CONDUCTOMETRIC INVESTIGATION OF ENZYMATIC UREA HYDROLYSIS IN A SELF BUFFERING SYSTEM

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abstract: The kinetics of urea hydrolysis in the presence of urease from sword beans was studied in a batch reactor in unbuffered aqueous media at high substrate conversions. The kinetic analysis was based on a conductometric method, at constant temperature. The variation of pH in the same experimental conditions was also recorded. The reaction rates were calculated by numerical derivation of the integral kinetic curves obtained from a conductance-concentration calibration curve. The kinetic analysis was based on both initial rate and extended progress curve methods. The selfbuffering of the system is a consequence of products accumulation which has also an inhibitory effect

Introduction

As a natural compound urea is a waste product created during protein metabolism. Today it is the most important solid fertilizer. When applied to soil it reacts with water in the presence of urease to produce plant-available ammonium. As a result of urea hydrolysis a fraction of ammonia is lost to the atmosphere [1], a process that greatly impacts its management as fertilizer. Numerous studies were consequently devoted to the controlled inhibition of urea hydrolysis.

On the other hand, urea and its hydrolysis product – ammonium ion – play an important role in analytical and clinical chemistry being linked to many processes such as blood and urine analysis, kidney failure and artificial kidney control, as well as in treatment of waste water in food and drug analysis.

To monitor the reaction progress, a number of experimental techniques were used. The most of them followed the ammonium concentration using an ammonium ion selective electrode, an acid-base titration, a spectrophotometric method in connection with Nessler's reagent or with the coupling to the glutamate dehydrogenase reaction, etc.

As an alternative, two nonspecific techniques – calorimetry [2,3] and conductometry[4,5] – were analyzed and tested as potential methods for kinetic studies of urea/urease system. However, except for several recent assay methods using the conductance changes[6,7], only few kinetic studies were reported on this subject.

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The present paper gives some preliminary results concerning the kinetics of urease catalyzed urea hydrolysis without prior addition of a buffer. The selfbuffering is subsequently ensured by the reaction products simulating the natural conditions for urea hydrolysis. The increase of the buffer capacity of the system during the reaction progress results also in a pH increase, from 8.75 to 9.15 which is then stabilized during a short period of the reaction course.

Experimental

The hydrolysis of urea p.a. purchased from Chimopar SA in the presence of sword bean urease from Merck was studied in an unbuffered system. The reaction was studied using a conductometric method. The initial urea concentration was ranging between 0.0125M and 0.125M and the initial concentration of urease was 2.18 10^{-7}M.

A known quantity of urea was completely hydrolyzed in a closed vessel in the presence of urease. The final solution was successively diluted and the corresponding conductances were measured using a Radelkis OK 102/1 Conductivity Meter and a standard cell with k=1.559 cm^{-1}.

The pH variation during a kinetic run was measured using a Corning Ion Analyzer 250. All measurements were performed in thermostated vessels open to atmosphere, at (298 ±0.1)K

For each kinetic run, the conductance(C) of solution was measured until its change became negligible.

Results and Discussion

While the common approach in enzyme kinetics makes use of constant pH conditions ensured by adequate buffers, for our investigation intended to simulate the natural hydrolysis conditions, the pH variation during a kinetic run at T=298K is dependent on both urea and enzyme initial concentration. This behavior is also different from other literature data when CO_2 was used to maintain a constant pH and to avoid the presence of buffer ionic species, also interfering with urease catalytic activity[8]. A typical result obtained in the absence of any other added buffering species is given in Fig. 1.

During the initial period the selfbuffering of the system is accompanied by a continuous pH increase stabilized at 9.15. This behaviour indicates that, for an easier kinetic analysis, the kinetic measurements must be either extrapolated to the initial conditions (pH=8.2) or analysed for pH=9.15

The calibration curve, conductance versus hydrolysis product concentration, is given in Fig.2. According to the most recent opinions, ammonium carbamate is the true product of urease catalyzed hydrolysis of urea, and the final products, bicarbonate and ammonium ions are formed by nonenzymatic and buffer-dependent decomposition of ammonium carbamate[8]. The complex equilibria involved in this system lead to a continuously changing composition dependent on the reaction extent. Consequently the hydrolysis

product concentration was taken as the corresponding equivalent concentration of the completely hydrolyzed urea.

