mixture, applied on an agarose gel, and seperated by electrophoresis. Gels were photographed and negatives scanned on a photometer. Amounts of DNA in the bands were processed by use of an integrated Michaelis-Menten equation (1) in order to determine the apparent kinetic constants K_M and V. In some cases a discontinuous (endpoint) method based on the same mathematical and experimental approach [2] was used. One enzyme unit is generally defined as that amount which digests 10^{-12} moles DNA per minute at V and 37°C in the following assay systems: BamHI, 10 mM Tris/Cl (pH 7.3), 13 mM $MgCl_2$; BglII, 25 mM Tris/Cl (pH 8.0), 80 mM NaCl, 13 mM $MgCl_2$, 3.6 mM dithioerythritol; ClaI, 10 mM Tris/Cl (pH 7.9), 10 mM $MgCl_2$, 7 mM dithioerythritol, 100 µg/ml bovine serum albumin; EcoRI, 10 mM Tris/Cl (pH 7.5), 125 mM NaCl, 5 mM $MgCl_2$, HpaII, 10 mM Tris/Cl (pH 7.5), 10 mM $MgCl_2$, 2.5 mM dithioerythritol, 125 µg/ml bovine serum albumin; PstI, 10 mM Tris/Cl (pH 7.0), 23 mM $MgCl_2$; SalI, 10 mM Tris/Cl (pH 8.0), 100 mM NaCl, 20 mM $MgCl_2$; SmaI, 15 mM Tris/Cl (pH 9.0), 15 mM KCl, 6 mM $MgCl_2$; XorII, 5 mM Tris/Cl (pH 7.3), 13 mM $MgCl_2$, 5 mM β-mercaptoethanol.

RESULTS AND DISCUSSION

Steady-state kinetics

All of the enzymes which could be treated according to eqn. (1) are compiled in Tab. 1.

$$[P] / t = V - K_m \ln \left(\frac{[S_o]}{[S]}\right) / t \qquad (1)$$

where [P] represents product concentration, $[S_o]$ and [S] are substrate concentrations at time zero and t.

Examples of progress curves for two enzymes together with plots according

Table 1 Kinetic constants of various site-specific endonucleases

Enzyme	Substrate	K_M / nM	V /nM · min^{-1}	
Bam HI	pBR322	0.4	0.54	
	pBR 325	0.4	0.34	
	pJC80	0.3	0.30	
	NTP14	not determined	0.21	
	λ (a)	0.04	0.06	
	λ/EcoRI fragments (a)	0.09	0.09	
Bgl II	pJC80	3	0.97	
Eco RI	pJC80	1	3.3	125 mM NaCl
	ColEI	3	9.6	125 mM NaCl
	ColEI (b)	0.5	1.3	50 mM NaCl
HpaII	SV40	4.2	0.7	
Pst I	pJC80	0.3	0.2	
Sal I	pBR322	0.4	1.2	
	ColEI	1.4	2.7	

V values are made comparable and refer to 50-fold dilution of enzyme stock solutions in the assay mixture.

(a) kinetic constants for λ-DNA are based on concentration of BamHI sites; they have been determined by discontinuous incubation.

(b) values obtained by continuous and discontinuous method.

to eqn. (1) are given in Figs. 1-2. The ratio $\frac{[S_o]}{[S]}$ is calculated from total substrate concentration (as monomer) at time zero and from product concentration at time t. Corrections are made, if there are linear substrate molecules present at time zero. The kinetic constants in Tab. 1 are calculated from at least three such experiments. The statistical error is about 20 %.

Our data for EcoRI with ColEI as the substrate are very similar to published values [16]. The validity of the method is therefore confirmed. It should be noticed that the kinetic constants are very sensitive to NaCl concentration. Generally the influence of ionic strength on dissociation reactions is more pronounced than on associations [17]. Enhanced dissociation explains the increase of K_M as well as of V, because the rate-limiting step of the whole reaction sequence is the dissociation of the final enzyme-product complex [16]. The same effect can be seen with BglII: The reaction rate is reduced tenfold when the reaction conditions of BamHI (low salt) are applied (data not shown).

