0273-2289/90/2303—0187$03.40

Thermodynamics of Industrially-Important, Enzyme-Catalyzed Reactions

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Received October 12, 1988; Accepted July 18, 1989

ABSTRACT

The thermodynamics of 10 industrially-important, enzyme-catalyzed reactions are examined. The reactions discussed are: the conversions of penicillin G to 6-amino-penicillinic acid using the enzyme penicillin acylase; starch to glucose using amylases; glucose to fructose using glucose (xylose) isomerase; cellulose to glucose using cellulase; fumaric acid and ammonia to L-aspartic acid using L-aspartase; transcinnamic acid and ammonia to L-phenylalanine using L-phenylalanine ammonia lyase; L-histidine to urocanic acid and ammonia using L-histidine ammonia lyase; lactose to glucose and galactose using lactase; and the reactions catalyzed by amino acylases and proteases. The selection of these processes was based on the economic value of the products and their intrinsic industrial importance. The available thermodynamic properties, such as equilibrium constants, Gibbs energies (ΔG°), enthalpies (ΔH°), and heat capacity changes (ΔC_p°) of these enzyme-catalyzed reactions, are reviewed and summarized. Recommendations are made for future research in this area.

Index Entries: Amino acids; amino acylase; amylase; aspartase; aspartic acid; cellulase; cellulose; enthalpy; Gibbs energy; glucose (xylose) isomerase; heat capacity; L-histidine ammonia lyase; lactase; lactose; L-phenylalanine ammonia lyase; protease; starch, thermodynamics; urocanic acid.

INTRODUCTION

In recent years, sales of biotechnological products have exceeded $15 billion (*1*). A variety of enzymes are now used in the food and pharma-

ceutical industries (2). In the food industry, for example, the most commonly used enzymes are α-amylase, glucoamylase, and glucose (xylose) isomerase (3). In the pharmaceutical industry, active antibiotics, such as penicillins and cephalosporins, are enzymatically produced (4). In general, biologically-active amino acids needed as medicinal agents and food additives are also enzymatically synthesized (5).

High product yield is important for successful commercialization of any conversion process. The factors influencing the product yields of these processes are temperature, ionic strength, pH, and concentrations of the substrates and cofactors (2). Thermodynamics plays an important role in the industrial process (6). Specifically, a knowledge of the thermodynamic parameters, i.e., the Gibbs energy (ΔG°), enthalphy (ΔH°), and heat capacity changes (ΔC_p°), can be used to calculate the optimal product yield which, in turn, is important in assessing the economic viability of a process. The need for thermodynamic data for the enzyme-catalyzed reactions becomes all the more apparent when one recognizes that, in addition to temperature and pressure as variables, one must consider the effects of pH, metal-ion concentrations, and ionic strength in the optimization of the performance of bioreactors. Thermodynamic data also provide information on the amount of heat evolved or absorbed in a process and define the energy costs for the process. Thus, a process can be carried out under optimal conditions to ensure maximum life and activity of the enzyme. An additional important feature of the utility of thermodynamic data is that it is now becoming possible, using genetic engineering techniques, to design enzymes for specific purposes. Since this is a time-consuming and expensive process, it is advantageous to use thermodynamic data to determine the economic benefits to be gained prior to embarking on such an effort.

An example of the importance of this data is illustrated by the recent use of thermodynamic data on evaluating the conversion of glucose to fructose at higher temperatures. High fructose corn syrup is produced from glucose using the enzyme glucose (xylose) isomerase (discussed later). The equilibrium data in the literature on this conversion process were highly scattered, and there were no calorimetric data available. Although all the available data indicated that the equilibrium constant and the product yield would increase with increasing temperature, the potential benefits and costs could not be assessed because of uncertainties in the data. Thus, industrial engineers could not make a decision on whether to proceed with the development of glucose isomerase enzyme that would operate successfully at high temperatures. As a consequence of both this need and the fundamental importance of the glucose/fructose equilibrium, a thermodynamic study of this process was undertaken at the National Institute of Standards and Technology (7,7a). This study was definitive, in that both the Gibbs energy and enthalpy changes were measured to resolve the issue of the effect of temperature on this process.

Specifically, the enthalpy and heat capacity data allowed for an accurate extrapolation of the equilibrium data to temperatures higher than at which measurements were performed. The data were used, in at least one industrial case, to decide that the payoff of going to higher temperatures was not sufficient to justify the currently high cost of the genetic engineering needed to develop the enzyme.