A possible source of errors regarding this system originates in the vapour-liquid equilibria involving CO_2 and NH_3. For the corresponding maximum ammonia and carbon dioxide concentrations, significant NH_3 and CO_2 partial pressures result (.046 and 11.76 torr, respectively)[9]. During a kinetic run, a significant quantity of NH_3 can leave the system, modifying the solution composition. Although this is an obvious loss, it was neglected in the published papers. Only recently the deviation of the experimental pH versus time curve from the calculated one was attributed to this effect [8].

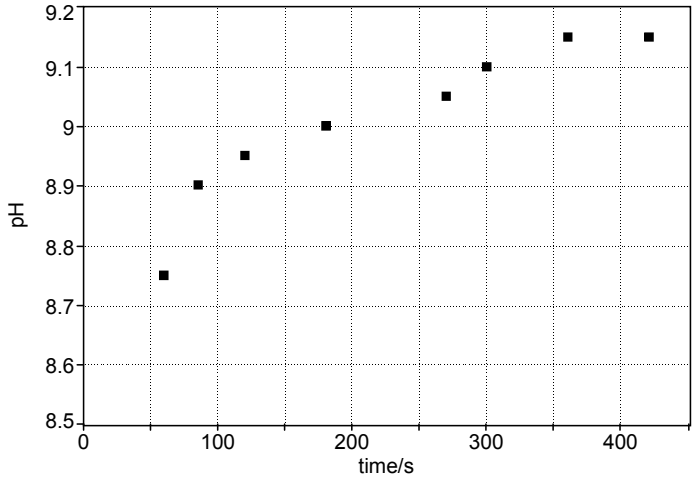

Fig 1. *pH variation for urea 0.091M hydrolysis in the presence of urease*

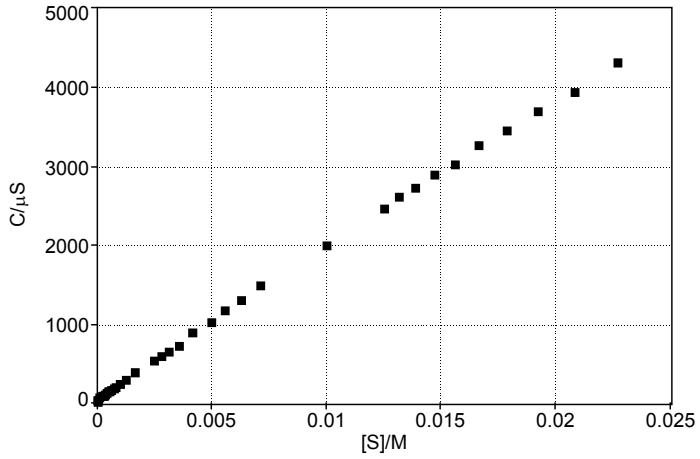

Fig 2. *The calibration curve*

As a first approximation, the calibration curve can be considered as linear ($C = a + b[S]$) within the given concentration range with the following regression parameters: $a = 15.6 \pm 5.2; b = (1.894 \pm 0.077)10^5; r = 0.9994$

However, safer results, especially for lower concentration range, can be obtained if the same data are used for a nonlinear regression of the form $C = a + b[S]^c$, with the estimated parameters: $a = 15.6 \pm 5.2;\ b = (1.53 \pm 0.29)10^5;\ c = 0.9441 \pm 0.0049;\ r = 0.9998$

This quasilinear dependence of the system conductance as a function of hydrolyzed urea was previously used to design various biosensors for urea determination or for heavy metals determinations based on their inhibitory effect on the rate of urea hydrolysis[10].

Using the calibration curve, the concentration of product ([P]) for each kinetic run was obtained and the substrate concentration ([S]) was calculated as:

$$[S] = [S]_0 - [P] \tag{1}$$

Several kinetic curves for different initial urea concentrations and the same urease concentration are given in Fig. 3.

For the estimation of the kinetic parameters corresponding to Michaelis – Menten equation:

$$v = \frac{v_{\max}}{1 + \frac{K_M}{[S]}} \tag{2}$$

two approaches were considered: the initial rate method and the extended progress curve method.

