The relation between enzyme, substrate and product

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The object of the investigation described in the following article is to gain a better insight into the catalytic action of an enzyme on its substrate. The investigation is still in an initial phase, in which an attempt is being made to crystallize an enzyme and a substrate in the form of a complex. The approach to this problem follows logically from a particular relation between enzyme, substrate and product, assumed on theoretical grounds, and confirmed by experiments on the enzyme, substrate and product chosen for investigation.

Introduction

The structure of an enzyme

Enzymes are the catalysts of the living cell. They make it possible for a wide variety of organic reactions to take place under the very mild conditions prevailing in the living cell.

Enzymes are proteins with a high molecular weight. This may at the very least be 10,000, but in most cases it is nearer 100,000 and sometimes considerably more. Their basic structure, however, is very simple. It is known that proteins are composed of some twenty kinds of amino acid having the general formula:

\[ R - H_2N - C - COOH. \]

By the repetition of what are called peptide bonds between the NH₂ group of one amino acid and the COOH group of the next, as illustrated in fig. 1, a main chain is formed which is long and flexible and has side chains at regular intervals (the R-groups of the amino acids). A side chain can be a non-reactive paraffin chain, but most contain a functional group with specific chemical properties, e.g. of an acid or base.

The presence of acid or base groups in the side chains has an important bearing on the state of charge of the protein; depending on the pH of the protein's environment, these groups have a negative or positive charge.

The activity of an enzyme depends lastly on its spatial structure. As a rule this structure is fairly compact, and is brought about by the folding or coiling of the main chain. It is a rather delicate structure which is stable only under definite conditions, e.g. provided that the pH remains within certain limits. To understand this one need only think of the influence of the positive and negative groups in the molecule on the folding of the chain, bearing in mind that the distribution of these groups can be radically altered.

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Fig. 2. Schematic and hypothetical representation of the way in which the enzyme chymotrypsin converts the substrate benzoylglycine methyl ester (BGME) into benzoylglycine (BG) and methyl alcohol. In the cavity (the active centre), a COO⁻ group can be seen on the left, an OH group at the top and an imidazole group on the right. (The groups present in an active centre need not belong to successive amino acids, as might be thought. As the protein chain is in a folded condition, certain amino acids which would be quite a long way from each other in the unfolded condition of the chain may now be quite close to each other. This is the case here with the OH and imidazole groups.)

a) Enzyme with "empty" active centre.
b) The enzyme has bound a substrate molecule.
c) Due to the action of the imidazole group the ester bond (C-O) has been broken and a new ester bond has been formed with the OH group, resulting in methyl alcohol (HOCH₃).
d) The methyl alcohol molecule has made way for a water molecule (HOH).
e) Due to the action of the imidazole group the ester bond has again been broken, resulting now in the formation of benzoylglycine.
f) The enzyme has returned to its starting point a).

by changing the pH. Generally speaking it is therefore necessary to be very careful about changing the normal environment of the enzyme, otherwise the spatial structure may be destroyed and the enzyme "denatured".

The action of an enzyme

The action of an enzyme can be described in the simplest case by the equation

\[ E + S \rightarrow ES \rightarrow E + P + P' + \ldots \]

A molecule of the enzyme E links up with a molecule of the compound to be acted on, the substrate S, and forms with it an enzyme-substrate complex ES. This complex can either decompose into the original components, or — and in this case the catalysis is completed — it can decompose into the enzyme and the reaction products P + P', and so on.

Enzymes are characterized by their high specificity to a greater extent than ordinary catalysts. For example, the enzyme urease hydrolyzes urea, but not the related compounds methylurea and thio-urea; and the proteolytic enzymes trypsin and chymotrypsin both break peptide bonds in a protein, but they do so at different sites in the molecule. Quite small changes in the spatial structure of the substrate are enough to stop the formation of the complex and thus to inhibit the catalytic action completely.