In this paper, the thermodynamics of 10 industrially-important, enzyme-catalyzed processes are discussed. The available thermodynamic data on these processes are examined and evaluated. Recommendations are made for future work in this area. We now turn our attention to a brief description of the thermodynamic formalism used to describe these processes.

DISCUSSION

Thermodynamic Representation of the Equilibria

A useful methodology for describing the thermodynamics of a biochemical process can be best described by means of an example. Let us first consider a simple example of the conversion of glucose to fructose using the enzyme glucose (xylose) isomerase (*7*). Here, ionic species are not involved. The reaction for the process is

$$\text{Glucose} \xrightarrow[\text{Isomerase}]{\text{Glucose}} \text{Fructose} \qquad \text{(A)}$$

and the corresponding equilibrium constant is:

$$K = [\text{Fructose}]/[\text{Glucose}] \qquad (1)$$

The percent conversion is given by

$$\% \text{ conversion} = \{K/(1+K)\}x100 \qquad (2)$$

Thermodynamic calculations frequently involve considerations of the effects of temperature and ionic strength. The temperature plays an important role in controlling percent conversion or the equilibrium constant. The temperature dependence (*8*) of the Gibbs energies or equilibrium constants and enthalpy changes can be calculated using

$$\Delta G^\circ_T = -RT \ln K_T = \Delta H^\circ_\theta + \Delta C^\circ_p(T-\theta) + T(\Delta G^\circ_\theta - \Delta H^\circ_\theta)/\theta - T\Delta C^\circ_p \ln(T/\theta) \qquad (3)$$

$$\Delta H^\circ_T \cong \Delta H^\circ_\theta + \Delta C^\circ_p(T-\theta) \qquad (4)$$

Here, T is the thermodynamic temperature and θ is the reference temperature of 298.15 K.

Now we consider a more complex example, the conversion of penicillin G (PGH_2) to phenylacetic acid (ϕAH) and 6-aminopenicillanic acid ($APAH_2$), which involves a multiplicity of ionic species (*8*). Note that hydrogen ions are included in the abbreviations to indicate that each of the substances can exist in various states of ionization. The overall reaction is

$$PGH_2 + H_2O \xrightarrow[\text{Acylase}]{\text{Penicillin}} \phi AH + APAH_2 \qquad \text{(B)}$$

The equilibrium constant for this process is

$$K_{obs} = [\Sigma\phi AH][\Sigma APAH_2]/[\Sigma PGH_2] \qquad (5)$$

Here, the square brackets denote concentrations. Hydrogens are included in the abbreviations to indicate that each of the substances can exist in various states of ionization. The "Σ" represents the total of the concentrations of the different ionic states for a given substance in solution. A reference reaction, involving specific ionic states of each of the substances, is introduced

$$PGH^- + H_2O = \phi A^- + APAH^- + H^+ \qquad \text{(C)}$$

The equilibrium constant for the reference reaction is

$$K_{ref} = (\phi A^-)(APAH^-)(H^+)/(PGH^-) \qquad (6)$$

In the above equation, the parentheses denote the activities, as distinct from concentrations, of the reference species in solution. The use of activities necessitates the use of a standard state which, in this paper, is the hypothetical ideal solution of unit molality (*8*). For this reaction the relationship between these two equilibrium constants is (*8*)

$$K_{obs} = K_{ref}\ f_{PG}/(f_{\phi A}\ f_{APA}) \qquad (7)$$

Here, the "f"s are the fractions of a given substance existing in a specified ionic state. Thus, f_{PG} is the fraction of penicillin G existing in solution as the ion PGH^-, i.e., $[PGH^-]/[\Sigma PGH_2]$. Values of the ionization constants are needed to calculate these fractions and explicit formulae can be introduced for this purpose [8]. Nonideal solution behavior can be accounted for (*8*) by the introduction of activity coefficeints (γ) for each of the species in solution

$$\ln \gamma_i = -Az_i^2 I^{1/2} / (1 + BI^{1/2}) \qquad (8)$$

Here, A is a Debye-Hückel constant, z_i is the charge of the *ith* species, B is an "ion-size" parameter, and I is the ionic strength.

