Specific-Enzyme Release of Cellulose-Bound Drugs, Experimental and Theoretical Study

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Synopsis

Various polymeric prodrugs of pholcodine were prepared by covalently linking a substrate ester of the hydroxylic drug to cellulosic derivatives. The ester derivatives of the drug were chosen so that the resulting insoluble cellulosic prodrugs became substrates of α-chymotrypsin. The hydrolysis of such compounds was studied for various spacer arms in conditions simulating the intestinal medium. A theoretical model was developed to describe the enzymatic catalysis of this hydrolysis, taking into account the adsorption of the enzyme onto the polymer, the hydrolysis reaction, and the enzyme denaturation. The resulting equations using independent ly determined parameter values such as the Langmuir adsorption constants, the enzyme denaturation constants, and the initial hydrolysis rate constant successfully correlated with the experimental data.

INTRODUCTION

Binding pharmacologically active compounds to macromolecules sometimes makes it possible to prolong the action of the drugs and to improve their specificity.

Several polymeric prodrugs of pholcodine (Fig. 1), whose general structure is represented in Figure 2, have recently been prepared.[1] The release of the active agent was expected to result from the hydrolysis of the ester function formed between the alcohol group of the drug and the carboxylic group of an oligopeptide immobilized on cellulose. When this oligopeptide was ended by an aromatic L-aminoacid, the ester bond was found to be a specific substrate of α-chymotrypsin,[2] a pancreatic enzyme: Indeed, no ester hydrolysis was observed, either with a terminal amino-acid of D-configuration or in the absence of α-chymotrypsin. Hydrolysis was significant only with an L-amino-acid, which probably meant that a complex between the enzyme and the polymeric substrate was necessary to catalyze hydrolysis.

The cellulosic prodrugs were not soluble in water, and the problem of the heterogeneous hydrolysis of the substrates was very complex, compared to that of water-soluble ones.[3] In fact, due to the diffusion and adsorption phenomena, it was not possible simply to apply the Michaelis–Menten equation, and we had to take into account the various processes involved, as follows:

Adsorption of the enzyme on the polymer:

$$E_S + \text{Polymer} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_P \qquad (1)$$

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Journal of Applied Polymer Science, Vol. 32, 2851–2866 (1986)
 CCC 0021-8995/86/012851-16$04.00

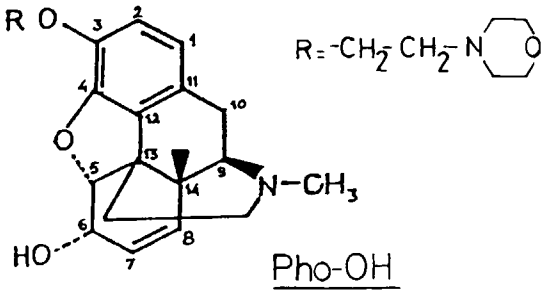


Fig. 1. Structure of pholcodine.

with E_S = enzyme in the supernatant and E_P = enzyme adsorbed on the polymeric phase.

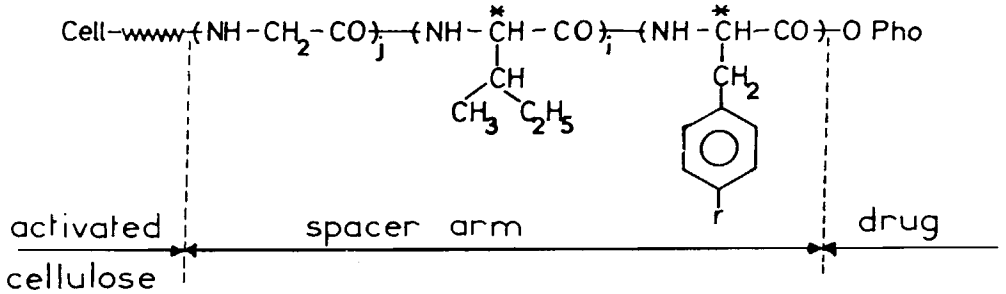
Conformational arrangement between the enzyme and the active site of the polymeric substrate:

$$E_P + S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (ES) \tag{2}$$

with S = substrate active site (ester bond) and (ES) = adsorptive enzyme–substrate complex.

Hydrolysis step:

$$(ES) \rightarrow E_P + P + P' \tag{3}$$



		Polymeric prodrugs	
		Symbol	Spacer arm
i = 0, j = 0	r = H	CEPP'	-L-Phe-O-Pho
		CEDPP'	-D-Phe-O-Pho
	r = OH	CETP'	-L-Tyr-O-Pho
i = 1, r = OH	j = 0	CEITP'	-L-Ile-L-Tyr-O-Pho
	j = 1	CEGITP'	-Gly-L-Ile-L-Tyr-O-Pho

Fig. 2. Chemical structures of pholcodine cellulosic prodrugs.

with P = produced alcohol and P′ = carboxylic acid created on the solid phase.

Diffusion of soluble products.

Various studies dealing with enzymatic hydrolysis involving an adsorption preequilibrium have already been reported.[4-6] Others have described enzymatic reactions controlled by Fick's diffusion.[7,8]

However, in the case of the α-chymotrypsin-catalyzed hydrolysis an additional phenomenon, the enzyme denaturation at the physiological temperature (37°C) must be considered. The study reported here concerns the α-chymotrypsin-catalyzed hydrolysis of insoluble prodrugs described in Figure 2; an original model is proposed, simultaneously taking into account the partial equilibria of enzyme adsorption and denaturation, and ester hydrolysis, and relying on the experimental results independently obtained from the study of the individual steps.