Most substrate molecules are small compared with the enzyme molecules. In the formation of a complex, therefore, only a small part of the enzyme molecule will be directly involved in the bond with the substrate molecule. This part of the enzyme molecule is called the active centre. The role played by the rest of the enzyme molecule is at present still uncertain; it may possibly have a stabilizing function.

The object of our investigation is to learn more about the action of enzymes; we want to obtain a more detailed and a three-dimensional picture of this process.

At the present stage we are primarily interested in what takes place in the active centre. Some insight into the processes concerned has already been gained in a few cases. As an example we give in fig. 2 a schematic representation of a possible way in which the hydrolysis of benzoylglycine methyl ester by chymotrypsin
can take place. We have already mentioned chymotrypsin as an example of a proteolytic enzyme (pro-
tease). The fact that it is also capable of hydrolyzing certain esters is not so surprising: the ester bond and the peptide bond show much resemblance and many enzymes are able to break both kinds of bond. A COO-group in the active centre of chymotrypsin has been found to be essential to the formation of the complex. Once the complex has been formed, an imidazole group in the centre exerts a catalytic action on the breakdown of the ester bond. Methyl alcohol is formed from the detached CH₃O group, while the other part separated from the substrate molecule temporarily attaches itself to an OH group present in the active centre. The last step in this process is straightforward hydrolysis, in which benzoylglycine is formed and at the same time the active centre becomes available again for a new reaction.

\[
\begin{align*}
\text{NH}_3 & \quad \text{IC=NH} \\
\text{NH} & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{C} & \quad -\text{N}-\text{C} + \text{OC}_{2}H_{5} \\
\end{align*}
\]

benzoylarginine ethyl ester

\[
\begin{align*}
\text{OH} & \quad \text{H} \\
\end{align*}
\]

water

\[
\begin{align*}
\text{NH} & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{C} & \quad -\text{OH} + \text{C}_{2}H_{5}OH \\
\end{align*}
\]

benzoylarginine ethylalcohol

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\end{align*}
\]

Fig. 3. Equation of the hydrolysis of benzoylarginine ethyl ester.

Although the way in which a reaction of this type takes place is broadly known, we would, for example, like to know more about the way in which the substrate binds with the enzyme, and in particular, how the reaction proceeds in three dimensions.

In order to study enzymic reactions in such detail it is desirable to have a very precise knowledge of the spatial structure of the active centre and of the substrate attached to it. The only method of acquiring this knowledge is by X-ray analysis, and so it became the first objective of our investigation to obtain a crystalline ES complex. Our attempts so far have not yet met with any success. Meanwhile Japanese investigators have been the first to report the crystallization of an ES complex \[21\]. The molecular weight of this complex, however, is too high for an investigation by X-ray analysis to have any chance of succeeding at the present time.

The following article is a report on the experimental and other considerations that preceded our attempts at crystallization \[3\]. Some account will be given of the kinetic methods which are often used in the study of enzymes. What is perhaps of greater interest, however, is the insight afforded into the characteristic relation that can exist between enzyme, substrate and product.

**The interaction of enzyme and substrate**

We decided to make the enzyme papain the subject of our investigations. Papain appeared suitable as it has a relatively low molecular weight (22 000), it is a fairly stable enzyme, and it can be prepared in large quantities with a high degree of purity. Like chymotrypsin, papain can hydrolyze both proteins and simple esters. The substrate we chose was the ethyl ester of benzoylarginine, which can easily be synthesized. The structure of this compound and the equation of the hydrolysis reaction are represented in fig. 3.

In practice the combination of this enzyme and substrate raises an important general problem, but we shall leave the discussion of this problem to the next section, in which we consider the relation between enzyme, substrate and product. We shall first discuss the determination of the "associative tendency" of enzyme and substrate and then the way in which it is affected by the pH. These data often make it possible to draw conclusions about the presence of certain functional groups in the active centre.

