Biochimica et Biophysica Acta, 614 (1980) 237—241

BBA 69043

TEMPERATURE EFFECTS WITH IMMOBILIZED YEAST ALCOHOL DEHYDROGENASE IN FLOW SYSTEMS

M.A. MAZID and K.J. LAIDLER

Department of Chemistry, University of Ottawa, Ottawa K1N 9B4 (Canada)

(Received December 5th, 1979)

Key words: Alcohol dehydrogenase; Activation energy; Immobilized enzyme; (Yeast)

Summary

Activation energies were determined for the flow kinetics of yeast alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) attached to nylon tubing, over a range of concentrations of the two substrates (NAD and ethanol) and at various flow rates. With NAD at saturating concentrations the activation energies showed little dependence on ethanol concentration and flow rate. Under these conditions the reaction shows hardly any diffusion control, and the activation energy for the chemical interaction at the surface is concluded to be about 9 kcal · mol^{-1}. At saturating ethanol concentrations the activation energies increased significantly with increasing NAD concentration and flow rate. The limiting value at high NAD concentration and flow rate is consistent with the 9 kcal · mol^{-1} deduced for complete chemical control. Extrapolation to zero NAD concentrations and flow rates suggested a value of 4.2 kcal · mol^{-1} for the reaction under conditions of full diffusion control.

Introduction

In the previous paper [1] the kinetics of the yeast alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) were studied at a single temperature, 25.5°C. In the present work kinetic results were obtained and analyzed at temperatures ranging from 5 to 45°C. Similar studies have previously been made with immobilized electric eel acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7) [2] and with immobilized lactate dehydrogenase (L-lactate:NAD^+ oxidoreductase, EC 1.1.1.27) [3].

Temperature studies on immobilized enzymes are useful in a number of ways. Activation energies for diffusion in water fall within a certain range of values, and may differ from the values for the chemical interaction; an analysis

of the temperature dependence under different conditions of concentration and flow rate can therefore provide mechanistic information. The studies also have a practical application in the design of enzyme-reactors for various purposes.

Materials and Methods

The procedures used were as described in the previous paper [1]. Temperature-controlled substrate solution was pumped through the tube, which was in a thermostatically-controlled waterbath having the same temperature as the substrate solution.

Rates were measured at five temperatures ranging from 5°C to 45°C, and at a variety of flow rates and substrate concentrations as specified in Tables I and II.

Theoretical

The main theoretical aspects that relate to temperature effects have been summarized by Daka and Laidler [3], and here we emphasize only the main points. Under the conditions of high substrate concentrations and flow rates the theory [4,5] leads to the result that the rate is given by

$$v = 2\pi\, rLk'_c[E]_s \tag{1}$$

where r is the tube radius, L its length, and $[E]_s$ the enzyme concentration at the surface. The rate constant k'_c is the inherent rate constant at the surface. The temperature dependence of the rate therefore reflects only that of this chemical rate constant.

At low substrate concentrations and low flow rates the rate is given by

$$v = 8.06\,(v_f D^2 r^2 L^2)^{1/3}[S] \tag{2}$$

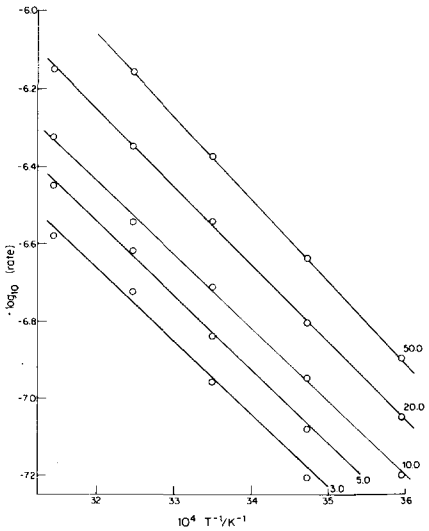
where v_f is the rate of flow and D the diffusion coefficient. Under these conditions the temperature dependence is controlled entirely by that of the diffusion coefficient, and the observed activation energy will be two-thirds that for the diffusion process.

Results and Discussion

Fig. 1 shows typical Arrhenius plots for NAD in excess and at various ethanol concentrations. Fig. 2 is for ethanol in excess and with variable NAD concentration. In all of our experiments the Arrhenius law was obeyed within the experimental error; there were no changes in slope such as were found with acetylcholinesterase [2] and which are attributed to changes in mechanism.