EXPERIMENTAL

Polymeric Prodrugs

Samples of cellulose linters (1 g) in pulver were treated with a 30% (w/w) NaOH solution (20 mL), at 9°C for 30 min. After filtration, the swollen cellulose was activated with epichlorhydrin and then allowed to react with the amino group of L-phenylalanine according to the methods previously described.[1,2] The carboxylic end of the cellulose-immobilized L-phenylalanine was then allowed to react with the previously prepared isourea of pholcodine,[2] to give the corresponding ester.

The other polymeric drugs, containing L-tyrosine as the COOH-terminal amino acid were prepared following the same procedure.[1] The amount of bound drug was determined after 0.01*N* NaOH hydrolysis by the UV absorption (280 nm) of pholcodine in the supernatant (ϵ_{280} = 1500 L mol^{-1} cm^{-1}).

Enzyme

α-Chymotrypsin from bovin pancreas, with an announced activity of 40.5 U/mg towards benzoyl-L-tyrosylethylester, was purchased from Sigma (U. S.). Its activity was checked using *N*-glutaryl-L-phenylalanine-4-nitroanilide (GPNA) as a substrate and the liberated 4-nitroaniline was measured by its absorption at 405 nm.

Drug Release

The drug release was investigated in conditions simulating the intestinal tractus, i.e., pH 7.9 phosphate buffer at 37°C. The hydrolysis of the ester bond was studied by potentiometric titration of the formed carboxylic acid, by means of a pH stat: The polymeric substrate was dispersed in a 5 mL thermostated vessel, under magnetic stirring; bubbling nitrogen saturated with water avoided water evaporation and introduction of atmospheric CO_2 into the vessel. After injection of a small volume (~ 50 μL) of the enzyme reserve solution, the reaction was initiated, and the pH of the medium was

kept constant by addition of 0.01*N* NaOH. The amount of added NaOH, automatically recorded as a function of time, was used as a measurement of the extent of the drug release. It was systematically checked that the sum of the amount of released drug measured by this technique plus the amount of drug remaining on the polymer and determined by UV titration was equal to the initial pholcodine content of cellulosic samples.

Denaturation of α-Chymotrypsin

α-Chymotrypsin in a pH 7.9, 0.05*M* phosphate buffer, with a well-known initial activity and concentration (concentration in the range 0.15–1 mg/mL, i.e., 7–46 μM) was incubated under stirring at 37°C, for periods between 0 and 2 h. The activity of the enzyme was then determined towards GPNA.

Adsorption of α-Chymotrypsin on the Cellulosic Substrates

A known amount of a cellulosic substrate was dispersed in 2.5 mL of pH 7.9, 0.05*M* phosphate buffer at 37°C. α-Chymotrypsin with a well-known initial concentration $[E_a]_0$ (between 0 and 40 μM) and activity, was then introduced at $t = 0$. After stirring (200 rpm) for a defined period (t), the sample was cooled and centrifuged, and the enzymatic activity of the supernatant towards GPNA was measured. From this determination, the value of $[E_S]$, which would be obtained if no denaturation occurred, was calculated as follows[1]:

$$[E_S] = \frac{\Delta A_t \cdot v_c}{\epsilon_{NA} \cdot v_e} \cdot \frac{1}{a \cdot h(t)} \tag{4}$$

ΔA_t was the absorbance variation per unit of time resulting from GPNA hydrolysis in the presence of the enzymatic mixture incubated during the period t, ϵ_{NA} was the 4-nitroaniline extinction coefficient, v_c and v_e, were, respectively, the volumes of the optical vessel and that of the sample used for the measurement of ΔA_t, a was the specific activity of α-chymotrypsin, and $h(t)$ the corrective factor taking into account the enzyme denaturation:

$$h(t) = \frac{f(t)}{[E_a]_0}$$

where $f(t)$ was the active enzyme concentration at t as defined in eq. (8). $[E_P]$ was then calculated from $[E_S]$ by

$$[E_a]_0 v_0 = [E_P] v_P + [E_S] v_S \tag{5}$$

where v_P was the volume of the polymeric phase and v_S that of the supernatant; v_0, the initial volume, was $(v_S + v_P)$. The value of v_P was determined from the pycnometric measurement of the polymer density and the estimation of the polymer void volume ϵ:

$$v_P = \frac{m_P}{d(1-\epsilon)}$$

with

$$\epsilon = \frac{1}{1 + 1/d(m-1)} \quad \text{and} \quad m = \frac{\text{wt wet polymer}}{\text{wt dry polymer}}$$

For all cellulosic derivatives, v_P/m_P was found to be about 7.4 mL·g^{-1}.

RESULTS AND DISCUSSION

Denaturation of α-Chymotrypsin

In a phosphate buffer simulating the intestinal juice (pH 7.9, 37°C), α-chymotrypsin was found to lose its activity rapidly and irreversibly.