**Determination of the association constant**

In a reaction of the form

\[
A + B \rightleftharpoons C + D, \\
\]

the rates of the forward and reverse reactions are respectively:

\[
v_{r} = k_{r} [A][B] \quad \text{and} \quad v_{-r} = k_{-r} [C][D],
\]

where \([A],[B],[C]\) and \([D]\) are the concentrations of
the substances concerned. The proportionality constants or reaction constants $k_1$ and $k_{-1}$ are a measure of the activation energy of the forward and reverse reactions. The effect of a catalyst is, of course, to lower the activation energy.

For our case we can write the equation:

$$k_1 \quad k_2$$
$$E + S \rightleftharpoons ES \rightarrow E + P + P'. \quad \cdots \quad (1)$$

The tendency of $E$ and $S$ to form the complex ES is expressed in the ratio $k_1/k_{-1}$, called the association constant. The substances $P$ and $P'$ are here benzoylarginine and ethyl alcohol. The reaction takes place in an aqueous environment. For simplicity, water has not been included in the reaction equation, because it is present in such large quantities that any changes in the water concentration due to the reaction process have no perceptible effect on the rate of the reaction.

It is not possible to derive exactly the reaction rate $v$ of the total reaction $(E + S \rightarrow E + P + P')$. In practice, however, the conditions are usually chosen in such a way as to permit certain simplifications, making it possible to write \[8\]:

$$v = \frac{ek_2 [S]}{[S] + 1/K}, \quad \cdots \quad (2)$$

where $e = [E] + [ES]$, i.e. the total amount of enzyme present, and $K = k_1/(k_{-1} + k_2)$. In most enzymic reactions of this type, $k_2$ is very much smaller than $k_{-1}$ and therefore $K$ is practically equal to $k_1/k_{-1}$; in this article we shall assume that this is the case. Equation (2) is usually written in the form:

$$\frac{1}{v} = \frac{1}{ek_2} + \frac{1}{ek_2 K[S]}, \quad \cdots \quad (3)$$

so that if measured values of $1/v$ are plotted against $1/[S]$ a straight line should be obtained. The intercept of this line and the $1/v$ ordinate is equal to $1/ek_2$, and the slope of the line is equal to $1/ek_2 K$. Both $k_2$ and $K$ can be calculated in this way from the results of the measurements (see fig. 4).

As a straight line can be plotted from the results of the measurements with our system it can be concluded that the enzyme reaction is in fact given by eq. (1) or to put it another way, that one molecule of papain attaches itself to one molecule of benzoylarginine to form a complex, and that one molecule

It has been concluded from the shape of the left-hand side of the curve that a COOH group must be present in the active centre and that this group must be in a negatively ionized state in order for the formation of the ES complex to be possible — just as for chymotrypsin. It can indeed be calculated that at pH = 3 only an extremely small number of such COOH groups are ionized, that at pH = 4 the fraction is roughly one half, and that at pH = 6 the ionization is virtually complete. This is therefore in very satisfactory agreement with the curve found for pH values lower than 6.

The identity of the group governing the form of the right-hand side of the curve is not yet known with any certainty. It is in any case not relevant to the investigation discussed here. We shall return later, however, to the possible existence of a COO\(^-\) group in the active centre of the enzyme.

**The relation between substrate and product**

What is the main problem in preparing an enzyme-substrate complex in crystal form? If we let papain in an aqueous environment react with benzoylarginine ester, the complex formed will hydrolyze much too quickly to allow an ES crystal to form, because of the excess of water. The obvious answer therefore seems to be to carry out the reaction with a very small quantity of water — or even entirely without water. Upon the formation of the ES complex the reaction then stops, which for our purposes can only be an advantage. The difficulty, however, is that papain, like all enzymes, must be in an aqueous environment if it is not to become denatured. In our work there is obviously no point in investigating the enzyme in its denatured form, in which it has lost its normal spatial structure and in which it cannot exercise its normal function.

