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A thermodynamic study of the lipase-catalyzed transesterification of benzyl alcohol and butyl acetate in supercritical carbon dioxide media

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Abstract

The equilibrium constant for the lipase-catalyzed transesterification reaction (benzyl alcohol + butyl acetate = benzyl acetate + 1-butanol), using supercritical carbon dioxide ($SCCO_2$) as the solvent, has been measured as a function of temperature ($T = 303.15$ to 318.15 K) at the pressure $p = 10.0$ MPa. At $T = 298.15$ K, the equilibrium constant $K = 0.238 \pm 0.020$ and the standard molar Gibbs energy change $\Delta_r G^\circ_m = 3.56 \pm 0.22$ kJ mol^{-1}; the values for the standard molar enthalpy and standard molar entropy changes $\Delta_r H^\circ_m$ and $\Delta_r S^\circ_m$, respectively, are zero within experimental error. The value of the equilibrium constant for this reaction in $SCCO_2$ was compared with values determined by carrying out the reaction in organic solvents. A time course study of this reaction has also been carried out in supercritical carbon dioxide, *n*-hexane, toluene, and neat media (no solvent added) at the temperature $T = 303.15$ K. The time course data show that the reaction proceeds more rapidly in $SCCO_2$ media than in the other three solvent systems and also that the reaction is at or near equilibrium within 3 h in the $SCCO_2$ solvent system. However, the value of the equilibrium constant for the reaction carried out in $SCCO_2$ is ≈30% smaller than the values of the equilibrium constants for the reactions carried out in hexane and toluene. Some advantages of the use of supercritical carbon dioxide as a solvent for enzyme-catalyzed reactions are discussed.

Keywords: Benzyl alcohol; Benzyl acetate; Biocatalysis; 1-Butanol; Butyl acetate; Equilibrium constant; Lipase; Rate of reaction; Supercritical carbon dioxide; Thermodynamics

1. Introduction

Lipases are of interest to the food and pharmaceutical industries because they are widely used to catalyze a number of important reactions [1,2]. Many of these reactions are carried out in non-aqueous media. However, when an organic solvent is used for the synthesis of a drug or food product, additional steps may be required for removal of residual solvent to meet the stringent requirements of the U.S. Food and Drug Administration [3]. During the past 20 years, supercritical carbon dioxide ($SCCO_2$) [4–9] has increasingly been investigated as an alternative solvent for biochemical reactions and these studies have documented the advantages that $SCCO_2$ has over commonly used organic solvents (e.g. hexane). Specifically, carbon dioxide is non-toxic, non-flammable, available in abundance, and is environmentally friendly and recyclable. The low viscosity and high diffusivity of $SCCO_2$ also serve to provide favorable mass transfer properties. It is now possible to design the synthesis of a compound via enzyme catalysis in $SCCO_2$ media, followed by the supercritical fluid extraction for separation of the reaction products. In the near future, biocatalysis in supercritical CO_2 media could become the preferable route for synthesis and separation of drug intermediates. There are several publications dealing with enzyme-catalyzed reactions in $SCCO_2$ [6,7,9–13]. Also, a number of studies [7,8,10,14] have reported an increased enantioselectivity of lipase-catalyzed reactions in $SCCO_2$ media compared to organic solvents. However, the amount of rate data is limited for certain lipase-catalyzed reactions and there are no thermodynamic results for any of

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these reactions in supercritical CO_2 media. This thermodynamic information is essential for both process design and optimization.

In previous publications [15–20] we have dealt with the thermodynamics of lipase-catalyzed reaction in non-aqueous solvents. In this study, equilibrium measurements for the following reaction were carried out in $SCCO_2$

$$\text{Benzyl alcohol(soln)} + \text{butyl acetate(soln)} = \text{benzyl acetate(soln)} + 1 - \text{butanol(soln)}. \quad (1)$$