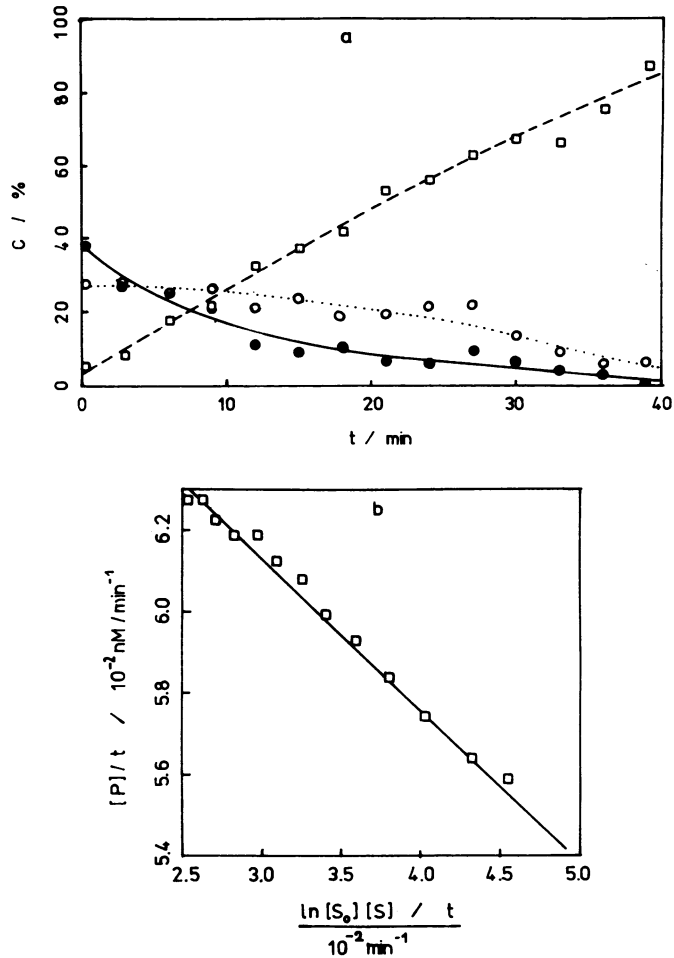

Fig. 1 Kinetics of PstI

a) relative concentrations of the conformers of pJC80 during the course of the reaction: superhelical (●), nicked circles (o), linears (□), difference to 100 % are oligomers (omitted for clarity). $[S_o]$ = 2.6 nM; [E] = 1/140 of stock solution.
b) Plot of data from the same experiment according to eqn. (1) for the determination of K_M (slope) and V (intercept).

The K_M-values of the six enzymes in Tab. 1 span one order of magnitude, if one disregards the data for λ-DNA. λ-DNA has five sites for BamHI and is thus not directly comparable to the other substrates with only one site. In the reaction with λ the enzyme may be trapped in the DNA coil and therefore finds