To explain the way in which we have tried to overcome this difficulty, it will first be useful to examine in more detail the manner in which a reaction can be influenced by a "true" catalyst, that is to say a substance that accelerates the reaction without itself being chemically changed in the process (as are enzymes).

The attainment of equilibrium in a reaction such as

\[
A + B \rightleftharpoons C + D
\]

\[
\frac{k_f}{k_r}
\]

can be treated in both kinetic and thermodynamic terms. From the kinetic standpoint the equilibrium is established as soon as the reaction rate in the forward direction \(k_f[A][B]\) is equal to the reaction rate in the reverse direction \(k_r[C][D]\). In thermodynamic terms the equilibrium is established as soon as the free energy (actually the free enthalpy) of the whole system is at a minimum. Since, by definition, nothing of the catalyst is lost in the reaction, the presence of the catalyst can have no influence on the free energy of the system, and therefore the catalyst cannot affect the position of the equilibrium. In other words, the ratio between the reaction constants of the forward and reverse reactions \((k_f/k_r)\) cannot be changed by the action of the catalyst. This means that a catalyst that accelerates the forward reaction must accelerate the corresponding reverse reactions by the same factor. Thus, the terms substrate and product have merely a relative significance: a compound that is the product in the forward reaction is the substrate in the (equally accelerated) reverse reaction. For example, using papain as enzyme we can hydrolyze benzoylarginine ethyl ester in a dilute aqueous solution, and we then obtain benzoylarginine and alcohol. But we can also start from benzoylarginine in a solution of high alcohol content and in this situation, under the action of the same enzyme, benzoylarginine ethyl ester is formed.

As we have seen, the formation of a complex between the enzyme papain and the substrate benzoylarginine ester precedes the conversion into benzoylarginine and alcohol. If a product becomes a substrate by reversal of the reaction, it seems likely that this product will also form a complex with the enzyme. The idea behind our attempts at crystallization is therefore to try an opposite approach, that is to say one starting from benzoylarginine. Our aim is therefore no longer the crystallization of an ES complex but of an EP complex. An EP complex must also exist in an aqueous environment, but the presence of water can now do no harm: the danger is no longer hydrolysis but esterification. This danger can easily be avoided by allowing the complex to form in the absence of alcohol, which, unlike the omission of water, does not cause denaturation. It appears therefore very logical to tackle the problem from the opposite direction [6].

It should be borne in mind, however, that there is as yet no certainty as far as the product is concerned that a complex is really formed. For the substrate the existence of the complex has been demonstrated in the manner described on page 169; this method cannot, however be adopted for the product because the condition that the environment should have an excess of water (and hence little alcohol) strongly limits such experiments.

The confirmation that we wanted, that benzoylarginine forms a complex with papain, has been found from the investigations described in the next section. In all the experiments concerned the substrate is hydrolyzed in an aqueous environment and in each
we study the effect of addition of certain quantities of product on the rate of the reaction.

The interaction of enzyme and product

Competitive inhibition

We begin by postulating that in a reaction accelerated by "true" catalysts the forward and reverse reactions pass through the same stages. Proof of this postulate will be found in the literature [6]. If, as we wish, both the product and the substrate react to form a complex with the enzyme, so that the reaction follows the equation:

\[ E + S + H_2O \rightleftharpoons ES + H_2O \rightleftharpoons EP + C_2H_5OH \]

the product and substrate will both necessarily be bound in the same active centre of the enzyme. The occupation of an active centre by substrate molecules will then prevent the occupation of the same site by product molecules, and vice versa. The result is that the presence of the one will have an inhibitive action on the conversion of the other: this effect is known as "competitive inhibition".