Here, "soln" denotes that the solvent is a non-aqueous solution such as supercritical carbon dioxide. The equilibrium constant for this reaction was measured in $SCCO_2$ as a function of temperature at the pressure $p = 10.0$ MPa. The results were then used to calculate the equilibrium constant K, the standard molar Gibbs energy $\Delta_r G^\circ_m$, enthalpy $\Delta_r H^\circ_m$, and entropy $\Delta_r S^\circ_m$ changes for this reaction at $T = 298.15$ K. To the best of our knowledge, this is the first thermodynamic study dealing with an enzyme-catalyzed reaction in supercritical fluid media. In addition, time course studies of this reaction have been carried out in *n*-hexane, toluene, $SCCO_2$ media, and in a solution without any solvent (the neat reaction mixture). The results show that under similar conditions the rate of reaction is faster in $SCCO_2$ than in organic solvents and also that the system reaches equilibrium faster in $SCCO_2$ media than in the other solvents. This confirms the findings of other workers [7,21], who also reported higher reaction rates in $SCCO_2$ media compared to the reaction rate in hexane.

2. Experimental

2.1. Materials

The substances used in this study, their Chemical Abstract Service (CAS) registry numbers, empirical formulas, molar masses, sources, and purity were reported previously [15]. The benzyl acetate used in this study was purchased from TCI America[1]; its mole fraction purity was >0.99. The solvents used in this study were toluene and *n*-hexane with mole fraction purities >0.99. The enzyme used in this study was lipase (E.C. 3.1.1.3) from *Candida antarctica* in the form of a lyophilized powder described earlier [15]. Carbon dioxide (SFC grade) was from Scott Specialty Gases, Plumsteadville, PA.

[1] Certain commercial equipment, instruments, or materials are identified in this paper to specify the experimental procedures adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

2.2. Chromatography

The analysis of the reactants and products was carried out with an HP 5890 gas chromatograph equipped with a flame ionization detector and a fused silica Phenomenex ZB-FFAP capillary column (30 m long, 0.53 mm i.d.). The method used for the quantitative analysis of the substrates and products was similar to the method described previously [15]. The injector and detector temperatures were 250 and 270 °C, respectively, and the head pressure of the helium carrier gas was 283 kPa. The initial column temperature of 60 °C was held for 3 min and then raised to 240 °C at a rate of 20 K min^{-1} and held at 240 °C for 15 min. The substance 1-decanoic acid was used as an internal standard for the quantitative determination of the compounds involved in this reaction. Thus, a standard solution of benzyl alcohol, benzyl acetate, 1-butanol, butyl acetate, and 1-decanoic acid was prepared gravimetrically in *n*-heptane and the ratios of their response factors were determined with reference to 1-decanoic acid. The retention times of butyl acetate, 1-butanol, benzyl acetate, benzyl alcohol, and 1-decanoic acid were, respectively, 2.41, 3.44, 8.40, 9.21, and 11.01 min. The response factor ratios of 1-butanol, butyl acetate, benzyl alcohol, and benzyl acetate with reference to 1-decanoic acid were 2.80 ± 0.08, 2.10 ± 0.06, 1.38 ± 0.04 , and 1.25 ± 0.04, respectively.

2.3. Supercritical fluid chromatograph

A modified supercritical fluid chromatograph (Hewlett Packard Model G1205A, Wilmington, DE), equipped with a temperature-controlled oven module, was used for carrying out the reaction in $SCCO_2$ media. The chromatographic system was modified by replacing the chromatographic column with a reaction vessel, which was connected via 2 three-way valves. One valve was used to isolate the reaction vessel from the rest of the system once the desired pressure was achieved. The second valve was used to release the CO_2 after completion of the reaction. The batch reactor vessel used in this study was similar to the reactor described by Hartmann et al. [8]. The reactor was fabricated from stainless steel at the National Institute of Standards and Technology. The reaction vessel had a volume of 15 ml and was sealed via a neoprene O-ring to a cover that had HPLC connections (outer diameter = 0.159 cm = 0.0625 in.) in it. The reaction vessel was tested for tightness up to a pressure of 25 MPa and no leaks were observed.