Thus, the treatment of biochemical equilibria requires the use of a formalism that is able to account for the effects of a multiplicity of charged and uncharged species in solution and the effects of temperature and ionic strength on the position of equilibrium and the enthalpy of the reaction. The reader is referred to references (*8–10*) for further details on calculations of this nature that make it possible to predict these effects in a systematic way. Consideration of the several processes of industrial interest follows.

Penicillin Acylase

Since the early 1940s penicillins have been used as antibiotics to control bacterial infections. Today antibiotics are used in plant protection, animal nutrition, and human medicine (*11*). Initially, these were naturally-occurring compounds but, over the years, considerable efforts have been made to extend the range of penicillins. Commonly-used antibiotics are semisynthetic ones that are prepared by chemical modifications of natural antibiotics. The basic structure of the penicillins is 6-aminopenicillanic acid (6-APA), which consists of a thiozolidine ring with a condensed β-lactam ring, shown in Fig. 1. The parent benzyl penicillin (known as penicillin G) or phenoxyl methyl penicillin (penicillin V) is produced by fermentation (*12*) and then treated with the enzyme, penicillin acylase (EC 3.5.1.1), to remove the side chain without degrading the activity of 6-APA. The reaction is

$$\underset{PGH_2}{\text{Penicillin G}} + H_2O \xrightarrow[\text{Acylase}]{\text{Penicillin}} \underset{\phi AH}{\text{Phenylacetic acid}} + \underset{APAH_2}{\text{6-aminopenicillanic acid}} \qquad \text{(B)}$$

The equilibrium constant for the overall process is

$$K_{obs} = [\Sigma\phi AH][\Sigma APAH_2]/[\Sigma PGH_2] \qquad (5)$$

A variety of synthetic penicillin antibiotics (*see* Fig. 1) are then synthesized from 6-APA by chemical substitution. In 1984, approximately 2500 tons of 6-APA (*12*) were produced by this method. Most of it was used to manufacture semisynthetic penicillins. The factors influencing the production of 6-APA and the semisynthetic penicillins are pH, temperature, and ionic strength of the reaction media. Recent work (*8*) shows that the extent of reaction for the conversion of penicillin G (or V) to 6-APA and phenylacetic acid (or phenoxyacetic acid) is highly pH dependent. The thermodynamic quantities for the reference reaction at 298.15 are (*8*) $K_{ref} = (7.35 \pm 1.5) \times 10^{-8}$, $\Delta G^\circ = 40.7 \pm 0.5$ kJ/mol, $\Delta H^\circ = 29.7 \pm 0.6$ kJ/mol, and $\Delta C_p^\circ = -240 \pm 50$ J/(mol K). The temperature dependence of the equilibrium

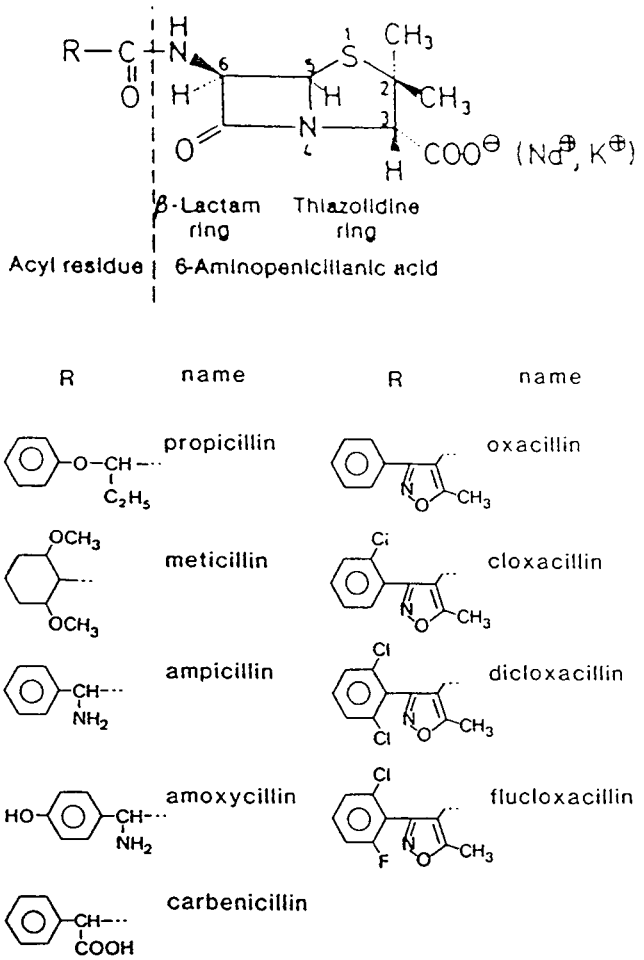


Fig. 1. Chemical structures of some semisynthetic penicillins.

