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Calorimetric investigations into enzyme catalysed glucose oxidation

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Abstract

The study presents the results of calorimetric investigations into enzyme catalysed glucose oxidation. It was shown that the convertible amount of glucose is limited by the availability of the oxidant or mediator: O_2, $K_3[Fe(CN)_6]$ and benzoquinone were used as mediators. The measurable glucose concentration range was much expanded by using benzoquinone. Independently of the mediator used, the enthalpy value for the oxidation of glucose in the presence of the enzyme glucose oxidase was found to be $\Delta_R H = -125 \pm 2\ kJ\ mol^{-1}$.

INTRODUCTION

The analytical determination of glucose is based upon its enzyme-catalysed oxidation to D-glucono-δ-lactone or D-gluconic acid and hydrogen peroxide; only β-D-glucose is capable of this reaction. Possible oxidants or mediators include benzoquinone and potassium hexacyanoferrate, in addition to oxygen. The reaction can be quantitatively indicated in three ways: amperometrically [1], calorimetrically [2], or by measuring the concentration of one reactant (usually by photometry) [3]. The accurate knowledge of the reaction conditions and parameters is an indispensable prerequisite for research activities such as the development of a glucose sensor.

This study communicates the results of calorimetric measurements at normal temperature as a basis for the development of a thermal sensor. The influence of various mediators, the presence of catalase in addition to the enzyme glucose oxidase and the equilibrium of mutarotation on the molar reaction enthalpy were investigated. Qualitative statements are made with

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respect to the conversion rate. A subsequent study [4] presents the results of a quantitative thermokinetic analysis.

MATERIALS AND EQUIPMENT

The glucose oxidase (E.C. 1.1.3.4) used was from *Aspergillus niger* (Serva), and had a specific activity of 250 U mg^{-1} (foreign activities 5 U mg^{-1} catalase; 0.05% α-amylase, 0.05% invertase and 0.05% maltase). The specific activity of the employed catalase (E.C. 1.11.1.6) from *Aspergillus niger* (Serva) was 1950 U mg^{-1}. Reagent-grade chemicals were used for all measurements. A test calibration buffer of pH = 6.86 (0.25 M KH_2PO_4–0.25 M Na_2HPO_4) was used.

The calorimetric measurements were carried out in an isoperibolic reaction calorimeter [5]. Usually about 62 ml of an aqueous solution of glucose, the mediator substance in different concentrations and phosphate buffer ($c = 0.1$ M, pH = 6.86) were mixed in the calorimetric cell.

The reaction was initiated by breaking an ampoule filled with enzyme solution. Because an evaluation of the temperature–time graphs in accordance with Dickinson involved a high degree of uncertainty, in view of the long reaction times (up to 100 min), the heat flow to the environment was first corrected on the basis of an electrical calibration (in accordance with Regnault–Pfaundler [6]). The resulting adiabatic temperature–time graphs allow the determination of both ΔT_{max} as required for the calculation of the reaction enthalpy and the functions $\Delta T = \Delta T(t)$ or

$$(\partial \Delta T/\partial t) = (\partial \Delta T/\partial t)(t)$$

which are necessary for kinetic analysis.

It holds that

$$x/c^\circ = \Delta T/\Delta T_{max}$$

where c° = total glucose concentration, x = converted glucose concentration at time t, and ΔT = adiabatic temperature difference at time t so that ΔT at time t is a measure for the conversion or the reaction rate.

$$\frac{\partial \Delta x}{\Delta T} = \frac{c^\circ}{\Delta T_{max}} \frac{\partial \Delta T}{\Delta t}$$

The object quantities were determined by weighing. All measurements refer to a thermostatic temperature of the calorimeter of 298 K. The glucose concentration was varied over the range 0.9–18 $mmol^{-1}$ corresponding to a ΔT_{max} value between about 16 and 250 mK. Thus, at a maximum reaction time of 100 min up to complete conversion and a

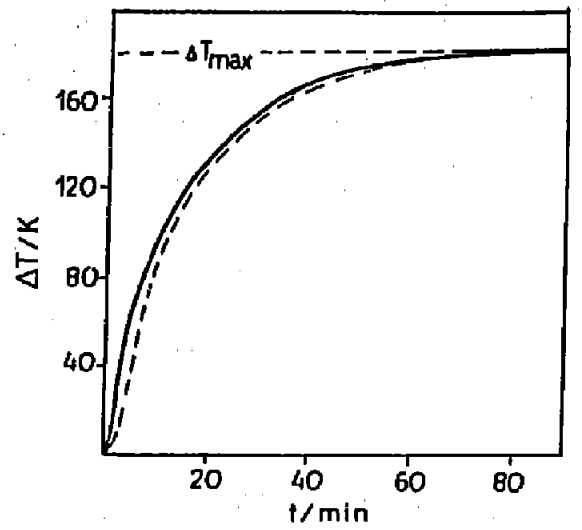

Fig. 1. ΔT–time graphs for glucose solution (—) and α-glucose (– – –) ($m = 100$ mg, $v = 62.15$ ml).

long-term stability of the calorimeter of ± 0.1 mK h^{-1}, the lowest glucose concentration was still far above the detection limit, which was assumed at some 0.05 mmol l^{-1} for an assumed error of ±2%. Oxygen, potassium hexacyanoferrate and benzoquinone were used as mediators.