2.4. Time course studies

A forward stock solution of an equimolar mixture of butyl acetate and benzyl alcohol {butyl acetate = 4.45 mol (kg soln)$^{-1}$ and benzyl alcohol = 4.47 mol (kg soln)$^{-1}$} was prepared and used for all rate measurements. For the rate studies in *n*-hexane and toluene, ≈0.20 g of forward stock

solution, ≈0.010 g of lipase, and 15 ml of solvent were added to a vial, and capped. This solution was placed in a thermostatted shaker bath at 30 °C (±0.05 °C) and allowed to react for a specified period of time. A clear portion of the reaction mixture (0.20–0.30 mL) was removed from the reaction mixture by using a syringe. This solution, ≈ 100 μL of internal standard solution {1-decanoic acid in *n*-heptane, concentration $c = 0.3646$ mol (kg soln)$^{-1}$}, and 10 ml of *n*-hexane were added to a vial, which was then capped tightly. The removal of a clear portion of the reaction mixture serves to remove the insoluble lipase from the reaction mixture and thus to freeze the concentrations of the reactants and products. The masses of all the aforementioned solutions were determined gravimetrically. The samples were then analyzed for 1-butanol, butyl acetate, benzyl alcohol, and benzyl acetate by using the GC method described above. The concentration of each substrate involved in the reaction was determined from its chromatographic peak area, its response factor ratio with reference to the peak area of the internal standard substance, 1-decanoic acid, which had a known concentration. In performing our calculations, we used a concentration which was expressed as mol (kg soln)$^{-1}$ and which was based on the entire reaction medium, namely the reactants, the products, and the solvent. This form of concentration is useful because it relates directly to the gravimetric determinations, which can be done very precisely. Also, these concentrations can be converted easily to concentrations expressed as mol L^{-1} by using the density of the solvent. Note that, for the reaction carried out in $SCCO_2$, the analytical procedure involved filling the reaction vessel with hexane and performing the GC analysis on this hexane solution. Thus, in this case, the appropriate density to use is that of hexane which was taken to be $\rho = 0.6548$ g cm^{-3} [22]. It is important to appreciate that, since reaction (1) is symmetrical in regards to reactants and products, both the reaction quotient Q and the equilibrium constant K are independent of the units used to express concentrations.

For the rate studies in the neat system ≈0.2 g of forward stock solution and ≈0.010 g of lipase were added to a vial and capped. The vial containing the reaction mixture was then placed in a thermostatted shaker bath at 30 °C and allowed to react. After a specified time, the vial was removed and ≈ 15 ml of *n*-hexane was then added to the reaction mixture. The resulting solution was then analyzed using the method described above for *n*-hexane. A fresh reaction mixture was used for each time point in the neat system.

For the rate studies in supercritical carbon dioxide media, ≈0.20 g of forward stock solution and ≈0.010 g of lipase were added to the reaction vessel. Following assembly, the reaction vessel was connected to the supercritical fluid chromatograph (SFC) system. The system was then filled with $SCCO_2$ by changing the three-way stainless steel valve (see Section 2.3) to the appropriate position. The pressure of $SCCO_2$ was set to the desired pressure, which was controlled by the back-pressure regulator of the SFC system. The reaction was then allowed to proceed for a specified period of time. The temperature ($T = 310.15$ K) was maintained by the oven module of the Hewlett Packard SFC system. After the specified reaction time, the vessel was depressurized by changing the position on the three-way valve and bubbling the escaping CO_2 into a 20 ml volumetric flask that contained ≈ 7 ml of hexane. This procedure served to collect any escaping products. When the pressure was ≈ 0.1 MPa, the vessel was disconnected from the SFC system and the cover of the reaction vessel was removed. The hexane in the 20 ml volumetric flask was poured into the reaction vessel. Additional hexane was also added to the reaction vessel to fill it completely. A clear portion (≈ 0.50 mL) of this diluted reaction mixture was promptly removed with a syringe. This also served to remove the lipase and to freeze the position of the reaction. This solution, ≈ 100 μl of the internal standard solution, and 10 ml of *n*-hexane were gravimetrically added to a vial, which was capped tightly. This sample was then analyzed for 1-butanol, butyl acetate, benzyl alcohol, and benzyl acetate (see Section 2.2). For each time period, a new sample of ≈0.2 g of forward stock solution and ≈0.01 g lipase was prepared in the reaction vessel and then allowed to react in the $SCCO_2$ media at $p = 10.0$ MPa. Generally, assembly and pressurization of the reaction vessel required 5–6 minutes. However, since the enzyme was added to the forward stock solution at the very first stage of assembly, it is clear that some reaction has already started before the introduction of any $SCCO_2$. Depressurization of the reaction vessel took ≈ 10 min. These issues serve to complicate the performance of a true kinetic study.