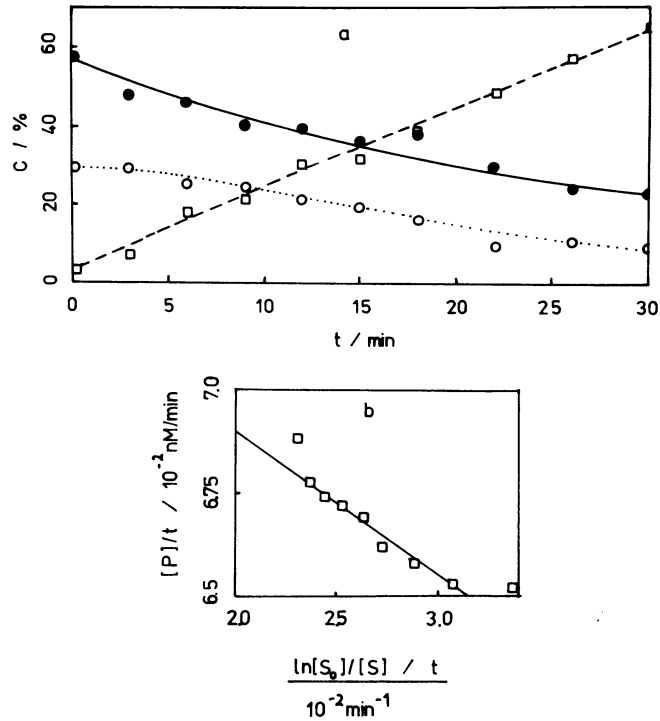

Fig. 2 Kinetics of SalI

a) relative concentrations of the conformers of pBR 322 during the course of the reaction: superhelical (●), nicked circles (o), linears (□), difference to 100 % are oligomers (omitted for clarity). $[S_o]$ = 3.1 nM; [E] = 1/667 of stock solution.
b) Plot of data from the same experiment according to eqn. (1) for the determination of K_M (slope) and V (intercept).

itself in an environment of apparently increased substrate concentration [3].

The similarity of K_M-values is not obvious, as the enzymes recognize sites of different length and base composition. The range of values for a single enzyme is comparable to the variation between different enzymes. It can therefore be concluded that all enzymes have comparable intrinsic rate constants of association and dissociation of their enzyme-substrate complexes. As already described for BamHI and EcoRI, the variation of K_M can be interpreted as a modulation of intrinsic constants by non-specific binding to the regions adjacent to the recognition sites [18,19].

The enzymes can further be compared by their efficiency in cleaving super-

coiled and relaxed substrates. In accordance with previous work by Ruben et al. [20] we observed an accumulation of nicked molecules only in the case of HpaII on SV40, which is caused by the dissociation of the nicked intermediate from the enzyme, before the second cut is introduced. In the case of EcoRI supercoils are preferentially attacked and nicked substrates do not accumulate. A similar behaviour is shown by PstI (Fig. 1). BglII and SalI on the other hand cleave both types of substrates with comparable efficiency (Fig. 2).

Burst reaction

The kinetics of ClaI are more difficult to analyze, as they do no fit eqn. (1). Progress curves show a biphasic behaviour (Fig. 3). After an initial burst of linear molecules a slow, relatively constant turnover is observed. To explain these findings we considered several possibilities.

First we made sure that the biphasic kinetics are not caused by a substrate heterogeneity as i) a variation of the ratio of enzyme to substrate leads to a varied extent of substrate consumption in the initial burst, and as ii) the same behaviour is seen with different substrates, namely ColEI and pJC80. We then tested, if the structural heterogeneity of the substrate could be responsible, but the progress curves had the same appearance, whether a mixture of supercoiled and relaxed molecules was used as normally or whether the DNA

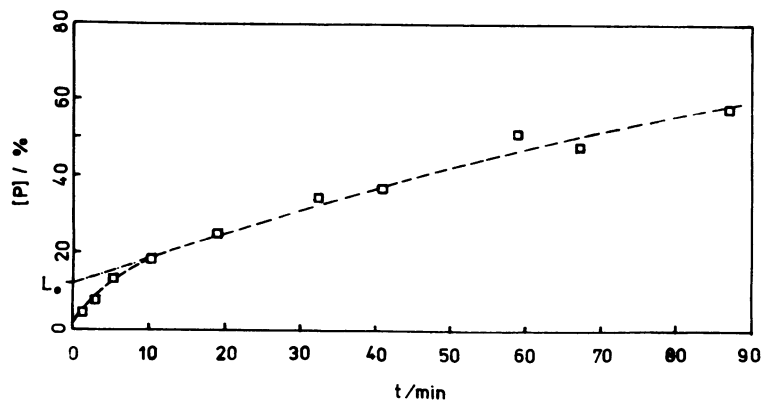

Fig. 3 Cleavage of linearized pJC80 by ClaI

pJC80 DNA was completely linearized by incubation with BamHI prior to the ClaI reaction.
Relative concentration of the large fragment during the course of the reaction (broken line). ClaI cuts pJC80 once.
$[S_o]$ = 12.5 nM; [E] = 0.62 nM. Extrapolation to an apparent initial concentration L_o of linear DNA is needed for the kinetic analysis (see text).

had previously been linearized with the endonuclease BamHI (Fig. 3). Next we considered product inhibition as the explanation for the unusual shape of the curves, but as the reaction rate remains constant after the initial burst, this possibility could be ruled out.