Tables I and II show the observed activation energies. These values were calculated using the method of least-squares [6,7], the errors being estimated at the 90% confidence level. The activation energies listed are the apparent values, obtained directly from the Arrhenius plots, without multiplication by the 3/2 factor.

The results in Table I, for NAD at saturating concentrations and [ethanol]



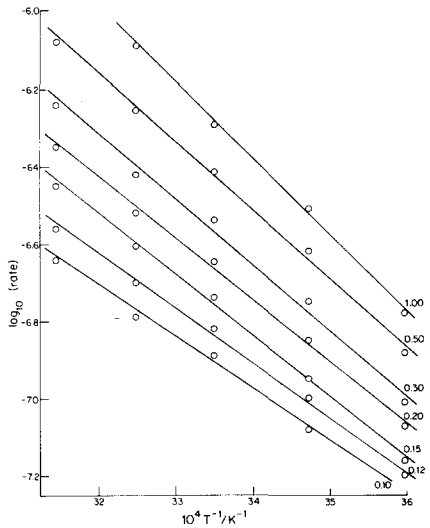


Fig. 1. Arrhenius plots for the rate of product formation with [NAD] at 5.0 mM and at the ethanol concentrations (mM) indicated. Flow rate, 0.50 cm · s^{-1}.

Fig. 2. Arrhenius plots for the rate of product formation with [ethanol] at 500 mM and at the NAD concentrations (mM) indicated. Flow rate, 0.50 cm · s^{-1}.

varied, show very little dependence of the activation energy on the ethanol concentration or on the flow rate. This result is consistent with the conclusion of the preceding paper [1] that the kinetics under these conditions are essentially diffusion free. The activation energies, particularly at the higher ethanol concentrations, therefore correspond to the chemical process occurring at the surface. Included in the table are values extrapolated to 1/[S] = 0 and therefore corresponding to very high concentrations of ethanol. It appears that the activation energy for the chemical processes when there is saturation by both substrates is about 9 kcal · mol^{-1}.

In contrast, the results with saturating concentrations of ethanol, listed in

TABLE I

ACTIVATION ENERGIES (kcal · mol^{-1}) WITH EXCESS NAD AT 5.0 mM

EtOH is used for concentration of ethanol.

Concentration of EtOH (mM)	Flow rates (cm · s^{-1})				
	0.10	0.50	1.00	2.00	3.30
3.0	8.5 ± 1.1	9.0 ± 1.6	10.2 ± 0.6	10.3 ± 1.0	10.3 ± 0.8
5.0	8.6 ± 0.9	8.9 ± 0.8	10.1 ± 0.5	10.1 ± 1.4	10.1 ± 1.0
10.0	8.5 ± 0.4	8.7 ± 0.4	9.8 ± 0.5	9.6 ± 0.5	9.2 ± 0.7
20.0	8.6 ± 0.8	9.1 ± 0.2	9.3 ± 0.2	9.4 ± 0.5	9.3 ± 0.1
50.0	8.7 ± 1.7	9.4 ± 0.3	9.1 ± 0.2	9.4 ± 0.7	9.2 ± 0.4
∞ (extrapolated)	8.7	9.1	9.2	9.3	9.1

TABLE II

ACTIVATION ENERGIES (kcal · mol^{-1}) WITH EXCESS ETHANOL AT 500.0 mM

Concentration of NAD (mM)	Flow rates (cm · s^{-1})					
	0.10	0.30	0.50	1.00	2.00	3.30
0 (extrapolated)	2.8	4.0	5.0	5.1	5.9	6.5
0.10	3.8 ± 0.5	5.4 ± 0.4	6.0 ± 1.1	5.9 ± 0.9	6.6 ± 1.0	6.9 ± 1.4
0.12	4.3 ± 0.3	6.1 ± 0.3	6.4 ± 0.5	6.0 ± 0.5	7.0 ± 0.6	7.0 ± 0.7
0.15	4.8 ± 0.3	6.5 ± 0.2	7.2 ± 0.5	6.2 ± 0.5	7.2 ± 0.4	6.9 ± 1.0
0.20	5.0 ± 0.3	7.1 ± 0.3	7.2 ± 0.5	6.7 ± 0.5	7.5 ± 0.6	7.3 ± 0.5
0.30	5.4 ± 0.4	7.5 ± 0.3	7.6 ± 0.9	7.3 ± 0.6	8.2 ± 0.6	7.5 ± 0.5
0.50	6.0 ± 0.4	8.1 ± 0.3	8.0 ± 0.6	8.3 ± 0.6	8.6 ± 0.4	7.9 ± 0.5
1.00	6.7 ± 0.9	9.0 ± 1.4	8.9 ± 0.7	8.9 ± 1.1	9.0 ± 0.9	8.5 ± 0.5
∞ (extrapolated)	7.0	9.2	9.1	9.4	9.4	8.6