2.5. Equilibrium studies

The position of equilibrium of reaction (1) in supercritical carbon dioxide media was approached from two directions, namely the forward and the reverse. For each forward reaction, the reaction mixture consisted of ≈0.20 g of an equimolar mixture of butyl acetate and benzyl alcohol and ≈0.010 g of lipase. Similarly, for the reverse reaction, the mixture consisted of ≈0.20 g of an equimolar mixture of 1-butanol and benzyl acetate and ≈0.010 g of lipase. The solution and the enzyme were placed in the stainless steel reaction vessel. Following assembly, the reaction vessel was connected to the SFC system and was pressurized with $SCCO_2$. Based on our time course studies, it was judged that a period of 3 h was required for the reaction to be either near or at equilibrium. Accordingly, all equilibrium measurements for the forward and the reverse reactions were carried out for 3 h at $p(SCCO_2) = 10.0$ MPa. The reaction vessel was shaken gently at approximately 15-min intervals during the reaction time. After the 3-h reaction period, the vessel was depressurized and the reaction mixtures were analyzed as described above.

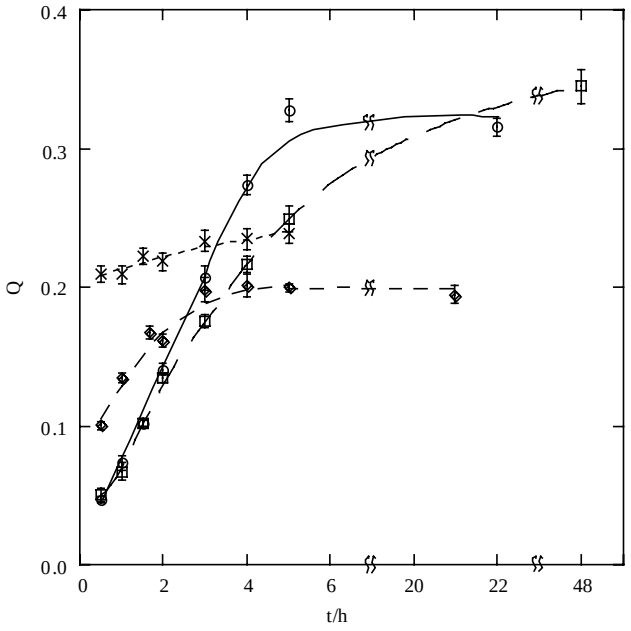

Fig. 1. The reaction quotients Q for the reaction (butyl acetate + benzyl alcohol = benzyl acetate + 1-butanol) in various solvents at $T = 303.15$ K and $p = 10.0$ MPa as a function of time. At time $t = 0$, the reaction mixture(s) consisted of ≈0.20 g of forward stock solution (an equimolar mixture of butyl acetate and benzyl alcohol) and ≈0.010 g of lyophilized lipase. The solvents used in these time studies and their respective symbols are: *n*-hexane, (○); toluene, (□); supercritical carbon dioxide, (×); and the neat system (without any solvent), (◇). The error bars correspond to two estimated standard deviations of the mean.