Since the burst was always complete after less than 15 minutes, it could also have been caused by an initial desactivation of the enzyme, because it is diluted into the reaction mixture or because the reaction mixture lacks an essential component. Several substances were tried to stabilize the enzyme. TritonX-100 at a concentration of 0.01 %, NaCl or KCl up to 100 mM, bovine serum albumin up to 0.5 g/l, ATP at 0.25 mM, β-mercaptoethanol instead of dithiothreitol, $MgCl_2$ up to 24 mM, and pH 7.0 instead of pH 7.9, only influenced the reaction rate of the second portion of the progress curve by less than 25 %, but could not eliminate the burst. A polynucleotide, poly(dG) · poly(dC), acted only inhibitory ($K_i \approx 40$ µM). The reaction rate was reduced to about one third, when mercaptoethanol or dithioerythritol were omitted. The enzyme appears to have a sensitive cystein necessary for activity; 1 mM $ZnCl_2$ completely inhibited ClaI. Reduced temperatures did also not eliminate the biphasic appearance.

The clearest evidence, that desactivation was not responsible for the unusual kinetics, was taken from the following experiment. A reaction mixture which was devoid of magnesium, but otherwise complete was incubated for up to 60 min before starting the reaction by the addition of $MgCl_2$. The biphasic shape was still observed, although there had been an appreciable loss of activity. But this was only displayed in a reduced reaction rate during the linear portion of the curve. Later we found, that V was reduced by about 50 % and K_M was not affected (method of determination see below). The biphasic behaviour was not unique to the enzyme prepared in our laboratory, but was also observed with a commercial ClaI preparation.

After these experiments we were left with the possibility that high ratios of enzyme to substrate are responsible for the burst, although we were not able to prove this directly by diluting the enzyme. The burst could thus be explained by the rapid initial reactions: formation of the enzyme-substrate complex ES (eqn. (2), step I),

(2) $$E + S \overset{I}{\rightleftharpoons} ES \overset{II}{\rightleftharpoons} EN \overset{IV}{\rightleftharpoons} EP \overset{V}{\rightleftharpoons} E + P$$
$$EN \overset{III}{\rightleftharpoons} E + N$$

subsequent cleavage reactions leading to the build-up of enzyme-product com-

plex EP via the nicked intermediate N. Finally the dissociation of the enzyme from the product (eqn. (2), step V) becomes rate-controlling, because it is the slowest reaction, leading to a fairly constant production of linear molecules. With this interpretation it is expected that the concentration difference of the burst is proportional to the enzyme concentration. When extrapolating the constant part of the reaction back to the concentration axis (Fig. 3) the difference between this value L_0 and the real initial concentration of linear molecules was indeed found to be proportional to the concentration of the enzyme in the assay. At 100fold dilution of the enzyme in the final assay mixture we found [E] = 0.31 ± 0.08 nM. This value of enzyme concentration is based on 20 experiments employing two different substrates, namely ColEI and pJC 80. As the value of [E] was, in certain limits (v.s.), independent of salt concentration, pH, and $MgCl_2$ concentration, putative different aggregated forms of ClaI do not influence the activity in a fairly broad range of reaction conditions.

The interpretation of the burst as pre-steady-state kinetics is further evidenced by a sequential incubation (Fig. 4). In the first part a reaction is allowed to proceed to about 95 % completion. Then new substrate is added (arrow). Turnover resumes without an initial burst, because the enyzme is

