A36 Michaelis-Menten kinetics: Hydrolysis of urea

Task:
Investigation of the kinetics of the decomposition of urea with urease

Basics:

a) Michaelis-Menten kinetics

In the field of biochemistry catalytic reactions play an important role. Such biocatalysts are called enzymes. Urease is an enzyme which cleaves urea (the diamine of the carboxylic acid) into ammonium carbonate in aqueous solution. The chemical equation for the gross reaction reads

$$H_2N - CO - NH_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad . \tag{1}$$

Although catalysts, in this case urease, are not changed after reaction and therefore, are not part of the chemical equation, they decisively influence the reaction rate. The simplest model of an enzyme-catalyst reaction is the Michaelis-Menten mechanism:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P. \tag{2}$$

E: enzyme
S: substrate
P: products

In the first step the enzyme-substrate complex ES is formed by a reversible reaction with rate constants k_1 for complex formation and k_{-1} for decomposition to precursors. In a second, irreversible step products are formed from ES and the enzyme is regained. Now, the time-dependent change of the concentration of the enzyme-substrate complex [ES] can be written as follows:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]. \tag{3}$$

The reaction rate v for product formation reads:

$$v = \frac{d[P]}{dt} = k_2[ES]. \tag{4}$$

Provided that the concentration of the enzyme-substrate complex is small throughout the reaction, Bodenstein's steady-state principle can be applied:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0. \tag{5}$$

Furthermore, mass balance

$$[E] + [ES] = [E]_0 \tag{6}$$

is valid with $[E]_0$ as initial enzyme concentration.

By rearranging equations (5) and (6) as well as insertion into equation (4), one obtains the so-called Michaelis-Menten equation:

$$v = \frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{[S]+K_M} \tag{7}$$

with

$$K_M = \frac{k_{-1}+k_2}{k_1}. \tag{8}$$

In the case that $[S]$ is significantly larger than K_M one can make use of the approximation $[S] + K_M \approx [S]$. The rate law of equation (7) then simplifies to

$$\frac{d[P]}{dt} = k_2[E]_0. \tag{9}$$

In the case that $[S]$ is significantly smaller than K_M the reaction is first order with respect to $[S]$:

$$\frac{d[P]}{dt} = k_2 \frac{[E]_0[S]}{K_M}. \tag{10}$$

At t = 0 the concentration of $[S]_0$ is known. Therefore, the constants K_M and k_2 can be determined by measuring the initial reaction rate v_0:

$$v_0 = \left(\frac{d[P]}{dt}\right)_{t=0} = \frac{k_2[E]_0[S]}{[S]_0+K_M}\,. \tag{11}$$

The reciprocal of equation (11) gives the Lineweaver-Burk plot:

$$\frac{1}{v_0} = \frac{1}{v_{max}} + \frac{1}{[S]_0}\frac{K_M}{v_{max}} \tag{12}$$

with $v_{max} = k_2[E]_0$.

Plotting of $\frac{1}{v_0}$ versus $\frac{1}{[S]_0}$ results in a straight line from where v_{max} and K_M can be determined (intersection with ordinate and slope).

b) Conductivity of electrolytes

In general, resistance is defined as the ratio of voltage and current. In an electrolyte it is proportional to the quotient of the electrode distance l and the surface of the electrode A. Therefore, one defines a specific resistance ρ, which is characteristic of an electrolytic conductor, as

$$\rho = R\frac{A}{l}. \qquad (13)$$

The specific conductivity κ is defined as the reciprocal of the specific resistance

$$\kappa = \frac{1}{\rho} = \frac{l}{R \cdot A} \qquad \left[\frac{1}{\Omega \cdot m} = \frac{S}{m}\right] \qquad (14)$$

and depends on the concentration of the electrolyte in solution.

Urea in aqueous solution is not conductive. Hence, the specific conductivity κ, which is measured in this experiment before addition of the urease solution, results from a weak intrinsic conductivity of water. If κ exceeds 7 µS/cm, impurities are too high and the glass devices needs to be cleaned as well as the solution must be prepared again. The increase of the conductivity after addition of the urease solution results from formation of ammonium carbonate by urea splitting of the urease. After a short mixing period (ca. 20 s) the expected linear rise of the specific conductivity with time can be observed.

Experimental procedure:

- Please bring a USB stick along -

1) Before starting the lab course, please calculate the necessary masses and volumes of the dilution series of urea and ammonium carbonate solutions.

2) Prepare a urease suspension with β = 2 g/l. Take care of proper mixing.

3) Based on 100 ml 0.1 M urea solution prepare a dilution series each consisting of 50 ml solution with the concentration (in mol/L): $2 \cdot 10^{-3}$; $2.2 \cdot 10^{-3}$; $2.5 \cdot 10^{-3}$; $2.8 \cdot 10^{-3}$; $3.4 \cdot 10^{-3}$; $4 \cdot 10^{-3}$; $5 \cdot 10^{-3}$; $8 \cdot 10^{-3}$; $2 \cdot 10^{-2}$; $5 \cdot 10^{-2}$.

4) Proceed as follows with each urea solution: Temper the urea solution at room temperature (can be tracked with “measure”) and stir with the magnetic stirrer (if κ > 7 µS/cm dispose this solution, flush thoroughly and prepare a new one). After reaching the temperature start the measurement and after few seconds add 5 ml urease suspension (please shake again before taking) using a volumetric pipette. After 3 minutes, stop the measurement and save the data (option: “Alle Daten an

measure übertragen" (Transfer all data to measure), then choose via Messung (measurement) "Messwerte exportieren" (export measured data) → export in data file.

5) Prepare the dilution series of ammonium carbonate solutions starting with a 50 ml 0.1 M stock solution and the following concentrations (in mol/L): $5 \cdot 10^{-4}$; $5 \cdot 10^{-3}$; $1 \cdot 10^{-2}$; $1.5 \cdot 10^{-2}$; $2 \cdot 10^{-2}$. Measure the conductivities of the corresponding solution (Attention: Ammonium carbonate must be completely dissolved).

Important: While adding urease take care that the enzyme actually reaches the solution and not the edge of the flask. In this case, small changes can lead to large errors.

Data analysis:

1) Plot the conductivity of the different ammonium carbonate solutions versus concentration and perform a linear regression. In this way, the calibration line is obtained.
2) Determine the initial reaction rates v_0 by plotting the measured conductivity of the urea-urease solution with time. While doing this, neglect approximately the first 20 s after urease addition and use the subsequent 120 s of the recorded measured data. (The corresponding tables do not have to be listed in the records).
3) From the initial reaction rates v_0 determine the constants K_M and k_2 by using the Lineweaver-Burk plot. Attention: By adding 5 ml of the aqueous urease solution there will be a non-negligible change of the initial urea concentration. Therefore, the concentrations need to be converted for the Lineweaver-Burk plot. For this purpose, assume ideal solution behavior with a total volume of 55 ml.
4) Discuss your results (not only by means of error analysis) and possible deviations from literature.

What one should know:

Pre-equilibrium, reactions of 0., 1. and 2. order, Bodenstein's steady-state approximation, temperature dependence of rate constants, basics of conducitivity measurements, conductivity of strong and weak electrolytes, Kohlrausch (square root) law, Ostwald's dilution law.

Mandatory supplementary questions:

- Qualitatively sketch a concentration-time profile including all species participating in this reaction.

- Explain how a catalyst works. In addition, sketch a potential energy diagramme for a reaction of your choice with and without the influence of a catalyst.