Table II, show a significant variation of activation energy with flow rate and with concentration of NAD. Increasing the flow rate and increasing [NAD] both produce an increase in activation energy. This result is also consistent with the conclusions of the previous paper, which showed that there is substantial diffusion control under these conditions, the extent of diffusion control being reduced as the flow rate and the substrate concentration are increased. The activation energies extrapolated to infinite concentrations and flow rates should therefore correspond to the diffusion-free value. Table II shows the values extrapolated to $1/[S] = 0$. These extrapolated values increase with increasing flow rate, and at the higher flow rates are consistent with the 9 kcal · mol^{-1} obtained from the values in Table I. Both sets of results therefore suggest that the activation energy for the chemical interaction at the surface is about 9 kcal · mol^{-1}.

Extrapolation of the values in Table II to low [NAD] and flow rate leads to a value for the fully diffusion-controlled process. The values extrapolated to [NAD] = 0 are shown at the top of the columns in Table II. In accordance with expectations these values fall as the flow rate is reduced and suggest a value of about 2.8 kcal · mol^{-1} for the apparent activation energy for full diffusion control. In the light of Eqn. 2 this value must be multiplied by 3/2 to give the activation energy corresponding to the diffusion coefficient. The result is 4.2 kcal · mol^{-1}, which is very reasonable for diffusion in water.

The results obtained in this work are somewhat similar to those obtained by Buchholz and Rüth [8] for immobilized trypsin. They also found a significant drop in activation energy with decreasing substrate concentration and concluded that the decrease was due to an increase in the extent of diffusion control. For alcohol dehydrogenase in free solution Gierer [10] found an activation energy of about 14 kcal · mol^{-1} for V; this is somewhat higher than our value of about 9 kcal · mol^{-1} for the immobilized enzyme. Gierer also found values of 3—8 kcal · mol^{-1} from the temperature coefficient of the Michaelis constant, these values being for association processes. Our results gave no values to compare with these, because of the changes of mechanism with change of substrate concentration.

In our present results and those of Buchholz and Rüth [8] the activation energy for the chemical process is substantially greater than that for the diffusion process. In these systems it is therefore easy to find conditions in which the processes are essentially diffusion-free. These results show an interesting contrast to those of Daka and Laidler [3] for lactate dehydrogenase attached to nylon tubing. There the activation energy for the chemical process, approx. 1 kcal · mol^{-1}, is very much less than that for diffusion, approx. 5 kcal · mol^{-1}. In that system it was therefore much easier to find conditions favoring diffusion control and diffusion-free behavior was only found under extreme conditions of substrate concentration and flow rate.

One consequence of the situation existing in our present work with alcohol dehydrogenase is that, because of the high activation energy for the chemical process, the reactions are very much slower than found in the system studied by Daka and Laidler [3,9].

References

1 Mazid, M.A. and Laidler, K.J. (1980) Biochim. Biophys. Acta 614, 225—236
2 Ngo, T.T. and Laidler, K.J. (1978) Biochim. Biophys. Acta 525, 93—102
3 Daka, N.J. and Laidler, K.J. (1980) Biochim. Biophys. Acta 612, 305—316
4 Kobayashi, T. and Laidler, K.J. (1974) Biotechnol. Bioeng. 16, 99—118
5 Laidler, K.J. and Bunting, P.S. (1979) Methods Enzymol. 64B, 227—248
6 Ostle, B. (1963) Statistics in Research, 2nd edn., pp. 159—221, Iowa State University Press, Ames, IA
7 Swinbourne, E.S. (1971) Analysis of Kinetic Data, pp. 17—43, Thomas Nelson and Sons Ltd., London
8 Buchholz, K. and Rüth, W. (1976) Biotechnol. Bioeng. 18, 95—104
9 Daka, N.J. and Laidler, K.J. (1978) Can. J. Biochem. 56, 774—779
10 Gierer, A. (1955) Biochim. Biophys. Acta 17, 111—121