KINETICAL COMPARISON OF THE ACIDIC, ALKALINE AND ENZYMATIC HYDROLYSIS OF STARCH*

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(Received June 10, 1969)

Reaction kinetical investigations are of similar, prominent importance both from theoretical and practical aspects. From theoretical point of view they furnish very valuable data for the elucidation of the mechanisms of reaction. Their practical significance manifests itself, in turn, in the field of following, directing and controlling the individual industrial processes.

All the data published thus far in respect to rates of splitting and of formation of glycosidic bonds conducted in various ways, to the orders of these reactions and to their dependence on temperature and other parameters are also very important in the case of starch. Thanks to these data of literature, the overall reaction mechanisms characterizing these processes are today already known, it is possible to make *distinctions between* the probability of splitting of the glycosidic bonds present in starch. Reaction kinetical knowledge and data are for the time being indispensable in the various industrial processes of starch hydrolysis as well.

In our Institute, quite a number of splitting and formation reactions of glycosidic bonds had been investigated by kinetical methods. The results of these investigations are particularly suited for drawing useful conclusions if the investigations had been conducted with the same substance and the kinetical constants obtained in the various processes can be compared. In the present case starch is this common substance or at least one of its well-defined fractions (e.g. amylose, amylopectin etc.).

The first investigations of this character had been carried out by the late professor of our Institute, Sigmond, more than 50 years ago, by comparing the acidic and enzymatic hydrolysis of glycosidic bonds. In the last 15 years, in turn, we dealt with the reaction kinetical investigation of the alkaline, acidic and enzymatic hydrolysis and synthesis of starch. In the followings, we desire to present a comparison of the results of these investigations.

* 73rd publication on polysaccharide research from this Institute. On the occasion of the 50th anniversary of Professor J. Holló, the Polysaccharide Research Group express their very best wishes.

1. The alkaline degradation of amylose

It is known from literature that in a medium free of oxygen the degradation of amylose follows the "peeling" reaction [1], and the end product is isosaccharic acid while in the presence of oxygen also the non-terminal bonds are split and also other acids (lactic acid, hydroxybutyric acid etc.) are formed [1, 2].

These reactions were investigated from kinetical aspects as well. It was found that the splitting of bonds can be described in both cases by rate equa-

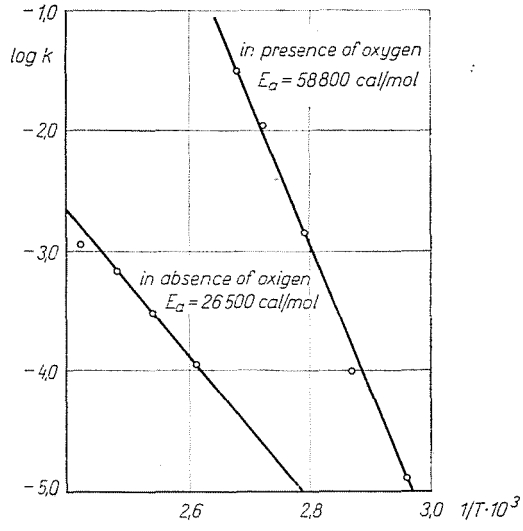

Fig. 1. Effect of oxygen on the Arrhenius diagram of the alkaline hydrolysis of starch

tions of first order [2, 3]. This is in accordance with the general experiences according to which, if the catalyst concentration is constant, the rate of reaction is determined only by the concentration of the decomposing substance.

Great differences exist between the rate of the "peeling" reaction and that of the reaction taking place in the presence of oxygen [2, 3]. However, the reactions of the two types differ from each other also in respect to the rate-increasing effect of temperature. On plotting the logarithmic values of the rate constants of first order against the reciprocal values of temperature, the two straights presented in Fig. 1 are obtained. From this it follows that the rise of temperature encourages the splitting of non-terminal bonds. The splitting of non-terminal bonds, in turn, results in the formation of newer reducing end groups. This process explains the essentially higher reaction rates experienced in the presence of oxygen. The same follows also from the values of the activation energy which proved to range 26 500 cal/mole and 58 800 cal/mole, respectively [3].