DISCUSSION

In accordance with the mutarotation equilibrium, α-glucose (38%) is present in solutions in addition to β-glucose (62%). Therefore the first question to be answered was whether only the β-fraction would react, or whether the mutarotation rate would be sufficient for a total conversion of the glucose. In contrast to a calorimetric measurement (solution of glucose ($c = 8.9$ mmol l^{-1}), benzoquinone ($c = 15.6$ mmol l^{-1}) and buffer + glucose oxidase (god) solution, a measurement was carried out in which solid α-glucose was added to a solution of equal concentrations of buffer, benzoquinone and god. The resulting endothermic solution effect was determined separately in an experiment without god ($\Delta_L H = 10.04$ kJ mol^{-1}); the previous measurement was then corrected to the pure reaction effect. Figure 1 shows the two calorimetric curves (for glucose solution and α-glucose).

It is evident that the ΔT_{max} value, and thus the molar reaction enthalpy, is independent of the initial composition (glucose solution or α-glucose). This means that the mutarotation rate is so high that α-glucose is converted as well, so that the reaction enthalpy must be related to the entire object quantity of the glucose. The ΔT–time graph (Fig. 1) for α-glucose shows the typical shape of that for a successive reaction, especially in the starting phase. On the whole, the conversion rate of α-glucose was lower than that of glucose solution, which was expected.

It is known [7], and will be confirmed in the following, that the convertible amount of glucose is limited by the available amount of oxidants or mediators. If oxygen is used as a mediator, the limit at 25°C (in dependence on the oxygen concentration) is 2.36×10^{-4} mol l^{-1} for air-saturated solutions and 1.22×10^{-3} mol l^{-1} for oxygen-saturated solutions. As can be seen from Fig. 2, these limits can be proved beyond doubt from the calorimetric curves. Sharp breaks can be observed in either case. With minor deviations, the ΔT_K values assigned to the break points exactly correlate with the above oxygen concentrations:

$c_K(\text{air}) 2.29 \times 10^{-4}$ mol l^{-1}; $\quad c_K(O_2) = 1.18 \times 10^{-3}$ mol l^{-1};

$c_K/c^\circ = \Delta T_K/\Delta T_{max}$.

The constant increase of the ΔT–t curve after the break (equivalent to a constant reaction rate) is independent of the glucose concentration, and is obviously controlled by the diffusion of the oxygen from the gas phase (air or oxygen) into the solution. It is understandable that in the case of an oxygen atmosphere (curve b in Fig. 2) this increase is greater than in the case of air (curve a in Fig. 2). The ratio of the increases b/a is about 4.8, i.e. exactly the same as the ratio of the oxygen partial pressures $p_b(O_2)/p_a(O_2)$.

The high-resolution calorimeter used in the experiments allows a reliable determination of the entire reaction effect and thus of $\Delta_R H$; nevertheless, the upper limit for the thermometric determination of the glucose concentration appears to be the limit fixed by the oxygen concentration in the solution. As discussed in refs. 8 and 9, this limit can be completely eliminated (1) for an addition of the enzyme catalase to the pair, or (2) for the use of a different mediator.

Among others, potassium hexacyanoferrate and benzoquinone [10, 11] are known as substitutes for oxygen. Calorimetric measurements with $K_3[Fe(CN)_6]$ as a mediator show that a vast excess of $K_3[Fe(CN)_6]$ in

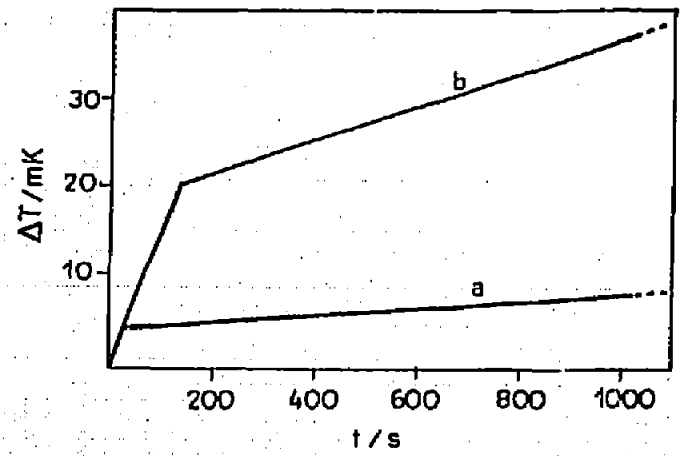

Fig. 2. Calorimetric curves, $c_{Gl} = 7.14$ mmol l^{-1}; curve a, air-saturated solution; curve b, oxygen-saturated solution.