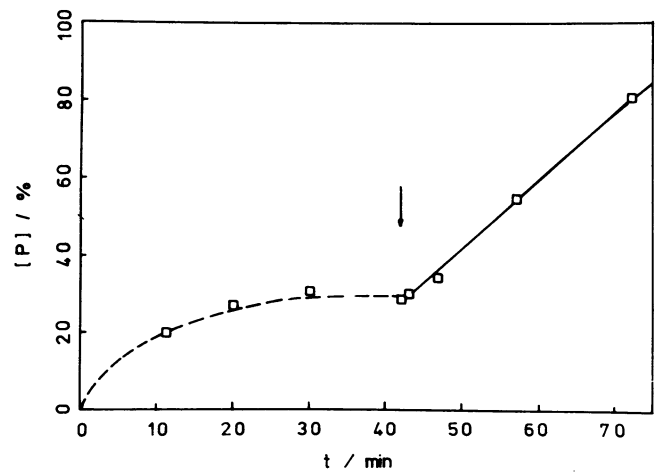

Fig. 4 Sequential cleavage with ClaI

Relative concentration of linear pJC80 DNA (reaction product) during the course of the reaction. The reaction was started at $[S_o]$ = 3.7 nM and [E] = 2.4 nM. After 40 min about 95 % of the substrate was cleaved and another 6.6 nM pJC80 was added (arrow).

still bound to the reaction products of the first round of incubation. The reaction indeed starts again at the rate of the rate-limiting step, which can be calculated using the parameters from Tab. 2. Interestingly there is no loss of activity compared to the first part of the reaction, which clearly rules out desactivation as a reason for the first burst.

In order to determine Michaelis parameters one would have to work at sufficiently low [E]/[S]-values. For ClaI this is impossible as the reaction becomes so slow (several hours reaction time) that non-specific nicking and desactivation of the enzyme would interfer with the analysis. But another method can lead to an acceptable approximation. We take L_0 as the origin of the steady-state portion of a progress curve and analyze this curve according to eqn. (1). An example is given in Fig. 5. In Tab. 2 we have compiled some parameters for the enzyme ClaI, which were thus calculated. Their statistical error is greater than for the values in Tab. 1, because the exact origin of the progress curve is unknown [21].

The values from Tab. 2 and the enzyme concentration as determined from the burst difference can now be used to calculate the catalytic rate constant k_5 according to eqn. (3)

(3) $$V = k_5 \cdot [E] \quad .$$

It is found to be 0.22 ± 0.08 min^{-1} for pJC80. This value is one order of magnitude lower than the values for BamHI and EcoRI [3,16]. The other rate constants (eqn. (2)) can be estimated from logarithmic plots of the curves given in Fig. 6. From this experiment it can be concluded that supercoiled molecules are better substrates than relaxed ones and that a steady-state is reached after approximately 3 minutes. The determination of Michaelis parameters from this part of the reaction is therefore justified.

From Fig. 7 a rough estimate of the rate constants k_2 and k_4 can be obtained. In Fig. 7 the total burst difference for each DNA species was taken as 100% and the decrease (supercoils) or increase (relaxed circles, linears) was read from Fig. 6 and plotted logarithmically. k_2 was about 10 min^{-1} and

Table 2 Kinetic constants of ClaI

Substrate	K_M/nM	V/nM • min^{-1}
ColEI	1	0.03
pJC80	2.0	0.13
linearized pJC80	2.4	0.28

V-values at 50fold dilution corresponding to 0.62 nM ClaI

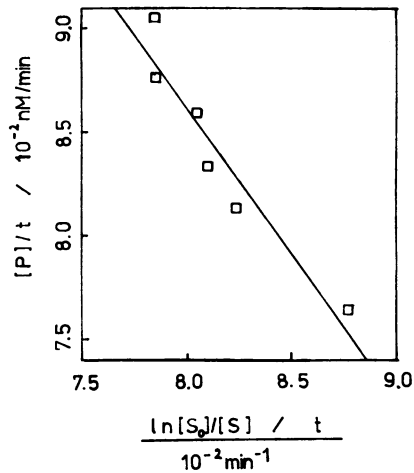

Fig. 5 Evaluation of Michaelis parameters for ClaI

The data of the steady-state part from the experiment in fig. 3 are plotted according to eqn. (1) for the determination of K_m (slope) and V (intercept). L_o is taken as the origin of the progress curve.