2. The acidic hydrolysis of starch

In the acidic hydrolysis of starch the end product is glucose. For a long time, the single glycosidic bonds had been characterized by identical probabilities of splitting. FREUDENBERG was the first to point out that the rate constants measured in the initial period of hydrolysis and at 50 per cent hydrolysis are not identical [4]. The latter value is the same as the rate constant experienced in the hydrolysis of maltose. According to our measurements [5]

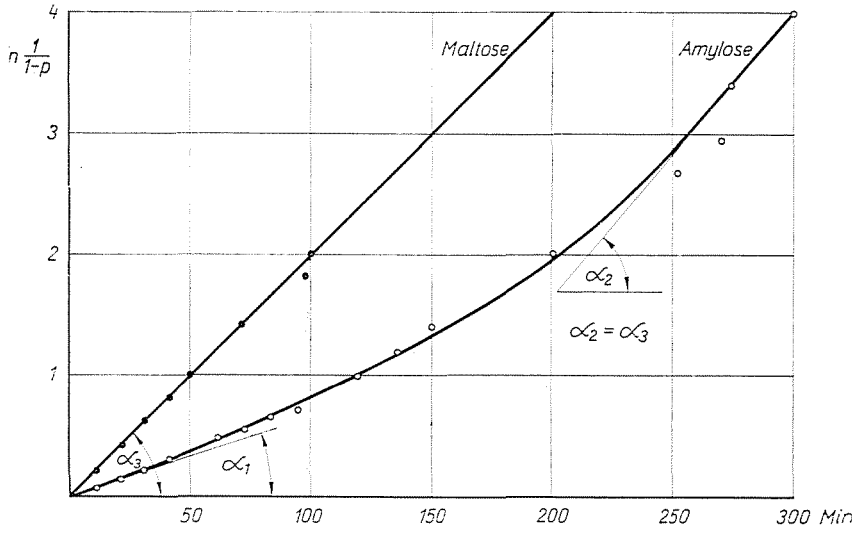

Fig. 2. Changes in the reaction rates of the acidic hydrolysis of starch

the rate constant measured in the initial period shows a continuous rise until it attains the level characteristic of maltose (Fig. 2). The rate difference follows from the quicker splitting of the terminal glucose units [6]. Of the reducing and non-reducing terminal glucose units, the non-reducing ones showed a higher probability of splitting [7]. These experimental facts may be explained by the following theories.

According to a generally accepted presumption [8], the acidic hydrolysis of glycosidic bonds takes place in two phases: in the first one, the activation complex is formed which consists of a glucoside component and of a proton component, then this complex undergoes decomposition simultaneously with the splitting of the glycosidic bond. This second step is a monomolecular reaction in which essentially the splitting of a water molecule is caused by the carbonium cation representing the activation complex. As regards the mechanism of the process two routes are actually possible (Fig. 3).