constant may be calculated using these values and Eqs. (3) and (4), with effects of nonideality being accounted for using Eq. (8). The pH dependence of the equilibrium constant (K_{obs}) is shown in Fig. 2. In a typical industrial environment, the production of 6-APA is carried out around pH 8, where more than 99% of the penicillins are cleaved to 6-APA. Generally, the semisynthetic penicillins are manufactured from 6-APA and a suitable acyl residue by lowering the pH of the reaction media to around 3. This favors the formation of semisynthetic penicillins, and more than 99% is converted to semisynthetic penicillins.

Amylases

Starch, a glucose polymer, is one of the most widely available plant polysaccharides. The starch-processing industries are the largest user of

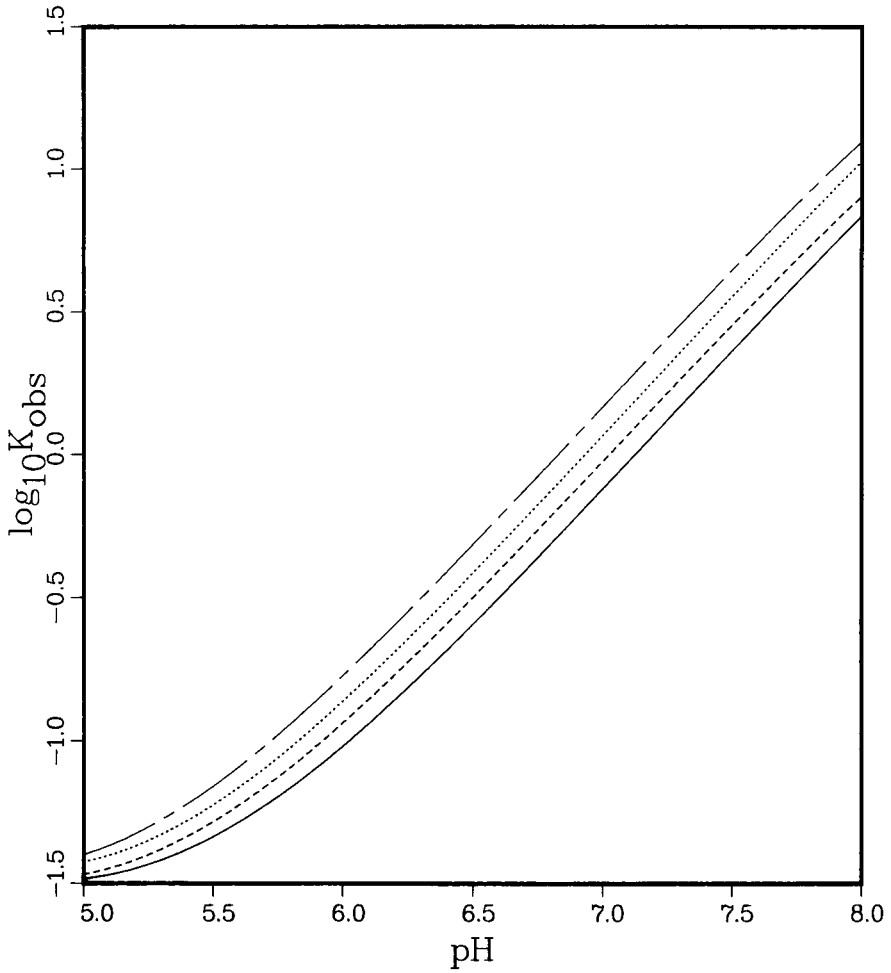


Fig. 2. Equilibrium constants (K_{obs}) for the conversion of penicillin G to phenylacetic acid and 6-aminopenicillanic acid. The equilibrium constant is shown as a function of pH at different temperatures and ionic strengths: $T=298.15$ K and $I=0.0$ mol/kg (———); $T=298.15$ K and $I=0.1$ mol/kg (------); $T=310.15$ K and $I=0.0$ mol/kg (• • • • • • •); and $T=310.15$ K and $I=0.1$ mol/kg (— — —). K_{obs} is equal to [ΣAPA] [ΣϕA[/[ΣPG].