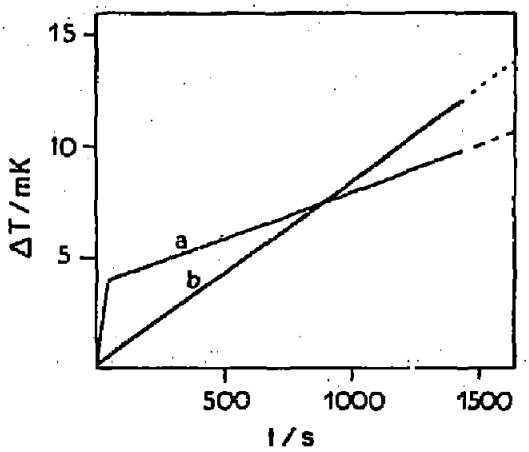

Fig. 3. Calorimetric curves, $c_{Gl} = 1.34$ mmol l^{-1}; curve a, air-saturated solution; curve b, N_2 atmosphere, with n(glucose): $n(K_3[Fe(CN)_6]) = 1{:}55$.

relation to stoichiometric conversion is needed to obtain reaction rates allowing an evaluation of the calorimetric curve. Figure 3 compares two calorimetric curves. It is evident that even for a vast excess of $K_3[Fe(CN)_6]$ the attainable reaction rates are in the same range as for an air-saturated solution. Hence $K_3[Fe(CN)_6]$ does not appear to be a suitable mediator.

A different situation is created when benzoquinone is used as a mediator. For equal glucose concentrations below the saturation concentration of oxygen, the calorimetric curves for oxygen and benzoquinone are absolutely identical within the error limits, i.e. the same reaction rates as with oxygen can be attained with benzoquinone.

These are some further conclusions from this experiment:

(1) the reaction rate is independent of the mediator concentration. This is also confirmed by measurements with different benzoquinone concentrations;

(2) the conversion of the reduced form of glucose oxidase to the oxidized form by means of the mediator is effected so rapidly that this reaction stage does not exert any influence on the overall reaction rate;

(3) for the given pH value of the solution (6.86), the oxidation potential of $K_3[Fe(CN)_6]$ is greater than that of benzoquinone; therefore the unexpectedly small effect of $K_3[Fe(CN)_6]$ can be explained only by the reaction kinetics. It must be assumed that the oxidation of the reduced glucose oxidase is connected with hydrogen transfer rather than electron transfer. Hydrogen transfer is excluded for $K_3[Fe(CN)_6]$, whereas it is possible for oxygen and benzoquinone.

Calorimetric measurements with benzoquinone as a mediator were performed in the glucose concentration range between 0.8 and 18 mmol l^{-1}. A kinetic analysis allows statements concerning the reaction order, the functional dependence of the reaction rate on glucose and enzyme concentration, the enzyme activity, and the determination of the Michaelis

TABLE 1

Enzyme	Mediator	Number of measurements	$\Delta_R H$ (kJ mol^{-1})
god	Benzoquinone	12	124.0 ± 4
god	Oxygen	12	127.1 ± 4.3
god	$K_3[Fe(CN)_6]$ + air	10	123.3 ± 5.6
god	$K_3[Fe(CN)_6]$ + air	10	124.9 ± 4.0
god	$K_3[Fe(CN)_6]$ + N_2	4	125.3 ± 1.8
god	$K_3[Fe(CN)_6]$ + O_2	4	127.4 ± 2.5
god + catalase	Benzoquinone	7	223.3 ± 1.9

constant K_M. These questions will be discussed in detail in a subsequent study [4].

All measurements were evaluated with respect to the overall reaction effect $\Delta_R H$.

An overview is given in Table 1. Independently of the mediator, the enthalpy value of the oxidation of glucose in the presence of the enzyme glucose oxydase was found to be $\Delta_R H = -125 \pm 2$ kJ mol^{-1}. As expected, this value was increased by some -100 kJ mol^{-1} in the presence of catalase. The difference is almost equal to the reaction enthalpy of the decomposition reaction.

$$H_2O_2(aq) \rightarrow H_2O + 0.5O_2(g) \qquad \Delta_R H^{\ominus}(298) = 100.06 \text{ kJ mol}^{-1}$$

All literature data originate from the measurements by Schmidt et al. [12]. For the oxidation of glucose in the presence of glucose oxidase and catalase, a value of $\Delta_R H = -207.2$ kJ mol^{-1} is given, i.e. a value 7% below that measured by us. Since Schmidt et al. [12] used immobilized enzymes, an incomplete reaction can be assumed, which would explain the lower value.

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