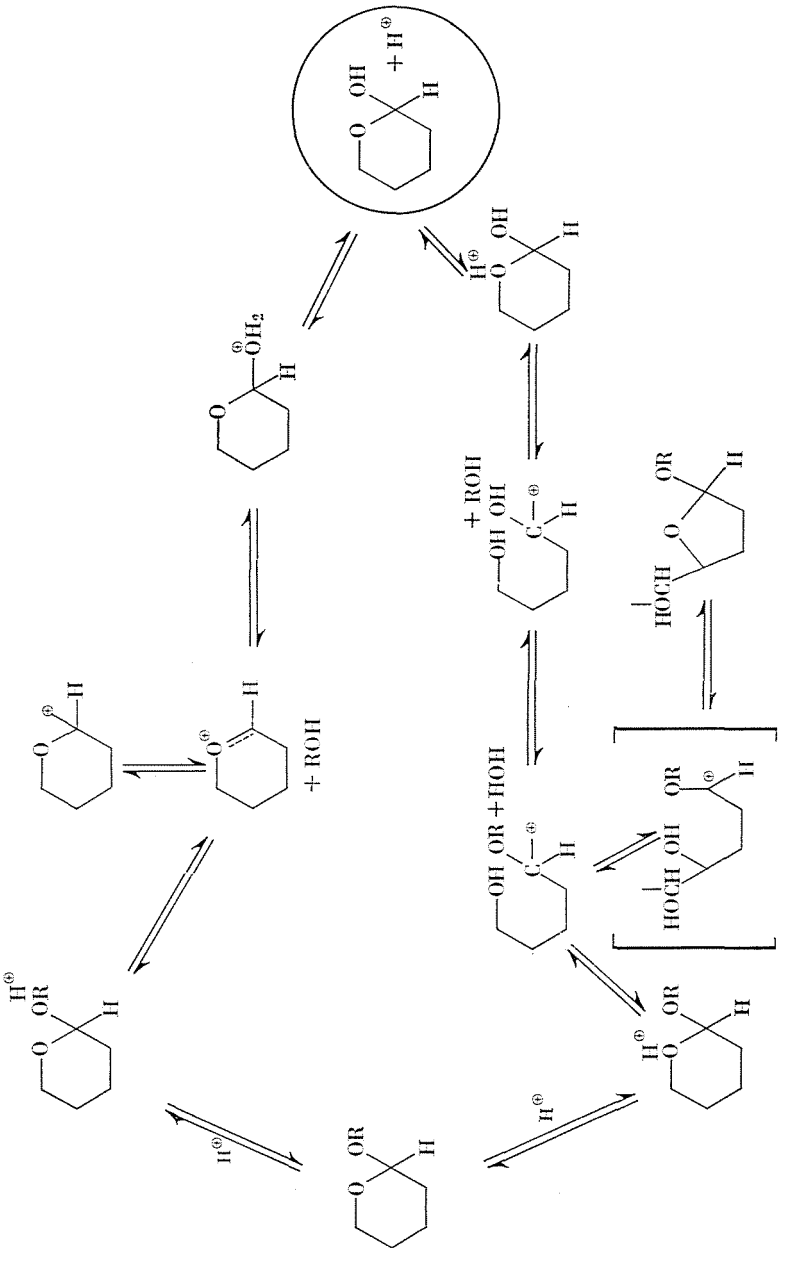

Fig. 3. Two-step and three-step schemes of the acidic hydrolysis of glucoside bonds

One of the possible routes of the reaction is a so-called "two-step" scheme [9]. According to that, the proton is accepted by the oxygen atom of the glycosidic bond. In this way, the activation complex is being formed. This complex, after the uptake of the required amount of energy undergoes splitting, and the created carbonium cation is stabilized by the hydroxyl group split off the water molecule.

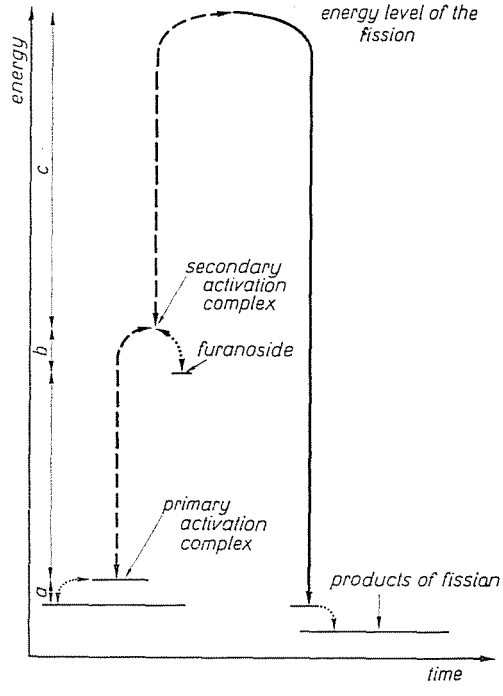

Fig. 4. Energy scheme of bond splitting in acidic starch hydrolysis

On the basis of the available experimental data, in our opinion however the other, so-called "three-step" scheme appears to be more likely.

According to this scheme, the hydroxonium ion and the proton, respectively, is built up on oxygen, the heteroatom of the ring. The equilibrium state of the formed so-called "primary activation complex" depends to a great extent on the charges present in its environment. This means that in the case of polyglucosanes the rings in the inside of chains undergo protonation more difficultly than the terminal rings [10].

The mentioned primary activation complex possesses only a relatively small energy excess in respect to the initial ring. The energetic scheme of bond splitting is shown by Fig. 4. Owing to the uptake of the "primary activation energy", the complex is lifted up to the energy level of acyclization.