In the experiments now to be described the benzoylarginine ester is hydrolyzed by the action of papain in the presence of a certain amount of benzoylarginine. For what we want to prove, the simplest course is to let the reaction take place without alcohol, and moreover to make the measurements only while the amount of alcohol formed during the reaction is negligibly small. This means in fact that we let the reverse reaction go no farther than the formation of the complex, so that we have the following reaction:

\[ E + S \rightleftharpoons ES \rightarrow EP \rightleftharpoons E + P, \]

where \( P \) represents benzoylarginine. The reason we want alcohol to play as little part as possible in the reaction is simply that we do not wish the reaction to be more complicated than is necessary for our experiments.

The rate \( v_1 \) of the reaction

\[ \frac{k_1}{k_2} E + S \rightleftharpoons ES \rightarrow E + P, \quad \ldots \quad (5) \]

which is competitively inhibited by the reaction

\[ \frac{k_1}{k_2} E + P \rightleftharpoons EP, \quad \ldots \quad \ldots \quad (6) \]

is found from a simple calculation [8] to be given by:

\[ \frac{1}{v_1} = \frac{1}{ek_2} + \frac{1}{ek_2K_8} (1 + K_P)[P] \frac{1}{[S]^l} \quad \ldots \quad (7) \]

where \( K_P = k_1/k_{-1} \) is the association constant of the enzyme-inhibitor complex \( EP \) and \( e = [E] + [ES] + [EP] \). To distinguish it more clearly, the association constant of the substrate will be represented by \( K_S \) in the following. (When \( [P] \) is zero the equation is identical with eq. 3.)

If we vary the concentration \( [S] \) of the substrate at a constant concentration \( [P] \) of the competitive inhibitor, then according to (7) we should find a straight line that intercepts the \( 1/v \) axis at \( 1/ek_2 \).

As already noted in fig. 4, the intercept on the \( 1/v \) axis is also \( 1/ek_2 \) in the absence of a competitive inhibitor. This is what one would expect, as given an infinitely high substrate concentration (\( 1/[S] = 0 \)) then the competition of the inhibitor molecules, which are present in a constant and thus limited concentration, can no longer be effective. This can be understood in another way: given an infinitely high substrate concentration all enzyme molecules contribute to the conversion of the substrate, whether an inhibitor is present or not, i.e. in both cases \( v = ek_2 \).

In fig. 6 the circles and crosses represent the results of two of our series of measurements to determine the rate of hydrolysis of benzoylarginine ester. One series

\[ 25 \times 10^{-3} M^{-1} min^{-1} \]

Fig. 6. Reciprocals \( 1/v \) and \( 1/v_1 \) of the rates of hydrolysis of benzoylarginine ethyl ester acted upon by papain, in the absence of benzoylarginine (circles), and in the presence of \( 3.35 \times 10^{-2} M \) benzoylarginine (crosses) at a pH of 4.6. The reciprocal of the concentration \( 1/[S] \) of benzoylarginine ethyl ester is plotted on the abscissa. The experimental points shown by circles are identical with those in fig. 4. From the fact that both lines intersect on the \( 1/v \) ordinate it may be concluded that benzoylarginine ethyl ester and benzoylarginine show competitive inhibition, which means that they are converted in the same active centre of papain.

[8] The same approach as ours has already proved successful with an enzyme other than papain; see the note at the end of this article.

of measurements was made in the absence of benzoylarginine and the other was made in the presence of a constant concentration of benzoylarginine. The two straight lines intersect on the \(1/\nu\) axis, which leads us to conclude that benzoylarginine ester and benzoylarginine bind with the same active centre of papain to form a complex. This confirms our prediction that the enzyme reaction concerned is in accordance with eq. (4).

The number of molecules involved in the complex formation

Let us now do just the opposite, and vary the concentration of the inhibitor, benzoylarginine, and we keep the concentration of the substrate, benzoylarginine ester, constant. Using eq. (3) we write eq. (7) in the form:

\[
\log \left( \frac{\nu}{\nu_1} - 1 \right) = \log \frac{[P]K_{p}\nu}{ek_2K_3[S]} = \log [P] + C, \quad (8)
\]

where \(C\) is a constant. If we plot \(\log(\nu/\nu_1 - 1)\) against \(\log [P]\), then according to eq. (8) we should find a straight line of slope equal to 1. This again enables us to test our assumptions.