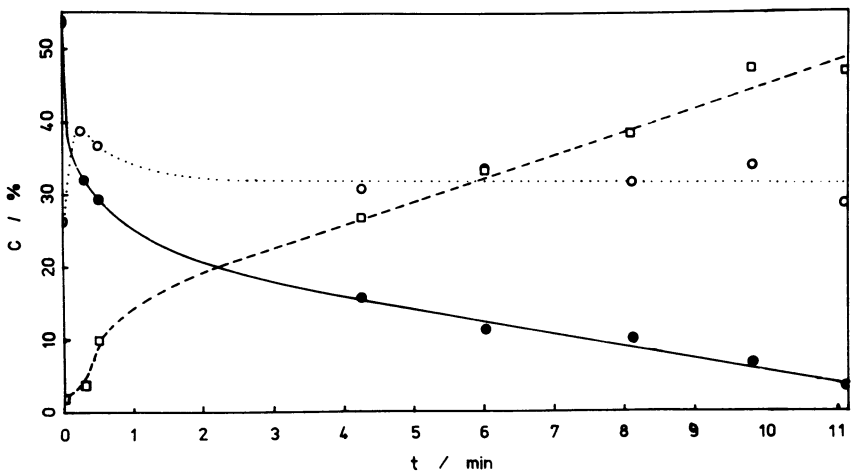

Fig. 6 Pre-steady-state kinetics of ClaI

Relative concentrations of the conformers of pJC80 during the course of the reaction: superhelical (●), nicked circles (o), and linears (□); difference to 100 % : oligomers (not shown). $[S_o]$ = 8.3 nM; [E] = 1.55 nM.

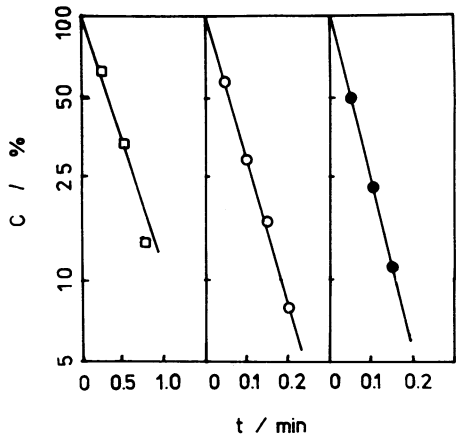

Fig. 7 Rate constants of ClaI from the pre-steady-state

Logarithmic plot of the steep increase of linear DNA (left; 100 % - linear concentration), of the steep increase of nicked intermediates (center, 100 % - intermediate concentration), and initial decrease of superhelix concentration (right) is time t. The total concentration difference of the burst was set to 100 %. All values from Fig. 6.

k_4 about 1.7 min^{-1}.

The ratio of rate constants namely $k_2/k_4 \approx 6$ and $k_4/k_5 \approx 8$ correlates well with values for EcoRI [16] for which $k_2/k_4 \approx 3$ and $k_4/k_5 \approx 20$ at 30°C. This means that the reaction of both enzymes proceeds similarily with the only difference of one order of magnitude in reaction velocity.

It must be emphasized, that the slow reaction rates of ClaI are an intrinsic property of the enzyme under the conditions employed in this investigation, because the determination of the enzyme concentration from the burst difference measures only fully active molecules. The reason for the slow reactions of ClaI is not clear. They could be caused by a substrate property which is common to ColEI, pJC80 and λ-DNA. This seems not unlikely after an inspection of the detailed experiments described by Wang et al.[7] for the cleavage of pBR322 with XorII. The unusual kinetics of this system, which was not observed, when Ad-2DNA was used as the substrate, appear to be caused by a very low k_5 and an appreciable dissociation of the enzyme from the nicked substrate prior to the second cleavage.

Experiments with XorII in our laboratory using pBR322 and pJC80 as substrates support this interpretation. Aside from the possibilities considered by

Wang et al. as an explanation for the kinetics of this system we checked, if product inhibition or desactivation could be responsible. The result were negative. An example of a progress curve is given in Fig. 8. The early part of the curves for each species was replotted logarithmically (not shown). The rate constants k_2, k_3 (dissociation of the enzyme from the nicked intermediate, of eqn (2)), and k_4 are in the range of 0.1 min^{-1}, k_5 was found to be about 0.003 ± 0.002 min^{-1}. The large uncertainly in these values is mainly due to the very low reaction rate.