However, the acyclic form is very unstable because a free carbonium cation is formed on the 1-positioned carbon atom. This is short-lived. It either loses the full amount of energy taken up and returns to its initial "state of primary activation complex" (eventually losing also its proton), or delivers only a small part of its energy and is stabilized by temporarily converting into a furanoside ring. Obviously, only the non-reducing type of terminal rings is capable of that (i.e., only the non-reducing end of polyglucosanes and, all other glycosides with a 4-positioned free hydroxyl group). The furanoside ring is readily formed from the acyclic form but it is rather labile. On taking up the energy amount designed by *b* in Fig. 4, the ring recovers again its acyclic form. This form of an extremely short life which represents the highest energy level of all the forms mentioned so far is the so-called "secondary activation complex". If it takes up in this state the energy surplus designed by *c*, it rises to the energy level of splitting. If it passes through this maximum value of the potential barrier, the bond is split, if however it cannot pass through this barrier, any of the already mentioned forms may be recovered. The essential feature of the mechanism of catalysis is just this partition of the otherwise high potential barrier in this way [10].

In the course of the investigation of the role of acid concentration it has been proved by both our experimental results and by data of literature that the rate constant depends exponentially on the hydrogen ion concentration. According to our measurements the value of the exponent ranges 1.070. (An exponent of this type but of a different value has been calculated also from the data of our experiments of the hydrolysis of inulin [12].

In this way we succeeded in making possible the comparison and precalculation of rate constants measured under various conditions. Namely, the so-called reduced rate constant k_r which is, at unit hydrogen activity identical with the theoretically observable rate constant, can be expressed by an equation.

At the investigation of the role of temperature it has been pointed out that it is not expedient to keep the concentration of the applied acid at a constant level because its hydrolysis-catalyzing activity depends on the temperature as well. Thus, the values of activation energy determined in this way are to be corrected. All the k values had been converted into to k_r values by means of the above mentioned equation, and the obtained values have been plotted against 1/T. The value of activation energy established in this way ranged 29 600 cal/mole [13].

3. Enzymatic hydrolysis of starch

On the basis of own experimental results and data of literature, the kinetical investigation of such enzymatic hydrolysis processes has been carried out which can be brought into some correlation with the afore-discussed chemical processes.

In respect to the probability of splitting of the glycosidic bonds, the alpha-amylolysis of starch can be compared to the acidic hydrolysis. It is gen-

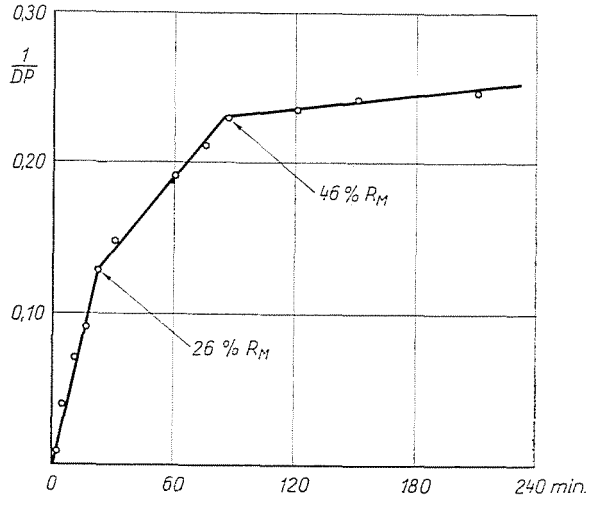

Fig. 5. Vink diagram of alpha-amylolysis of starch

erally accepted that in the case of substrates of high molecular weight, the splitting of bonds is statistical, independently of the origin of the enzyme type applied [14].

However, enzymatic hydrolysis essentially differs from acidic hydrolysis in that in the case of alpha-amylolysis, the glycosidic bonds of the transitionary decomposition products of low molecular weight decompose slower than the bonds of similar type in the large molecules [14]. This difference reflects reliably the deviating mechanisms of the catalysts of two types though actually quite identical processes take place in both cases. The decreased affinity of the enzyme to the maltodextrins is responsible for the rate decrease observed in the amylolysis of maltodextrins of low molecular weight. In the case of alpha-amylases of various type this affinity may show variations, and thus also the amount of transitionary products accumulating during hydrolysis may undergo changes.