Equation (8) is based on the reaction equations \(E + S \rightleftharpoons ES \rightarrow E + P\) and \(E + P \rightleftharpoons EP\), and this implies the assumption that the components in both complex formations \(E + S\) and \(E + P\) occur in the ratio of one to one. The first assumption has already been confirmed by the results presented in fig. 4. The second is now confirmed by the fact that a plot of \(\log(\nu/\nu_1 - 1)\) does indeed give a straight line of unit slope, as shown in fig. 7. (It is easily verified that a reaction equation \(E + nP \rightleftharpoons EP_n\) would have given a slope \(n\), as the concentration \([P]\) then occurs in eq. (8) in the \(n^{th}\) power.)

In the above, then, we have established that papain forms a complex both with the product and with the substrate through the action of one molecule per active centre. This is our second indication that the enzyme reaction is as given in eq. (4).

Influence of pH on the associative tendency

Fig. 8 shows the association constant \(K_P\) of the complex of papain and benzoylarginine as a function of the pH. The way in which this association constant is calculated at a given pH can be seen from equations (3) and (7) and fig. 6. From eq. (7) the slope of the upper line is equal to

\[
\frac{1}{ek_2K_3} (1 + K_P[P]),
\]

and from eq. (3), that of the lower line is equal to

\[
\frac{1}{ek_2K_3}.
\]

In these determinations the concentration \([P]\) of benzoylarginine is known, and therefore \(K_P\) can be directly calculated for any pH from the ratio of the two slopes. The curve thus found, which is shown in the figure, has a maximum at \(pH = 4\), and is quite different from the bell-shaped curve with its maximum at \(pH = 6\) which was found for the complex of papain and benzoylarginine ester (fig. 5). A reasonable explanation for this difference is given in the following.
We have already remarked that the shape of the latter curve in the pH interval from 3 to 6 may be attributed to the ionization of a COOH group at the active centre to a COO⁻ group. The ester group of the substrate remains neutral in this pH interval. The situation with respect to the action if the enzyme on the product is quite different. In addition to the COOH group of the active centre of the enzyme the COOH group of the product can also be ionized.

At pH = 8 the ionization of both COOH groups is complete. At this pH value the COO⁻ groups of the product, having like charges, repel each other, with the result that the tendency to form a complex is very slight. At pH = 6 the product is still largely in its ionized form, and there is no appreciable increase in the fraction of the product molecules having a neutral carboxyl group until there is a further decrease in pH. Only then may we expect the association constant to increase. This state continues until the COO⁻ group of the active centre is also neutralized. We already know that this has the effect of inactivating the active centre. Owing to the presence of the COOH group in the product molecule the maximum in the association curve shifts towards a lower pH. This explains, in qualitative terms, the difference between the curves in fig. 5 and fig. 8. A more quantitative comparison can however also be made.

Plainly, for quantitative comparison of the association curves of the substrate and the product, the product with the uncharged carboxyl group (which we shall call P₁) must be set against the substrate with its likewise uncharged ester group. We shall do this in the pH region from 3.7 to 5.5, where the inhibition is mainly attributable to P₁. At pH = 8 the inhibition is very much less and is almost entirely due to product molecules with an ionized carboxyl group (which we shall call P₁I).