amylases, mainly owing to success of α-amylase/amyloglucosidase-based processes for forming glucose syrups. Success has continued with high fructose corn syrups, formed by addition of glucose isomerase (discussed later), to the above processes. Currently, the main source of raw material is corn starch. The conversion of starch (2) to high fructose corn syrups or ethanol involves several steps: liquification of the starch, saccharification of starch, and isomerization of glucose to fructose (or fermentation of glucose to ethanol). During the conversion processes, the byproducts of corn oil and gluten are removed, and a starch slurry of about 30–40% concn. is

prepared. The slurry is then hydrolyzed by amylases to short-chain polymer units known as dextrins. This step is the liquification of starch. The dextrins are then hydrolyzed by glucoamylase (or amyloglucosidase) to glucose units according to the following reactions

$$\text{Starch} \xrightarrow{\alpha\text{-Amylase}} \text{Dextrin} \xrightarrow{\text{Glucoamylase}} \text{Glucose} \qquad \text{(D)}$$

The overall process is known as the saccharification of starch. Since glucose is not as sweet as its isomer fructose, it is converted to fructose using the enzyme, glucose isomerase (*see* next section). For ethanol production, glucose is fermented by yeast, such as *saccharomyces cerevisiae.*

Generally there are two types of glucosidic linkages between the glucose units composing the starch polysaccharides (*13*). The most predominant one is the α-1,4 linkage, which is involved in the formation of long-chain molecules, and the other one is the α-1,6 linkage, which participates in crosslinking of the individual glucose chain. During hydrolysis processes, these linkages are cleaved. The important enzymes (*13*) in the starch saccharification process are α-amylases, β-amylases, glucoamylases, pullulnase, and isoamylases. These hydrolyze the α-1,4 and α-1,6 glucosidic linkages. The α-amylase and glucoamylase (also known as amyloglucosidase) are the most commonly used enzymes in the starch industry.

Ono and Takahashi (*14*) have examined the available literature data on the hydrolysis of tri- and oligosaccharides. Based upon their own measurements on panose (one α-1,4 and one α-1,6 linkage), maltotroise (two α-1,4 linkages), and amylose ($\sim$6000 α-1,4 linkages), they present evidence that the enthalpy change corresponding to the hydrolysis of an individual glucosidic linkage is independent of the number of glucosidic linkages in a carbohydrate. Van Beynum et al. (*15*) report equilibrium data on the hydrolysis of α-1,4 and α-1,6 linkages. They state that the equilibrium constants for the hydrolysis of α-1,4 and α-1,6 linkages are independent of chain length. More recently, we have carried out hydrolysis studies of these glucosidic linkages using maltose (for α-1,4) and isomaltose (for α-1,6) as substrates (*16*).

Let us now consider the hydrolysis of the disaccharide maltose

$$\text{Maltose} + H_2O = 2\ \text{Glucose} \qquad (9)$$

The equilibrium constant for this process is (*16*)

$$K = [\text{glucose}]^2/[\text{maltose}] = [2x]^2/(m-x) \qquad (10)$$

Here, the square brackets indicate molalities (mol/kg), m is the initial molality of the maltose, and the quantity $2x$ is the molality of the glucose at equilibrium. The percent conversion for such an asymmetrical reaction is given by

$$\% \text{ conversion} = [(-K + \sqrt{(K^2 + 16Km)})/8] \cdot 100 \quad (11)$$

The recommended thermodynamic parameters at 298.15 K for the hydrolysis of the α-1,6 glucosidic linkage in isomaltose are (*16*): $K = 17.25 \pm 0.7$, % conversion = 83.74 (at m = 1 mol/kg), $\Delta G^\circ = -7.06 + 0.10$ kJ/mol, $\Delta H^\circ = 5.86 \pm 0.54$ kJ/mol, and $\Delta C_p^\circ \approx 13$ J/(mol K). For the hydrolysis of the α-1,4 glucosidic linkage in maltose, the recommended parameters are: $K \geq 513$, % conversion ≥ 99.23 (at m = 1 mol/kg), $\Delta G^\circ \leq -15.5$ kJ/mol, $\Delta H^\circ = -4.02 \pm 0.15$ kJ/mol, and $\Delta C_p^\circ \approx 43$ J/(mol K).