The changes in the transitionary products are demonstrated by our results obtained on using pancreas-amylase [15]: on investigating the course

of amylose hydrolysis in time according to VINK, it has been decomposed to three sections (Fig. 5). The distribution of molecular weights in the samples characteristic of the various sections has been established by means of Sephadex gel filters. On the basis of the elution diagrams, the amounts eluted at the points of the degree of polymerization 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2 and 1, were plotted against the percentages of R_M (Fig. 6). It can be seen that the

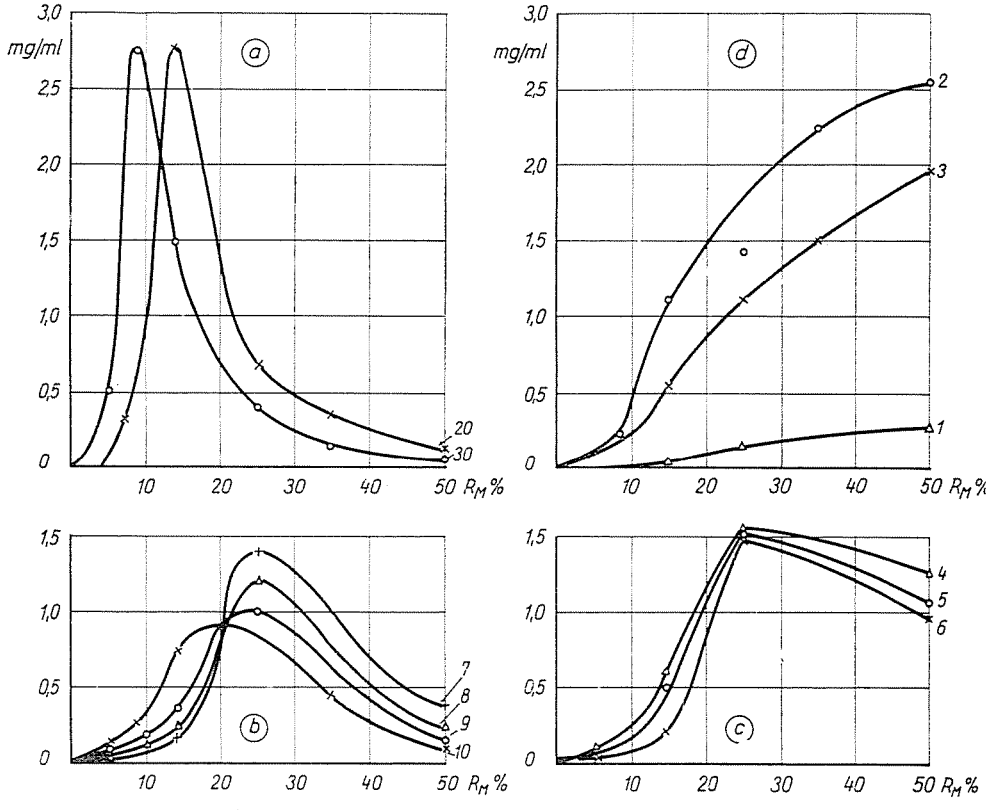

Fig. 6. Changes in the distribution of molecular weights in alpha-amylolysis

first section of Fig. 5 is actually characterized by the formation of intermediates (of a degree of polymerization from 4 to 10) from amyloses of higher degrees of polymerization. The second section, in turn, is characterized by the formation of products of degrees of polymerization of 4, 5, 6 and of end products (of degrees of polymerization of 3, 2, 1), respectively, from a part of intermediates (from the decomposition of those of degrees of polymerization of 7 to 10). Lastly, in the third section, the products of degrees of polymerization of 4, 5, 6 are decomposed to end products.

Thus, the products formed in the three different sections of hydrolysis can be classified into the following groups:

a) Substances with a degree of polymerization over 20: the higher are their degrees of polymerization, the more are they formed in the beginning of hydrolysis (ranging from 0 to 15 per cent) in maximum amounts; later their amount quickly decreases (Fig. 6a).

b) Substances with a degree of polymerization from 7 to 10: these appear in maximum amounts at 20 to 25 per cent of hydrolysis; later their amount decreases (Fig. 6b).

c) Substances with a degree of polymerization from 4 to 6: they occur in maximum amounts at 25 to 30 per cent of hydrolysis; later their amount very slowly decreases (Fig. 6c).

d) End products with a degree of polymerization of 3, 2 and 1: these show a steadily rising tendency. Maltose is formed in the greatest amount, then, from 15 per cent of hydrolysis, also triose and small amounts of glucose are detectable (Fig. 6d).