To calculate the association constant Kᵣ of P₁ we should write eq. (7) in a modified form. In the first place Kᵣ, which relates to both inhibitors together, has to be corrected for the inhibition of P₁I. A rough but sufficiently accurate correction is to subtract the amount Δ indicated by the dashed line in fig. 8: instead of Kᵣ we now write Kᵣ + Δ. Secondly, we must substitute for the concentration [P₁] the concentration [P₁I] of the non-ionized form. We calculate [P₁I] for each pH value with the aid of the known value of the dissociation constant of the relevant COOH group:

\[ K_{\text{diss}} = \frac{[\text{COO}^-][\text{H}^+]}{[\text{COOH}]} = \frac{[\text{P}_1][\text{H}^+]}{[\text{P}_1]} \]

The association constant Kᵣ of benzoylarginine as a function of pH are shown by crosses and circles respectively. The agreement may be described as very satisfactory.

Investigation using other substrates

Finally, we shall mention two results, found with other substrates. Instead of using benzoylarginine ester as a substrate, other investigators have used benzoylglycylglycine [7], a compound which, like benzoylarginine, contains a COOH group. In agreement with our assumptions the association constant was found to decrease as pH increased from 4 to 9 (fig. 10), as we found for benzoylarginine. If the investigations had been extended to pH values lower than 4, a corresponding decrease would presumably have been established.

We would also like to mention an investigation of our own, which used benzoylglycine ester as substrate. This substrate is converted by papain much more slowly than benzoylarginine ester. The question of interest here was whether the product also might be a poorer inhibitor than benzoylarginine (which, on the basis of our assumptions, was possible but not neces-

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Fig. 10. Association constant $K_s$ of benzoylglycylglycine as a function of pH in arbitrary units. (Due to E. L. Smith, V. J. Chavré and M. J. Parker [7].)

sarily so). This proved to be the case, the inhibitor action being six times smaller. Thus, the specificity of papain with respect to the substrate is found here

to be reflected, as it were, in its specificity with respect to the product.

We may summarize the results of our investigation by saying that we have established a certain degree of agreement between the chosen substrate and product. It would certainly be interesting to go into the question of whether this is a general rule, but for the time being it remains a pure speculation. For our immediate purposes it is sufficient to have found a number of indications that we have in fact chosen an appropriate "product" as substrate for the formation of a crystalline enzyme-substrate complex.

**Note upon going to press:** When this article was being prepared for publication, a report appeared on the successful crystallization of EP complexes as discussed in the foregoing, using the enzyme lysozyme (E. N. Johnson and D. C. Philips, Nature 206, 761, 1965). From the X-ray analysis results obtained so far it was possible to localize the active site and also to determine with a very high degree of certainty which groups of the enzyme lysozyme are involved in the formation of the complex. Efforts are being made to refine the procedure in order to obtain a more exact picture of the binding mechanism. From the experimentally determined fact that the relevant products are competitive inhibitors of the enzymic action, it may be concluded that the active centre investigated is also the site where the substrate is broken off by the enzyme.

Summary. Like all true catalysts, enzymes accelerate not only a forward reaction, but also a corresponding reverse reaction. The product of the forward reaction will therefore be the substrate of the reverse reaction, and it is to be expected that the interaction of enzyme and substrate will agree in certain respects with the interaction of enzyme and product. This theory was put to the test with the enzyme papain, use being made of a simple synthetic substrate (benzoylarginine ester) and the corresponding product (benzoylarginine). Five indications in support of the hypothesis were deduced from kinetic experiments.

This investigation was necessary as a preparation for attempts now being made to crystallize an enzyme-substrate complex, with a view to making possible an X-ray analysis of such a complex. The difficulty encountered when using benzoylarginine ester as substrate is that the complex hydrolyzes too quickly. The experimental indications make it seem likely that the product benzoylarginine can be made use of, in a suitable way, to obtain the required complex.
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D. Alma: Het gebruik van doorzichtkaarten bij het vastleggen en analyseren van gegevens. Sigma 11, 105-109, 1965 (No. 5).


J. van den Broek: Contact barriers in red lead monoxide. 
Philips Res. Repts. 20, 674-683, 1965 (No. 6).  

K. H. J. Buschow: Phase relations and intermetallic compounds in the systems neodymium-aluminium and gadolinium-aluminium. 
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