Glucose Isomerase

The isomerization of glucose to fructose using the enzyme glucose isomerase (EC 5.3.1.5) is one of the important large scale industrial processes. Production of high fructose syrups by this process is now over 4.3 million tons per year in the US (*18*), and the total market value is approximately one billion dollars per year. The conversion of glucose to fructose is

$$\text{Glucose} \xrightarrow[\text{Isomerase}]{\text{Glucose}} \text{Fructose} \quad (A)$$

The corresponding equilibrium constant for the process is

$$K = [F]/[G] \quad (1)$$

Presently, corn is the main source of fructose-rich syrup. As discussed in the preceding section, in this conversion process, corn is first hydrolyzed to monomeric hexose sugars, principally glucose. Since fructose is about three times sweeter (*16*) than glucose, conversion to fructose increases its value. This conversion is carried out using the enzyme glucose (xylose) isomerase. Using a combination of HPLC and microcalorimetric measurements (*7,7a*), the thermodymainc quantities for process (A) at 298.15 K are: $K = 0.87 \pm 0.02$, % conversion = 46.52, $\Delta G^\circ = 0.35 \pm 0.05$ kJ/mol, $\Delta H^\circ = 2.78 \pm 0.20$ kJ/mol, $\Delta C_p^\circ = 76 \pm 30$ J/(mol K). The temperature dependence of the equilibrium constant can be calculated using Eq. (3).

Cellulase

Cellulose, a crystalline glucose polymer, is the most abundant source of carbohydrates and renewable energy. Nearly 40% of plant cellulosic material is cellulose. It can be hydrolyzed to glucose using the enzyme cellulase (EC 3.2.1.4). The main linkage involved between the glucose units composing the cellulose polysaccharides is the β-1,4 glucosidic bond (*19*). During the hydrolysis of cellulose, the β-1,4 linkage is cleaved. The thermodynamic parameters for the hydrolysis of the β-1,4 linkage have recently (*16*) been reported using cellobiose as the substrate. The thermodynamic

parameters for the hydrolysis of the β-1,4 glucosidic linkage in celllobiose at 298.15 K are: $K \geq 155$, % conversion ≥ 97.54, (at m = 1 mol/kg), $\Delta G^\circ \leq -12.5$ kJ/mol, $\Delta H^\circ = -2.43 \pm 0.31$ kJ/mol, and $\Delta C_p^\circ \approx 17$ J/(mol K). There is no data in the literature on higher oligomers involving β-1,4 linkages.

Plant cellulosic materials consist mainly of three components: cellulose, hemicellulose, and lignin (*20*). Hemicellulose is a polymer of xylose with β-1,4 glucosidic linkages and side chains of arabinose and other pentose sugars. In order to develop an economically feasible process, it is necessary to utilize all these components. Hemicellulosic materials can be enzymatically hydrolyzed to pentose sugars, mainly xylose. Xylose cannot be easily fermented to ethanol, so it is isomerized to xylulose using glucose (xylose) isomerase (*21*), which is then fermented to ethanol (*22*). The lignin constitutents, amounting to 18–30% of plant mass, are the slowest to undergo biodegradation. These are used in glues, resins, adhesives, and several other industries (*5*).

L-Aspartase

L-Aspartic acid is widely used in medicine and as a food additive and is one of the principal ingredients in a low calorie sweetener, known as "aspartame" or "Nutrasweet" which is a dipeptide, L-aspartyl-L-phenylalanine methyl ester. Aspartame is about 200 times as sweet as sucrose (*2*). It has an annual market value of about one billion dollars in 1988. Since 1973, L-aspartic acid has been produced enzymatically from fumarate and ammonia, using the enzyme aspartase (L-asparatate ammonia lysase, EC 5.3.1.1)

$$\underset{\text{Fum}}{\text{Fumaric acid}} + \underset{\text{Amm}}{\text{Ammonia}} \xrightarrow{\text{Aspartase}} \underset{\text{Asp}}{\text{L-aspartic acid}} \quad \text{(E)}$$

The overall equilibrium constants of the process is

$$K_{obs} = [\Sigma Asp]/\{[\Sigma Fum][\Sigma Amm]\} \tag{12}$$

The thermodynamic parameters for the reference reaction at 298.14 K are (*8*): $K_{ref} = 676 \pm 46$, $\Delta G^\circ = -16.15 \pm 0.15$ kJ/mol, $\Delta H^\circ = -24.5 \pm 1.0$ kJ/mol, and $\Delta C_p^\circ = 147 \pm 100$ J/(mol K). Here, the reference reaction is

$$\text{fumarate}^{2-}(aq) + NH_4^+(aq) = \text{aspartate}^-(aq)$$

In industrial conversion processes, the ammonia concentration is kept high to force the formation of aspartic acid. Under typical operating conditions (pH 8.5, 10 m*M* $MgCl_2$, 37°C, and 1*M* ammonium fumarate solution), a 95% conversion to aspartic acid is conveniently obtained (*2*). The temperature and pH dependence of the equilibrium constant (K_{obs}) are given in Figs. 3 and 4, respectively.