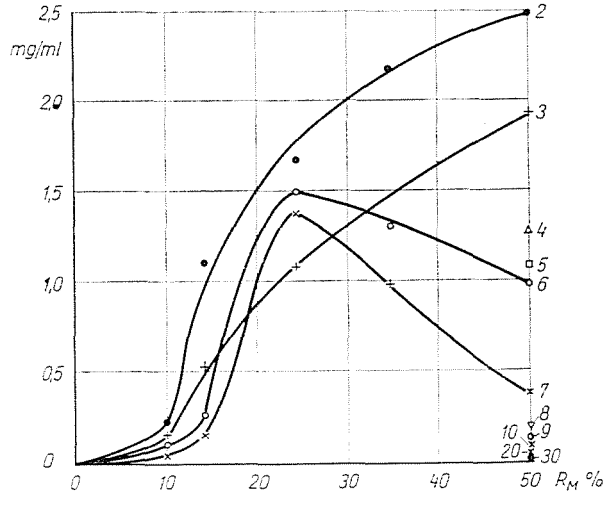

Fig. 7. Changes in the amount of transitionary products in alpha-amylolysis

A representant of each of these substances has been shown in Fig. 7 where also the amount of the other products at a hydrolysis value of 50 per cent is also given. From that it can be seen that the products with a degree of polymerization of 4 to 6 decompose at a rate much slower than the products with a degree of polymerization over 7. Great differences were observed in the decomposition rates of the products with a degree of polymerization 6 and 7. Consequently, also the accumulation of the product with a degree of polymerization 7 is slower. Also the other intermediate products with odd

degrees of polymerization decompose at higher rates than the corresponding products with an even number of polymerization degree. Still, the most striking difference appears between malto-hexaose and malto-heptaose.

For purposes of comparison with the activation energy of acidic hydrolysis, the results of ONO et al. [16] concerning the alpha-amylase enzyme of

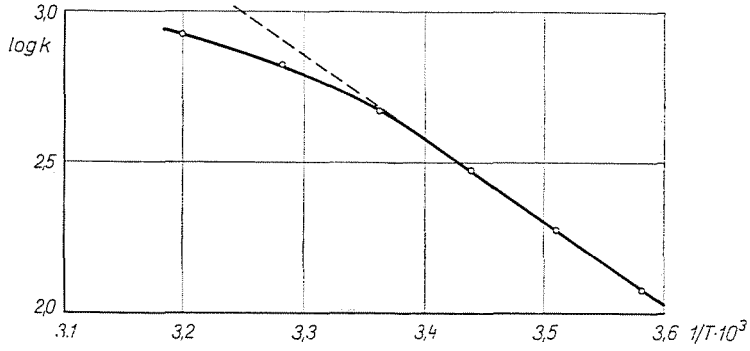

Fig. 8. Arrhenius diagram of the decomposition of amylose by alpha-amylase

Bac. subtilis are mentioned here. In enzymatic hydrolysis, the rate constants k_3 calculated from the rates show a linear change in the temperature range from 6 to 25 °C while at higher temperature this linearity disappears (Fig. 8). The activation energy calculated for the temperature range from 6 to 25 °C is 12,5 kcal./mole, in contrast to acidic hydrolysis where the dependence on

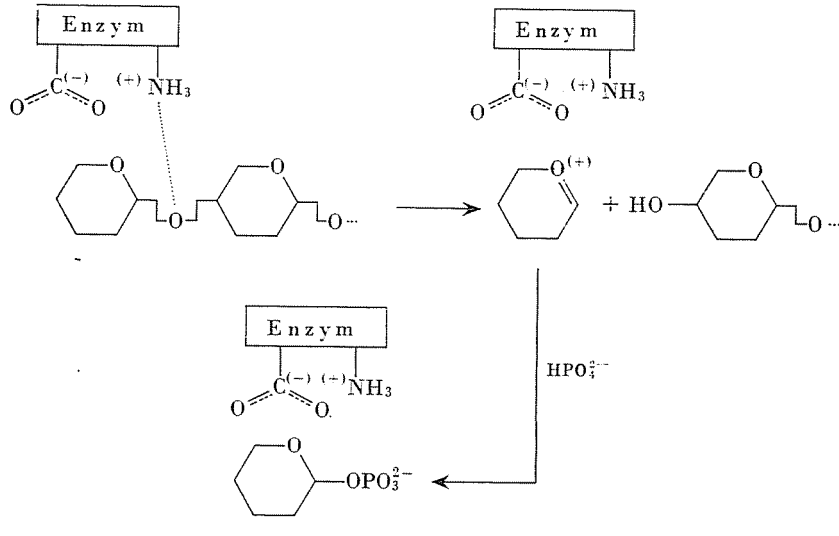

Fig. 9. Scheme of bond splitting in phosphorolysis according to NAKAMURA and KOSHLAND,

temperature is linear in the entire temperature range, and the activation energy, as mentioned above, is 29600 cal./mole.