That these ClaI-type kinetics are not so exceptional, is further evidenced by experiments with the enzyme SmaI and ColEI as the substrate. A pre-steady-state burst is observed, when the concentrations of enzyme and substrate are chosen in a "normal" range, e.g. 2000fold enzyme dilution corresponding to 0.15 nM and $[S_o]$ = 1.9 nM. k_5 was found to be 0.02 ± 0.01 min^{-1} and K_M in the order of nanomolar. Of course it can not be excluded, that reaction conditions for ClaI, SmaI, and XorII were suboptimal. But on the other hand the dissociation rates of 0.22 - 0.003 min^{-1} are not so exceptional, if one compares with the lac repressor, which dissociates from the operator at a rate of about 0.036 min^{-1} [17].

CONCLUSIONS

Several site-specific endonucleases have been compared with respect to their kinetics. Michaelis constants were found to be very similar. Therefore

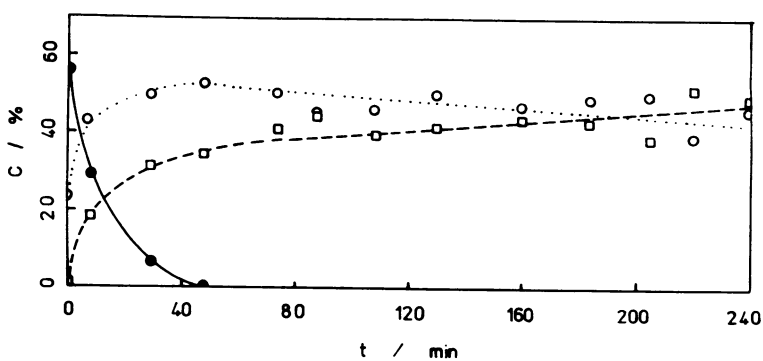

Fig. 8 Burst reaction of XorII

Relative concentrations of the conformers of pJC80 during the course of the reaction: superhelical (●), nicked circles (o), and linears (□); difference to 100 % are oligomers (omitted for clarity). $[S_o]$ = 6.25 nM; [E] ≈ 1.5 nM (60 BRL-Units/ml).

it may be concluded that aside from minor influences from DNA sequences adjacent to the recognition sites the intrinsic binding constants and thus type and number of interactions are comparable.

The observed differences in V are mainly caused by different molar concentrations of the enzymes in the particular preparation. This is true for BamHI and EcoRI, which have rate constants in the order of minutes. For BglII, HpaII, PstI, and SalI lower limits for these constants can be given. In the assays enzyme concentrations were always chosen to give V-values around 0.1 nM/min, while substrate concentrations ranged from 2 to 4 nM. As no burst was observed under these conditions it follows that $[S_o]/[E]>20$. From eqn. (3) we can then calculate $k_5>1\ min^{-1}$. For ClaI, SmaI, and XorII this rate-limiting step is much slower.

Some of the enzymes employed in this investigation also differ in their reactivity towards supercoiled as compared to relaxed DNA. This is astonishing, because the rate limiting step is the dissociation of the enzyme-product complex. It means that the initial structure of the substrate is conserved at least in the region of enzyme substrate interaction throughout the whole reaction sequence.

It must be emphasized that we did not attempt to work with DNA preparations, which are homogenous with respect to different conformers. It is an advantage of our kinetic method, that we can assess kinetic properties under those conditions, which are commonly applied, when using restriction endonucleases as tools in molecular biology. This is also very important, if there is more than one cleavage site for a particular enzyme on its substrate, which is often the case. Even then our method is applicable and gives unambiguous kinetic parameters, which can be used to calculate reaction times for any restriction digest. This can be seen from the data for BamHI with λ-DNA as the substrate (Tab. 1), which where determined by the discontinuous method as described earlier [2].