The first studies concerning this problem[9-12] described the denaturation as the transformation of one reactive species, the protein itself, according to a unimolecular pathway, but our experimental results were not consistent with a single order reaction (1,2 or intermediate). According to other authors,[13-17] the mechanism should be more complicated, involving the formation of two kinds of products without enzymatic activity: Products of thermal denaturation (E_d) and products of enzyme autolysis (P_A). The models proposed for describing denaturation were different according to the nature of the species involved in the various equilibria: The optimization of our experimental values was achieved with all proposed models[13-17] and the best agreement was obtained with the model described by Kumar and Hein[14]:

$$\begin{array}{ccccccc} E_iH & \overset{K_3}{\rightleftharpoons} & E_i & + & H^+ & \text{then} & E_i \xrightarrow{h_1} E_d \\ K_4 \uparrow\downarrow & & K_2 \uparrow\downarrow & & & & \\ EH & \overset{K_1}{\rightleftharpoons} & E & + & H^+ & & \end{array} \qquad (6)$$

and

$$EH + E_i \overset{K}{\rightleftharpoons} [EH \cdot E_i] \xrightarrow{h_2} EH + P_A$$

with EH = E_a = active form of the enzyme and E_i = initial inactive form of the enzyme.

The consumption rate of the active form can be expressed by

$$-\frac{d}{dt}[EH] = h_1[E_i] + h_2[EH \cdot E_i] \qquad (7)$$

or

$$-\frac{d}{dt}[E_a] = k_1'[E_a] + k_1'k_2'[E_a]^2 \qquad (7')$$

with $k_1' = h_1K_1K_2/[H^+]$ and $k_2' = (h_2/h_1)K$. By integration, we obtained

$$[E_a] = \frac{[E_a]_0}{(1+k_2'[E_a]_0)e^{k_1't} - k_2'[E_a]_0} = f(t) \qquad (8)$$

The optimization upon k_1' and k_2' of the error function F defined by

$$F = \sum_t (f(t) - [E_a]_{exp}(t))^2$$

where $[E_a]_{exp}$ was the experimental value of $[E_a]$, was carried out by the Rosenbrock method.[18] The results of this optimization were

$$k_1' = 0.242\ h^{-1} = 6.72 \times 10^{-5}\ s^{-1}$$

$$k_2' = 0.0811\ \mu M^{-1}$$

The values of k_1' and k_2' thus obtained were in good accordance with those reported by Kumar and Hein.[14] When compared, the values of $[E_a]$ calculated from the mathematical equation (6) and from experimental data (Fig. 3) showed good agreement, as the difference between them was not

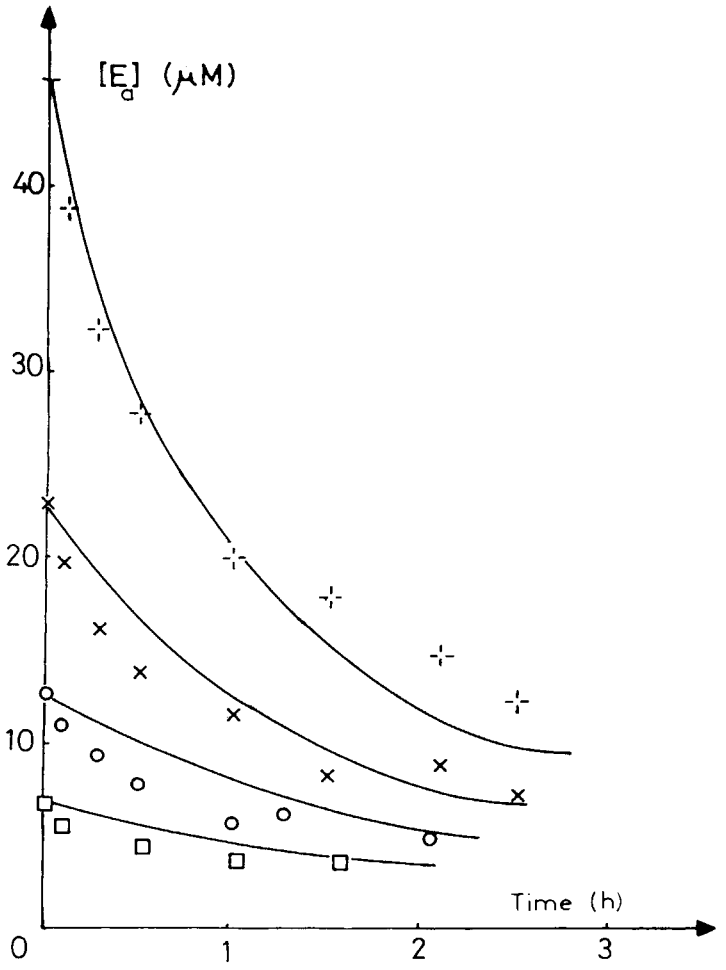


Fig. 3. Denaturation of α-chymotrypsin. Comparison of theoretical results (-) with the experimental data obtained in a 0.05*M* phosphate buffer, pH 7.9, at 37°C: $[E_a]_0$ (μM): (+) 45.8; (×) 22.9; (○) 12.75; (□) 6.9.

greater than 10%. The advantages of this model over the other ones are first that it describes the physical process by only two parameters and second that it takes into account the active and inactive forms, as well as the various ionization states of the protein at the considered pH.