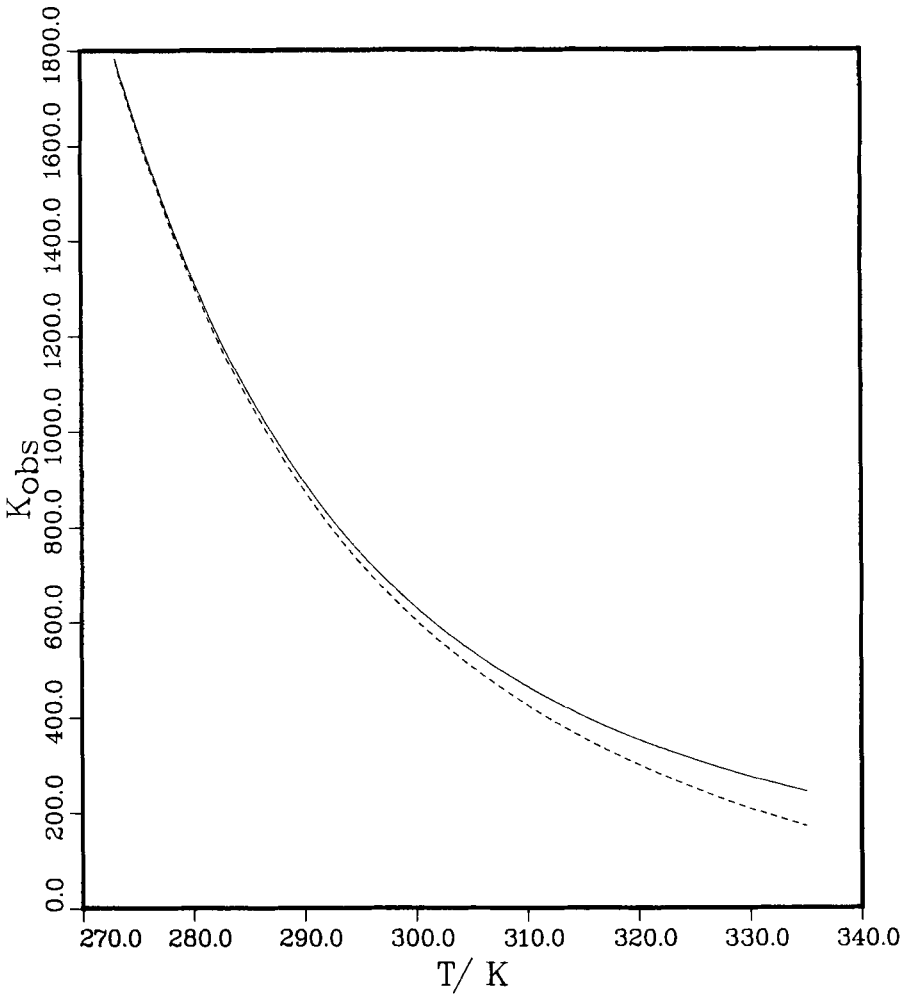


Fig. 3. The equilibrium constant (K_{obs}) for the conversion of fumaric acid and ammonia to aspartic acid as a function of temperature at zero ionic strength and at pH 7.0 (———) and 8.0 (-------). K_{obs} is equal to $[\Sigma Asp]/\{[\Sigma Fum][\Sigma Amm]\}$.