The burst reaction as described for ClaI can be used as an easy method for the determination of molar enzyme concentrations. These are otherwise difficult to access, because preparations of site-specific endonucleases are only functionally purified and usually very unstable in highly purified preparations. Aside from ClaI-type enzymes we have successfully tried the same method with BamHI (data not shown). Previously published kinetic parameters [3] could be confirmed by this method. It could further be deduced, that the active form of this enzyme is the tetramer. EcoRI can be treated similarily, which can be seen from Modrich & Zabels paper [16: Fig. 7].

In applying this method care must be taken, not to use lower $[S_o]/[E]$ ratios than about 5. At higher concentrations more than one native enzyme could be bound to one substrate molecule. Properly employed this method has the advantage that only active enzyme molecules are measured and that the values are inherently independent of the nature of the substrate and any other reaction conditions. This is thus the only way to get reproducible and comparable values for the concentration of site-specific endonucleases, e.g. to compare the quality of different preparations of a particular enzyme.

When comparing different enzymes for their efficiency in cleaving DNA, not only their concentrations and activities i.e. V-values, should be considered but also K_M is important. BamHI e.g. has a much lower activity (Tab. 1) than EcoRI, but its higher affinity for substrate assures sufficiently fast reactions. It must further be mentioned that the low rate constants of enzymes with ClaI-type kinetics do not impede the application of these enzymes for the cleavage of DNA. The only consequence is, that one works in the pre-steady-state (burst reaction) to achieve complete cleavage within reasonable time (1-2 hrs.). Example for ClaI: In order to cleave 1 µg of λ-DNA in one hour at 37°C in a reaction volume of 25 µl (= common unit definition) one has to work at a $[E]/[S_o]$ - ratio of about 1.

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REFERENCES

1 Roberts, R. J. (1981) Nucleic Acids Res. 9, r75-r96
2 Hinsch, B. and Kula, M.-R. (1980) Nucleic Acids Res. 8, 623-633
3 Hinsch, B., Mayer, H., and Kula, M.-R. (1980) Nucleic Acids Res. 8, 2547-2559
4 Michaelis, L. and Menten, M. L. (1913) Biochem. Z. 49, 333-369
5 Berkner, K. L. and Folk, W. R. (1979)J. Biol. Chem. 254, 2551-2560
6 Smith, H. O. (1979) Science 205, 455-462
7 Wang, R. Y. H., Shedlarski, J. G., Farber, M. B., Kuebbing, D. and Ehrlich, M. (1980) Biochim. Biophys. Acta 606, 371-385
8 Mayer, H. and Schütte, H. (1978) Patentschrift 2610 958, Deutsches Patentamt
9 Hinsch, B., Schütte, H., and Mayer, H. (1978) 12th FEBS Meeting, Dresden, Abstract 647
10 Goebel, W. and Bonewald, R. (1975) J. Bacteriol. 123, 658-665
11 Bøvre, K. and Szybalski, W. (1971) in Methods Enzymol., Colowick, S. P. and Kaplan, N. O. Eds., Vol. XXI, pp 350-383, Academic Press, New York
12 Sutcliffe, J. G. (1978) Nucleic Acids Res. 5, 2721-2728

13 Hershfield, V., Boyer, H. W., Yanofsky, C., Lovett, M. A., and Helinsky, D. R. (1974) Proc. Nat. Acad. Sci. USA 71, 3455-3459
14 Smith, H. R., Humphreys, G. O., Willshaw, G. A., and Anderson, E. S. (1976) Molec. Gen. Genet. 143, 319-325
15 Szybalski, E. H. and W. (1979) gene 7, 217-270
16 Modrich, P. and Zabel, D. (1976) J. Biol. Chem. 251, 5866-5874
17 Livshitz, M. A., Gursky, G. V., Zasedetelev, A. S., and Volkenstein, M. V. (1979) Nucleic Acids Res. 6, 2217-2235
18 Hinsch, B. (1980) PhD Thesis, Universität Marburg
19 Thomas, M. and Davies, R.W. (1975) J. Mol. Biol. 91, 315-328
20 Ruben, G., Spielman, P., Tu, C. D., Jay, E., Siegel, B. and Wu, R. (1977) Nucleic Acids Res. 4, 1803-1813
21 Atkins, G. L. and Nimmo, I. A. (1973) Biochem. J. 135, 779-784