Adsorption of α-Chymotrypsin on the Cellulosic Derivatives

After introduction of α-chymotrypsin into the suspension of the cellulosic prodrug in a phosphate buffer, a rapid decrease of the enzyme concentration could be observed in the supernatant within the first 5 min; afterwards the enzyme concentration remained constant, which meant that, after this period, the adsorption equilibrium was attained. It was assumed that this phenomenon occurred according to the Langmuir adsorption model[19] represented by the equilibrium (1) and described by the following equation:

$$[E_P] = K_{max} \frac{[E_S]}{K_a + [E_S]} \tag{9}$$

where K_{max} and K_a are constant values, respectively expressing the maximum adsorption capacity of the enzyme on the polymer and the enzyme concentration in the supernatant, in equilibrium with an adsorbed enzyme concentration $[E_P] = (K_{max}/2)$.

The Langmuir approach appeared in fact to be adequate as it could fit the experimental adsorption phenomena, without requiring suppositions about the chemical or physical nature of the process. It was frequently used for similar problems, such as, for example, the adsorption of α-chymotrypsin on polysaccharidic substrates,[5,20] or the adsorption of cellulases on cellulosic derivatives.[4,21]

The values of $[E_S]$ and $[E_P]$ at equilibrium (after 5 min) were determined for various initial concentrations of enzyme $[E_a]_0$ and plotted corresponding to the linear form of a Langmuir isotherm as follows:

$$\frac{[E_S]}{[E_P]} = \frac{1}{K_{max}}[E_S] + \frac{K_a}{K_{max}} \tag{10}$$

The variations of $[E_S]/[E_P]$ vs. $[E_S]$ were linear for the five polymeric substrates tested (Fig. 4) and the resulting Langmuir parameters are shown in Table I. Also reported is the value of E_f which represents the maximum molar amount of adsorbed enzyme per mole of ligand,[5] in this case bound pholcodine. In a way, this expression can be considered as an estimate of the specific affinity of the enzyme towards a bound ligand. In fact, further experiments relative to CEDPP′ for which no esterolysis was observed, pointed out that the enzyme concentration in the polymeric phase, was the same as in the supernatant, which meant that the enzyme adsorption indeed depends on the configuration of the bound ester.

The molar ratio of adsorbed enzyme to bound ligand (α_P) was calculated for various concentrations of substrate $[S]_0$, and the results for two samples of CEPP′ are reported in Figure 5. They show that, in the range of substrate concentrations used (0.1–0.6 mM), α_p remained almost constant close to

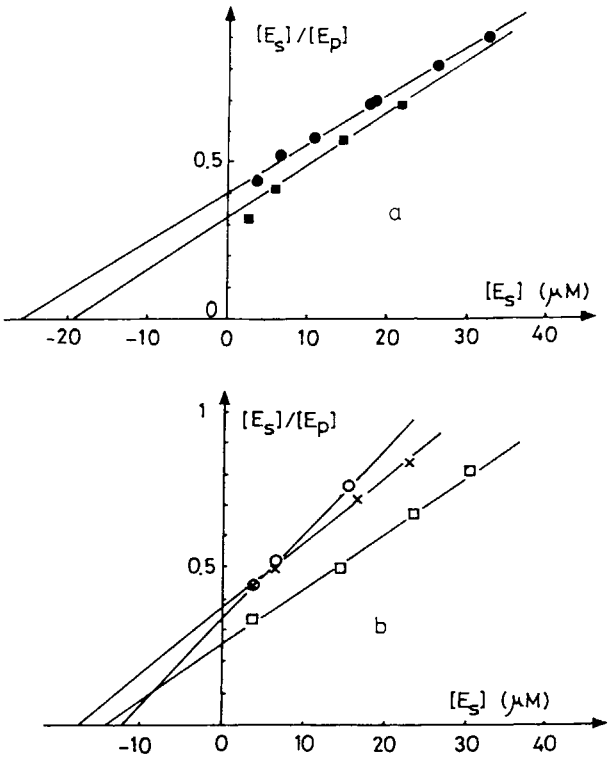


Fig. 4. Adsorption Langmuir isotherms for α-chymotrypsin on the various cellulosic prodrugs (20 mg of substrate; 2.5 mL of 0.05*M* phosphate buffer, pH 7.9, 37°C; determination after 5 min of incubation): (●) CEPP'1; (■) CEPP'2; (×) CETP'; (□) CEITP'; (○) CEGITP'.

0.6% and lower than E_f which could thus be considered as a limit of α_P for an indefinitely decreasing substrate concentration or for an enzyme concentration that would tend to infinity.

The very low values of E_f (0.3–1.4%) are consistent with the limited adsorption efficiency already mentioned by other authors in similar studies[5,22] and attributed to steric phenomena. On the other hand, the value of E_f decreased with increasing spacer arm length, which was attributed to a possible bending back of the spacer.[23]

The K_a values deduced from eq. (9) can be correlated with the affinity of the enzyme for a given substrate. From Table I, it can be concluded that

TABLE I
Langmuir Constants of the Adsorption of α-Chymotrypsin on the Cellulosic Prodrugs[a]

Cellulosic prodrugs	Pholcodine content (μmol/g)	K_{max} (μM)	K_a (μM)	E_f (%)
CEPP'1	31	62.5	26	1.4
CEPP'2	40	60	19.5	1.1
CETP'	50	48	17.8	0.71
CEITP'	97	57	14.4	0.43
CEGTP'	84	36.4	12.4	0.32

[a] Experimental conditions as in Figure 4.