3. Results and discussions

3.1. Time course results

The time course data for reaction (1) are shown in Fig. 1. The values of the reaction quotient Q, defined as $Q = c\,(1-\text{butanol}) \cdot c\,(\text{benzyl acetate}) / \{c\,(\text{benzyl alcohol}) \cdot c\,(\text{butyl acetate})\}$, were calculated from the measured concentrations of reactants and products. It is seen that the reaction quotient Q has a value of 0.21 for $SCCO_2$ media at $t = 30$ min. This value is not far from the value of the equilibrium constant $K = 0.238$ (see Section 3.2). In fact, the reaction comes to equilibrium more rapidly when carried out in $SCCO_2$ than when carried out using the other solvents (*n*-hexane, toluene, and neat media). It is important to appreciate that the experimental arrangement used in the $SCCO_2$ experiments did not permit for a continuous mixing of the enzyme and substrates. Thus, the measured time course data for the reaction in $SCCO_2$ media, unlike the studies carried out with the solvents *n*-hexane, toluene, and the neat solution, are very likely an underestimate of "true" rate data. Therefore, the results obtained for the $SCCO_2$ media should not be considered as truly quantitative kinetic data. A detailed rate study using a $SCCO_2$ device capable of rapid mixing is planned. However, the large improvement of reaction rate seen for the $SCCO_2$ media indicates a much more rapid reaction (by a factor of at least 2–3) than that obtained with the other solvents that were studied.

3.2. Equilibrium constants and thermodynamic reaction quantities

The equilibrium constant of reaction (1) is

$$K = \frac{c(1-\text{butanol}) \cdot c(\text{benzyl acetate})}{\{c(\text{benzyl alcohol}) \cdot c(\text{butyl acetate})\}}. \quad (2)$$

In this equation, c is a concentration, which can be expressed as mol L^{-1} or as mol (kg soln)$^{-1}$. As discussed previously [15], this is a symmetrical reaction with respect to reactants and products, and the equilibrium constant is a dimensionless quantity and is independent of the units used to express concentrations. The substrates involved in this reaction are in unionized form in non-aqueous media and the concentrations are small. Hence, it can be assumed that their activity coefficients are close to unity. Therefore, the calculated equilibrium constants can be identified as the thermodynamic equilibrium constants defined in terms of activities of the reactants and the products.

The results of the equilibrium measurements are given in Table 1. The values of the reaction quotient Q, defined as $Q = c\,(1-\text{butanol}) \cdot c\,(\text{benzyl acetate}) / \{c\,(\text{benzyl alcohol}) \cdot c\,(\text{butyl acetate})\}$, were calculated from the measured concentrations of reactants and products. Ideally, the values of Q obtained from both directions of reaction should be in agreement if equilibrium has been fully achieved. Thus, the results show that the reaction mixtures were very close to equilibrium and the values of Q obtained from both directions of reaction have been averaged to obtain the values of the equilibrium constant K given in Table 1.

The uncertainties given in Table 1 for the equilibrium constants K are the random errors associated with the measurements expressed as two estimated standard deviations of the mean. These uncertainties do not include possible systematic errors in the measurements, which are now considered. The possible systematic errors are estimated to be ±0.05 K in the GC measurement of concentrations of reactants and products, ±0.02 K due to sample impurities, and ±0.03 K for a failure to reach true equilibrium. These estimated possible errors are combined in quadrature together with the statistical uncertainties in the measured values of Q expressed as one estimated standard deviation of the mean to obtain combined standard uncertainties [23]. These uncertainties are then multiplied by 2 in order to obtain the final uncertainties in the values of K given in Table 1.

The standard molar Gibbs energy $\Delta_r G^\circ_m$, enthalpy $\Delta_r H^\circ_m$, and entropy $\Delta_r S^\circ_m$ changes for reaction (1) at $T = 298.15$ K have been calculated from the measured equilibrium constants by using the model of Clark and Glew [24]. In performing this calculation, it was assumed that that the standard molar heat-capacity change $\Delta_r C^\circ_{p,m}$ for this reaction was zero. Thus, the calculated values for these thermodynamic quantities for reaction (1) at $T = 298.15$ K are:

Table 1
Results of equilibrium measurements for the transesterification reaction: (butyl acetate + benzyl alcohol = benzyl acetate + 1-butanol) in supercritical carbon dioxide at the pressure $p = 10.0$ MPa