Similar correlations can also be established between the enzymes acting on the terminal groups and the chemical catalysts. The stepwise alkaline degradation, though the end product is not carbohydrate, can be compared on reaction kinetical basis with the stepwise enzymatic degradation, e.g. with degradation by amyloglucosidase, beta-amylase, phosphorolysis etc.

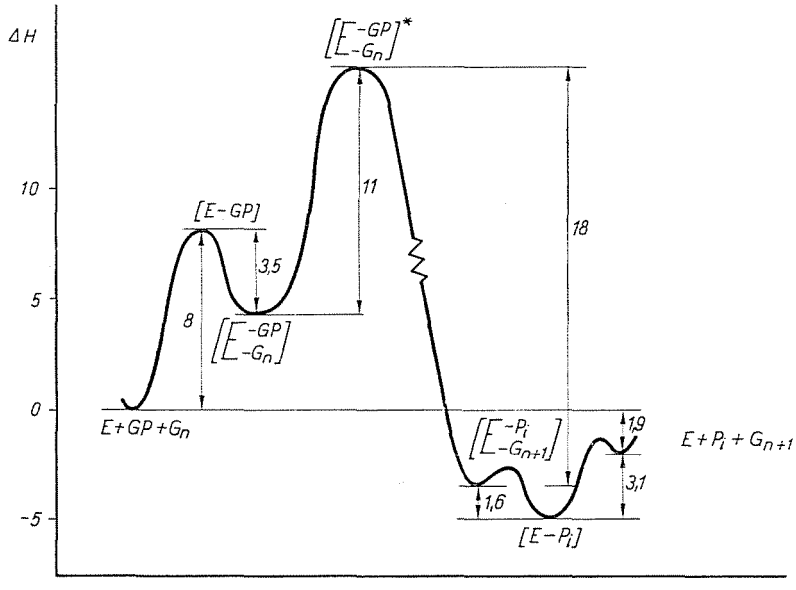

Fig. 10. Energy scheme of phosphorylase

According to NAKAMURA [17] and KOSHLAND [18], the hypothetical mechanism of the phosphorolysis of the alpha-1,4-glycosidic bonds is as follows (Fig. 9). It is linked to the hemiacetalic oxygen of the substrate through the carboxyl group of phosphorylase combined with the proton, the ring is split and an octet deficiency is created on the carbon atom of the carbonyl group. A HPO_4^{2-} ion is coupled to this carbonylic carbon atom in a non-enzymatic process, the hemiacetal ring is reformed, and the glycosidic bond is split, due to the rearrangement of electrons.

This hypothesis has not been completely proved by our experimental results. Namely, in the case of potato phosphorylase we have found that also the inorganic phosphate is coupled to the enzyme molecule [19], i.e., not only chemical linkage is present as mentioned above. (The bonding of inorganic phosphate takes place through a group with a pK value of 5.9.) On the other hand, the pK value of the group responsible for the bonding of polymers has been determined from reaction kinetical data (7.18 at 30 °C). Its heat of ioni-

zation ranged 6700 cal/mole [20], thus it is likely that the site of bonding is an imidazole group (and not a carboxyl group as stated in the above mentioned hypothesis).

On utilizing the thermodynamical data calculated from our experimental results, the energy scheme of the equilibrium reaction catalyzed by potato phosphorylase has been constructed [21]. It can be seen in Fig. 10 that the activation energy of the synthesis of the alpha-1,4-glycosidic bonds is 11.6 kcal./mole while the activation energy of the phosphorolysis of the same bonds ranges 18.6 kcal./mole. In the literature, activation energies similar to that given for phosphorolysis may be found also for the other two enzymes (e.g. 16 200 cal. for beta-amylase at 0—20 °C and 5530 cal. at 20—50 °C), which values are similarly lower than those calculated in the acidic and alkaline degradation.