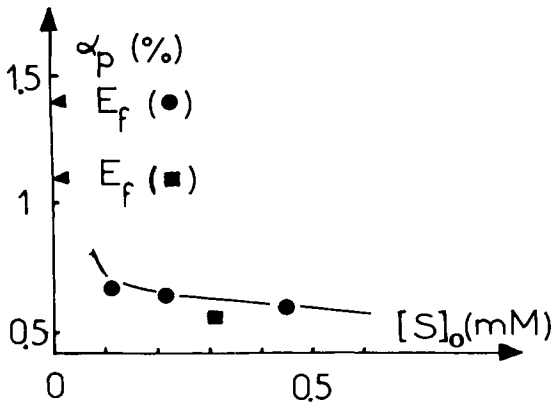


Fig. 5. Molar ratio α_P of adsorbed enzyme to bound ligand as a function of the substrate concentration $[S]_0$; $[E_a]_0$ = 40 μM; other conditions as in Figure 4.

increasing the amount of drug bound on cellulose improves the affinity of the enzyme, as does increasing the length of the spacer arm. This second property can either be attributed uniquely to the improvement of the accessibility of the bound ligand towards the enzyme or to the nature of the spacer arms also. In fact, the various spacer arms were chosen in relation with the known nature of the α-chymotrypsin active center, and the values of K_a obtained for the corresponding polymeric prodrugs were in good conformity with the primary, secondary, and tertiary specificity already described[24] for the specific substrates of the enzyme.

Enzymatic Hydrolysis of Polymeric Substrates

When α-chymotrypsin was added to an aqueous suspension of a polymeric prodrug, no significant hydrolysis was observed within the first few minutes; one can assume that there was a latency period of slow adjustment between the enzyme and the insoluble substrate. After that, the hydrolysis rate became high, remained almost constant for more than 1 hr, and then slowly decreased. The hydrolysis was never complete, since the total amount of pholcodine released even after 15 h was lower than the initial amount of bound drug. The possible reasons are either that some ester bonds were not accessible to the enzyme because of a steric effect or that, after 15 h, the enzyme was totally inactive.

The initial rate of hydrolysis was experimentally determined as the molar amount q_0 of carboxylic functions liberated during the first hour, in the presence of the enzyme whose activity was decreasing with time. For this determination, the approximation was that during the first hour, the concentration of the active adsorbed enzyme was constant and equal to the average value $\overline{E}_P$ calculated for this period:

$$\overline{E}_P = \frac{1}{t_0}\int_0^{t_0} [E_{Pa}]\, dt \qquad \text{with } t_0 = 1 \text{ h} \tag{11}$$

$[E_{Pa}]$ was the concentration of active adsorbed enzyme at t (h), correlated

with that of active free enzyme in the supernatant $[E_{Sa}]$ by eq. (9), and $[E_{Sa}]$ was obtained from the denaturation equation (8):

$$[E_{Sa}] = \frac{[E_S]_0}{1 + k_2'[E_S]_0 e^{k_1 t} - k_2'[E_S]_0} \tag{12}$$

By integration of eq. (9), $\bar{E}_P$ was determined as a function of $[E_S]_0$ and thus of $[E_a]_0$ via eqs. (5) and (9).

The variation of the initial rate q_0 of CEPP' hydrolysis as a function of the enzyme concentration (Fig. 6) is linear vs. $\bar{E}_P$ but not vs. $[E_a]_0$, which proves again that the adsorption of the enzyme on the polymer is indeed correlated with the specific hydrolysis process.

With regard to the influence of the initial substrate concentration $[S]_0$ on the initial hydrolysis rate q_0 of CEPP', it was found that q_0 increased with $[S]_0$ (Fig. 7). On the other hand, for a given substrate concentration, it was also shown that q_0 increased with the pholcodine substitution rate θ (θ = mol bound drug per g dry cellulosic derivative).

Since for low substrate concentrations, q_0 is proportional to $[S]_0$ (Fig. 7), one can deduce from equilibrium (2), the following equation:

$$v = -\frac{d[S]}{dt} = K[E_{Pa}][S] \tag{13}$$

where K is a constant value, experimentally determined by

$$K = \frac{q_0}{\bar{E}_P[S]_0} \tag{14}$$

With CEPP'2 as an example, K is the common slope of lines q_0 vs. $\bar{E}_P$ (Fig. 6) and q_0 vs. $[S]_0$ (Fig. 7). Figure 7 also shows similar diagrams drawn for the polymeric prodrugs containing L-tyrosyl in their spacer arms and per-

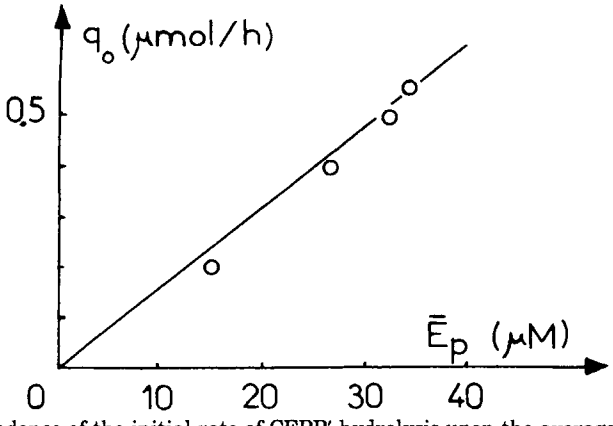


Fig. 6. Dependence of the initial rate of CEPP' hydrolysis upon the average concentration of active adsorbed enzyme $\bar{E}_P$ (○). 50 mg of CEPP'2 (2 μmol of bound pholcodine); 5 mL 0.05*M* phosphate buffer, pH 7.9, 37°C. $[S]_0$ = 0.4 mM.