T (K)	Direction	c(BuAc) (mol L^{-1})	c(BzAl) (mol L^{-1})	c(1-butanol) (mol L^{-1})	c(BzAc) (mol L^{-1})	Q	K
303.15	Forward	0.04533	0.04494	0.01997	0.02375	0.233 ± 0.005	0.248 ± 0.03
	Reverse	0.04073	0.04084	0.02061	0.02128	0.264 ± 0.006	
308.15	Forward	0.04292	0.04197	0.01987	0.02057	0.227 ± 0.002	0.236 ± 0.03
	Reverse	0.04163	0.04183	0.02063	0.02065	0.245 ± 0.008	
313.15	Forward	0.04608	0.04603	0.02258	0.02209	0.235 ± 0.003	0.245 ± 0.03
	Reverse	0.03620	0.03660	0.01844	0.01828	0.254 ± 0.007	
318.15	Forward	0.04137	0.04208	0.02010	0.02058	0.238 ± 0.004	0.256 ± 0.03
	Reverse	0.03707	0.03717	0.01932	0.01950	0.273 ± 0.006	

The temperatures T are given in column 1. Each reported concentration c (columns 3–6) gives the concentration of the substrate in $SCCO_2$ and is an average of three to five measurements. Abbreviations are: BuAc, butyl acetate; BzAl, benzyl alcohol; and BzAc, benzyl acetate. The forward reaction mixture consisted of ≈0.20 g of an equimolar mixture of butyl acetate and benzyl alcohol and ≈0.010 g of lyophilized lipase; the reverse reaction mixture consisted of ≈0.20 g of an equimolar mixture of 1-butanol and benzyl acetate and ≈0.010 g of lyophilized lipase. The equilibration time for the reaction was 3 h. The reaction quotient Q (column 7) is defined as c(benzyl acetate)·c(1-butanol)/{c(butyl acetate)·c(benzyl alcohol)}. The values of the equilibrium constant K (column 8) are the respective averages of the reaction quotients obtained from the forward and reverse directions of reaction. The uncertainties in the values of Q are equal to two estimated standard deviations of the mean. The basis for the uncertainties in the values of K is discussed in the text (see Section 3.2).

$K = 0.238 \pm 0.021$; $\Delta_r G^\circ_m = 3.56 \pm 0.22$ kJ mol^{-1}; $\Delta_r H^\circ_m = 2.1 \pm 5.2$ kJ mol^{-1}, and $\Delta_r S^\circ_m = -4.9 \pm 17$ J K^{-1} mol^{-1}. The value of $\Delta_r H^\circ_m$ and $\Delta_r S^\circ_m$ are zero within experimental error and, if one chooses to do so, one could justifiably set these two quantities to this value.

3.3. Related observations and summary

A significantly higher rate of reaction in $SCCO_2$ has also been reported for esterification [9], acylation [7], and interesterification reactions [21]. This higher conversion rate in $SCCO_2$ media compared to organic solvents may, as pointed out by Catoni et al. [9], be due to enhanced transport properties and a higher diffusivity of the solute in the supercritical fluid media. There are several other studies [10,25–28] dealing with the catalytic behavior of enzymes in $SCCO_2$. Most recently, Leitner [29] has emphasized the fact that a catalyst dissolved in a liquid phase can be recovered easily if the reaction products are captured in an adjacent supercritical phase.

The average value of the equilibrium constant for reaction (1) in $SCCO_2$ media is $K = 0.246$. This value is contrasted with the average value of $K = 0.29$ for this reaction in several organic solvents [15]. Thus, the thermodynamics of reaction (1) are not substantively changed by carrying it out in supercritical carbon dioxide media. This is an important result in that it provides a *tentative* basis for making estimates of the equilibrium constants for other reactions by using a known result(s) for the equilibrium constant for a given reaction in an organic solvent. Clearly, it would be desirable to have additional results available for several other representative reactions to test this hypothesis. In summary, supercritical carbon dioxide provides kinetic and environmentally friendly advantages for carrying out lipase-catalyzed reactions. This makes this substance a viable and potentially valuable alternative for use as a solvent in the biotechnology and pharmaceutical industries.

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