The initial rates of reaction were calculated as the slopes of the linear portion of [S]=f(t) curves by a method of linear regression. The results, as initial rates versus initial urea concentrations, are given in figure 4. The presence of a maximum on this curve suggests a substrate inhibition. However, the model of a noncompetitive substrate inhibition cannot be fitted satisfactorily on the experimental data. This can be attributed to the pH changes of the medium during the early stages of reaction, which have a strong effect on the extrapolation procedure for initial reaction rates. At the same time the pH changes are the result of product concentration increase, which acts itself as inhibitor for urease, even during the early stages of the reaction [8].

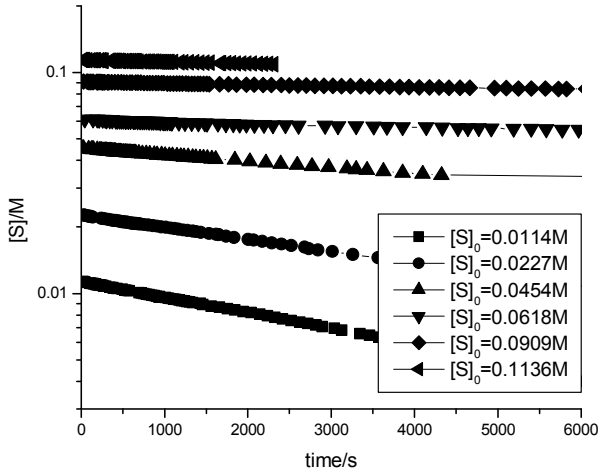

Fig 3. *Integral kinetic curves for urea hydrolysis in the presence of urease*

The results indicate that at initial concentrations of urea greater than 0.040M some inhibition phenomena occur, in agreement with literature data.

If the entire progress curve was used, the function $[S] = a \cdot \exp(-b \cdot t)$ was fitted on the experimental [S]=f(t) data by a method of nonlinear regression and the instant rates were calculated as the first derivative of this function. For initial urea concentration less than 0.04M, equation (2) gave good estimates. For larger initial concentrations, Michaelis-Menten equation cannot describe properly the experimental data. An illustrative example obeying the Michaelis Menten equation is given in Fig. 4. This result is in agreement with the initial rate data, indicating again that the product accumulation in a buffer free system has a stronger inhibition effect on urease activity.

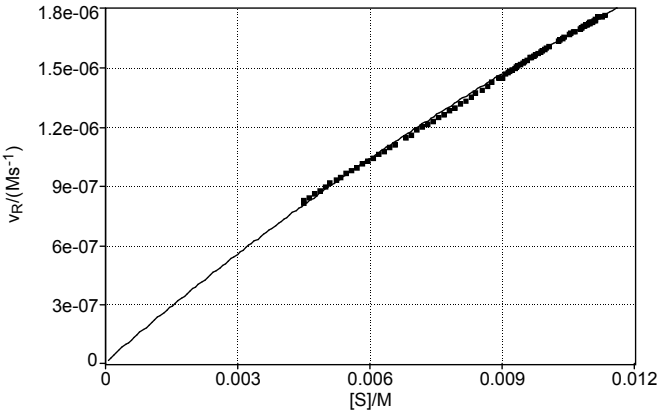

Fig 4. *Variation of reaction rate with urea concentration ($[S]_0$=0.01136M) and the result of nonlinear regression on Michaelis – Menten equation:* $v_{\max} = (8.66 \pm 2.1)10^{-6} Ms^{-1}$;

$$K_M = (4.44 \pm 0.13)10^{-2} M; r = 0.9987$$

The obtained value for K_M for sword bean urease is within the range of previously reported values [8] for urease from jack beans, which are pH and buffer dependent[11]: 0.125 10^{-2}M with no buffer in a pH-stat at pH=7 and 60.73 10^{-2}M in 0.1M phosphate buffer at the same pH.

Conclusions

The conductometric method can be used for the kinetic investigation of urease catalyzed urea hydrolysis.

The initial rate method and the analysis of the extended progress curve were applied for the investigation of the kinetics of enzymatic hydrolysis of urea. The results are concordant, showing that at initial substrate concentration greater than 0.04M supplementary inhibition phenomena occurs

The estimated kinetic parameters, obtained from the extended progress curves are in agreement with other reported data.

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