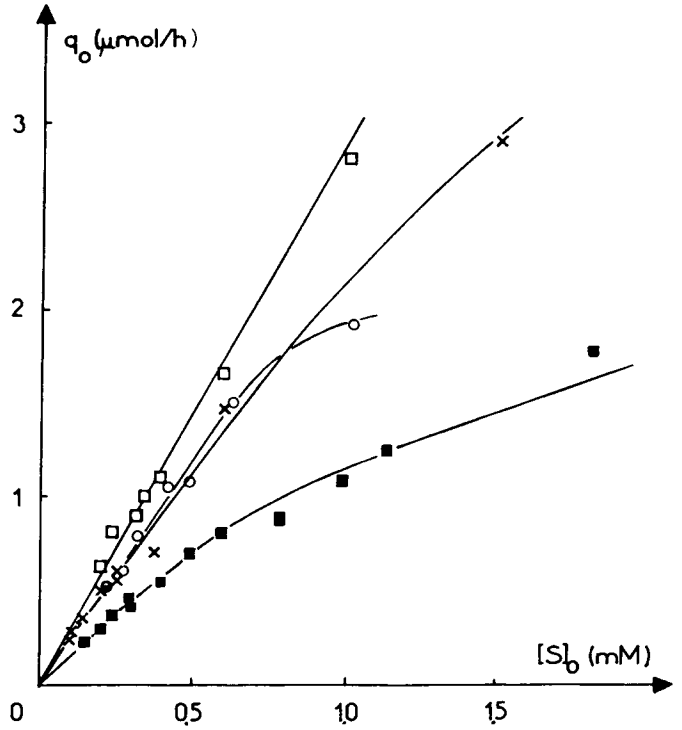


Fig. 7. Influence of the initial substrate concentration $[S]_0$ on the initial hydrolysis rates of various cellulosic prodrugs. $[E_a]_0$ = 40 μM; 0.05*M* phosphate buffer, pH 7.9; 37°C; other conditions as in Figure 4.

mitting the determination of the corresponding values of K. It must be mentioned that the hydrolysis reaction represented by equilibrium (3) and the diffusion of soluble products were supposedly so fast that the adsorption (1) and the conformational arrangement between enzyme and substrate (2) were rate-determining steps.

Taking into account eqs. (9) and (12), eq. (13) was transformed into

$$-\frac{d}{dt}[S] = \frac{K_{max}K[E_S]_0[S]}{(1-K_ak_2')[E_S]_0 + (1+k_2'[E_S]_0)K_ae^{k_1't}} \tag{15}$$

After integration, the theoretical concentration of released drug [P] at time t could then be expressed by the following relations:

$$[P] = [S]_0 - [S] \tag{16}$$

$$\frac{[P]}{[S]_0} = 1-\exp\left(\frac{K_{max}K}{K_ak_2'-1}\left(t + \frac{1}{k_1'}\ln\frac{[E_S]_0+K_a}{[E_S]_0(1-K_ak_2')+K_a(1+k_2'[E_S]_0)e^{k_1't}}\right)\right) \tag{17}$$

Values of $[P]/[S]_0$ experimentally determined for the hydrolysis of CEPP' at various concentrations and those deduced from eq. (17) with the formerly determined values of K, K_a, K_{max}, k_1', k_2' are plotted as a function fo time in Figure 8. From this, it can be observed that eq. (17) is a fairly good model

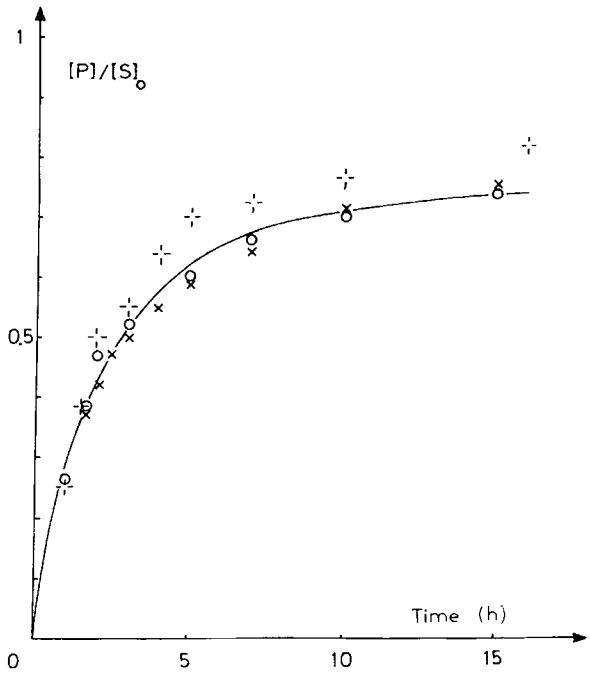


Fig. 8. Fraction of drug released from CEPP'2 as a function of time. Comparison between the theoretical model (-) and the experimental results obtained with $[S]_0$ = 0.2 mM (×), 0.4 mM (+), 0.6 mM (○). Experimental conditions as in Figure 7.

of experimental results within an error range of about 10%. The application of the same model to various enzyme concentrations $[E_a]_0$ (Fig. 9) leads to curves which are also in good agreement with the experimental data.