4. Comparison of various catalysts of the decomposition of starch

An interesting reaction kinetical comparison can be made between the above-discussed chemical and enzymatic catalyses. According to the Arrhenius-type way of plotting, diagrams were prepared from the rate values obtained under identical conditions (identical temperature range, nearly identical substrate concentrations, identical catalyst concentrations etc.) (Fig. 11). Also the afore-mentioned requirements of activation energy can be read in this figure. However, even more interesting data are obtained on investigating the rate constants observed with various catalysts at a constant temperature (e. g. at 25 °C), recalculating them to an identical catalyst concentration, e.g. 1 mole. It must be noted that the rate constants of the acidic and alkaline hydrolysis have been obtained by extrapolation because the reaction periods would be too long at 25 °C.

Table I

Degradation of starch with different types of catalysts at 25 °C

Catalyst	Calculated constant of velocity min^{-1}	Relative velocity
KOH (N_2)	$3.5 \cdot 10^{-9}$	11
KOH (O_2)	$3.2 \cdot 10^{-10}$	1
H_2SO_4	$3.2 \cdot 10^{-6}$	10^4
HCl	$3.2 \cdot 10^{-6}$	10^4
Phosphorylase	$7.9 \cdot 10^{-3}$	$2.5 \cdot 10^{13}$
Alpha-amylase	$2.5 \cdot 10^{-4}$	$8 \cdot 10^{13}$

It can be seen in Table I. that in a medium free of oxygen, the stepwise alkaline degradation takes place at a rate lower by about 3 orders of magnitude than that of the acidic hydrolysis characterized by the statistical splitting. This ratio is approximately corresponding to the degree of polymerization of amylose. Between the rates of the acidic hydrolysis and of alpha-amylolysis of similar character a difference of 9 orders of magnitude exists while the difference between the rates of alkaline degradation and phosphorolysis ranges 12 orders of magnitude.

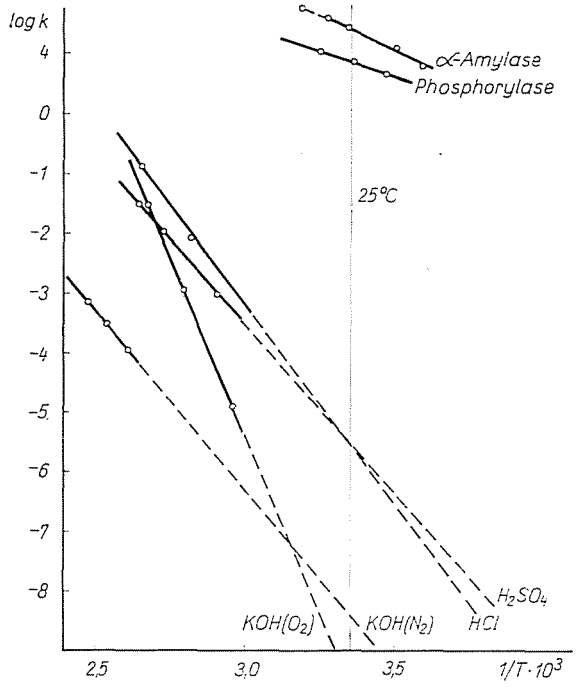

Fig. 11. Combined Arrhenius diagram

Accordingly, these enzymatic catalysts possess an activity greater by 10^9—10^{12} than the chemical catalysts. This massive rate increase had also been observed with proteolytic enzymes, and attempts were made to explain it by various theoretical presumptions, e.g. by presuming that the substrate molecules are expanded on the enzyme molecules, and thus the bonds become more sensitive against protons, or that the protons may accumulate around the bond to be split, or that the ratio of efficient collisions increases since in the enzyme several reaction partners are locally fixed, due to functional groups [22].

The elucidation of the problems is extremely important from theoretical and practical aspects. This is the cause why we have recently dealt with these

problems and why we attempted to clear up the correlations between the starch hydrolyses catalyzed in various ways. The scope of the present discussion was to offer a short survey.

Summary

On the basis of our experimental results and of literature data, we compared the hydrolysis of alpha-1,4-glucosidic bonds in proton-, hydroxyl- and enzyme-catalyzed reactions. That for identical catalyst concentration, proton catalysis was found to take place at a speed 10^4-times, and enzyme catalysis at 10^{13}-times the velocity of hydroxyl catalysis.

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