Other Applications of the Model

As the model described by eq. (17) seemed to be a good representation of processes certainly more complex than those taken into account in the equations, it was used to calculate the kinetic hydrolysis curves of compounds for which only few experiments had been carried out. For example, this was done with polymeric derivatives containing L-tyrosyl in their spacer arms, and for which only the variation of q_0 vs. $[S]_0$ was known. Figure 10 shows that the initial hydrolysis rate is faster for CETP' than for CEPP'2, which affords a more complete drug release. It can also be seen that a spacer arm prolonged by introduction of an isoleucyl residue notably accelerates and improves the release, but that including an additional residue (glycyl) does not increase the hydrolysis rate any further.

The model of eq. (15) can also be used to anticipate the behavior of polymeric drugs under conditions that cannot be produced *in vitro*, such as, for example, in the presence of a constant concentration of α-chymotrypsin. In fact, inside the duodenum and jejunum, this enzyme has a constant activity—corresponding to a concentration of about 40 μM[25,26]—which cannot be simulated *in vitro* due to its rapid denaturation. Modelling the pholcodine

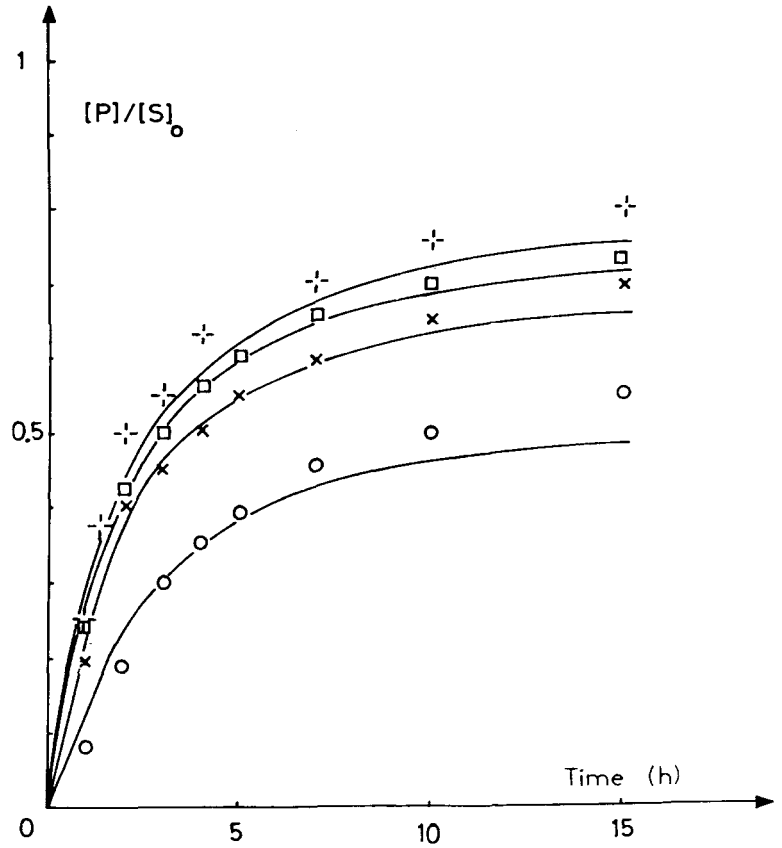


Fig. 9. Fraction of drug released from CEPP'2 as a function of time and $[E_a]_0$. Comparison between the theoretical model (–) and the experimental results obtained with $[E_a]_0$ (μM): (○) 9.1; (×) 22.7; (□) 34.6; (+) 40. Other conditions as in Figure 6.

release at this constant enzyme concentration led to curves (Fig. 11) from which it can be seen that, as expected, for every cellulosic substrate the ester hydrolysis is faster and more complete than in the presence of enzyme denaturation (Fig. 10). So, after about 10 h of hydrolysis, the theoretical fraction of released drug is greater than 95%. The true *in vivo* yield could, of course, be smaller due in particular to steric hindrance phenomena, but we can, however, assume that the polymeric products described could release pholcodine to a large extent during the intestinal transit since the duration of the drug disposal is of the same order as the mean residence time in the intestine (i.e., 5–10 h).

CONCLUSIONS

From the various studies carried out on the insoluble polymeric prodrugs described, it could be concluded first that a complex, like those observed beteween enzymes and soluble substrates, was formed between α-chymotrypsin and the polymeric substrates. The close connection between complex

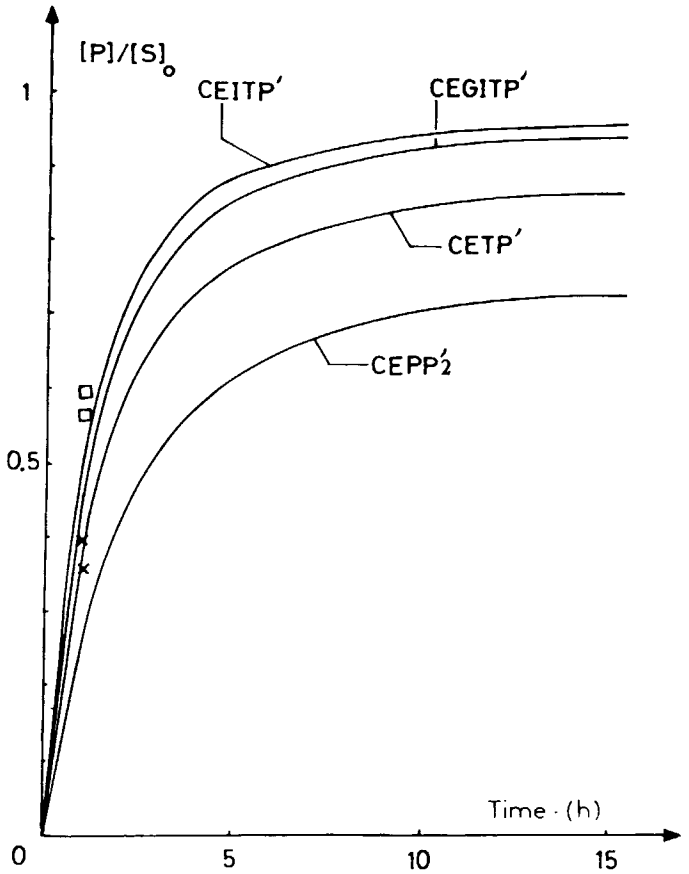


Fig. 10. Fraction of released drug for the various cellulosic prodrugs, as calculated from the theoretical model (–). Experimental results for CETP' (×) and CEITP' (□) under conditions as described in Figure 7.

formation and hydrolysis was proved and the Langmuir isotherm parameters (K_a, K_{max}) were determined.

However, at the considered temperature (37°C), α-chymotrypsin became inactive to a large extent, and this aspect could not be disregarded for the evaluation of the kinetic hydrolysis values. This process was described using the Kumar model,[14] which allowed the introduction and calculation of two other parameters, k_1' and k_2'.

Finally, the initial rate of hydrolysis was found to be proportional to the concentration of adsorbed enzyme and to the initial concentration of substrate: The proportionality constant K was determined.

From these five independently determined parameters (K, K_a, K_{max}, k_1', k_2') corresponding to elementary steps of the overall process, a mathematical model was developed in order to describe the drug release. A good agreement was observed between the experimental results and the values deduced from the model. On the other hand, the latter made it possible to estimate the release kinetics in circumstances under which no enzyme den-

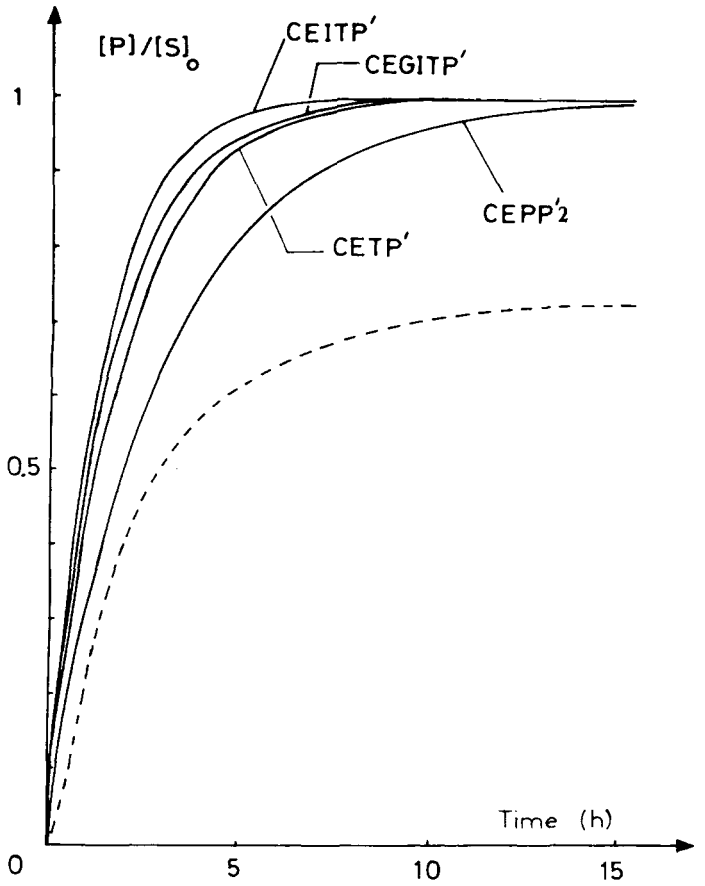


Fig. 11. Theoretical fraction of released drug calculated from eq. (17) for a constant α-chymotrypsin concentration: (- - -) experimental curve obtained for CEPP' (see Fig. 10).

aturation occurred. However, it would be hazardous to assert that these results would be identical in the intestine, as they take into account neither the mucosal absorption nor the fractionation of the prodrug dosage by the gastric chyme: *In vivo* tests are, of course, necessary to determine the relative importance of the different phenomena.

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Received July 22, 1985
Accepted December 10, 1985