L-Phenylalanine Ammonia Lyase

L-Phenylalanine is used as a food additive and medicinal agent. It is also one of the principal ingredients in the sweetener "aspartame." At present, L-phenylalanine is enzymatically produced from trans-cinnamic acid and ammonia using the enzyme L-phenylalanine ammonia lyase (EC 4.3.1.5)

$$\underset{\text{Tca}}{\text{Trans-cinnamic acid}} + \underset{\text{Amm}}{\text{Ammonia}} \xrightarrow[\text{Ammonia-lyase}]{\text{Phenylalanine}} \underset{\text{PhAla}}{\text{L-Phenylalanine}} \qquad \text{(F)}$$

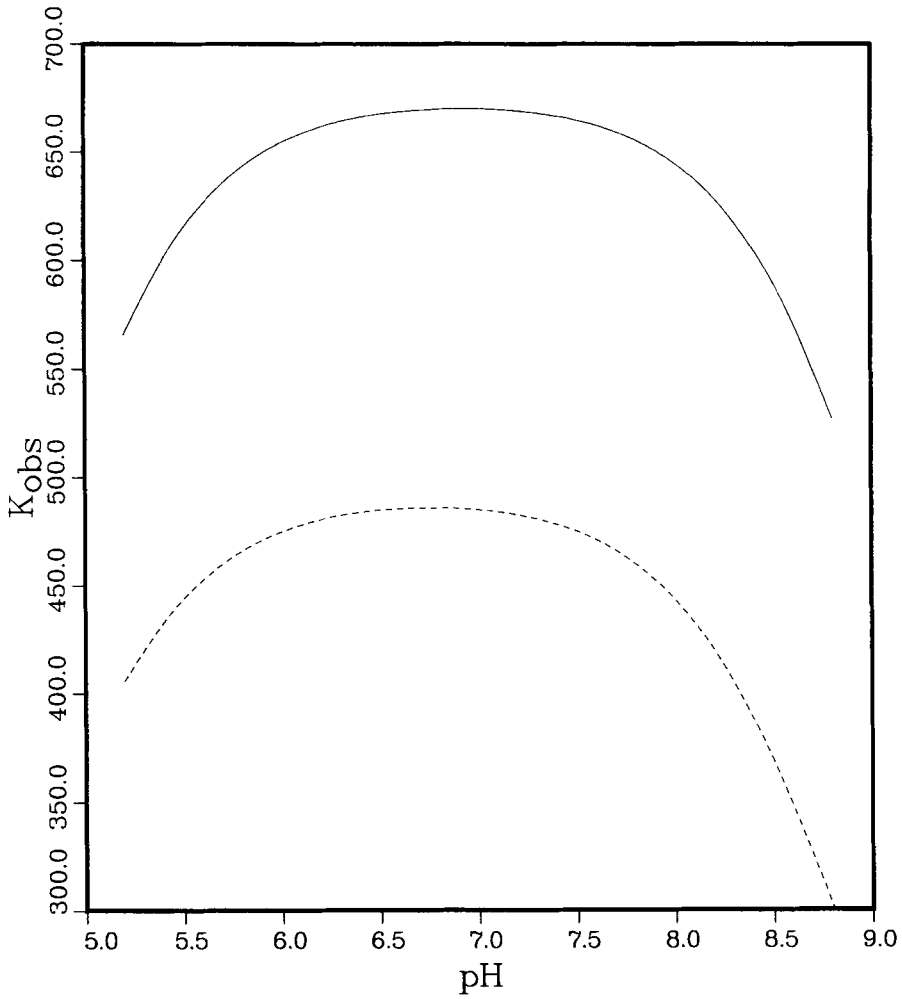


Fig. 4. The equilibrium constant (K_{obs}) for the conversion of fumaric acid and ammonia to aspartic acid at 298.15 K as a function of pH at ionic strengths of 0.0 mol/kg (———) and 0.1 mol/kg (•••••••). K_{obs} is equal to [ΣAsp]/{[ΣFum][ΣAmm]}.

The reference reaction for this process is

$$\text{Trans-cinnamic acid}^{-}(aq) + NH_4^+(aq) = \text{L-phenylalanine}^0(aq)$$

The overall equilibrium constant (K_{obs}) for the process is:

$$K_{obs} = [\Sigma PhAla]/\{[\Sigma Amm][\Sigma Tca]\} \quad (13)$$

The thermodynamic parameters for the reference reaction at 298.15 K are (*9*): $K_{ref} = 0.862 \pm 0.24$, $\Delta G^\circ = 0.368 \pm 0.57$ kJ/mol, $\Delta H^\circ = -24.8 \pm 2.0$ kJ/mol, and $\Delta C_p^\circ \approx -50$ J/(mol K). The optimal product yield under a given set of conditions can be calculated from the knowledge of the equilibrium con-

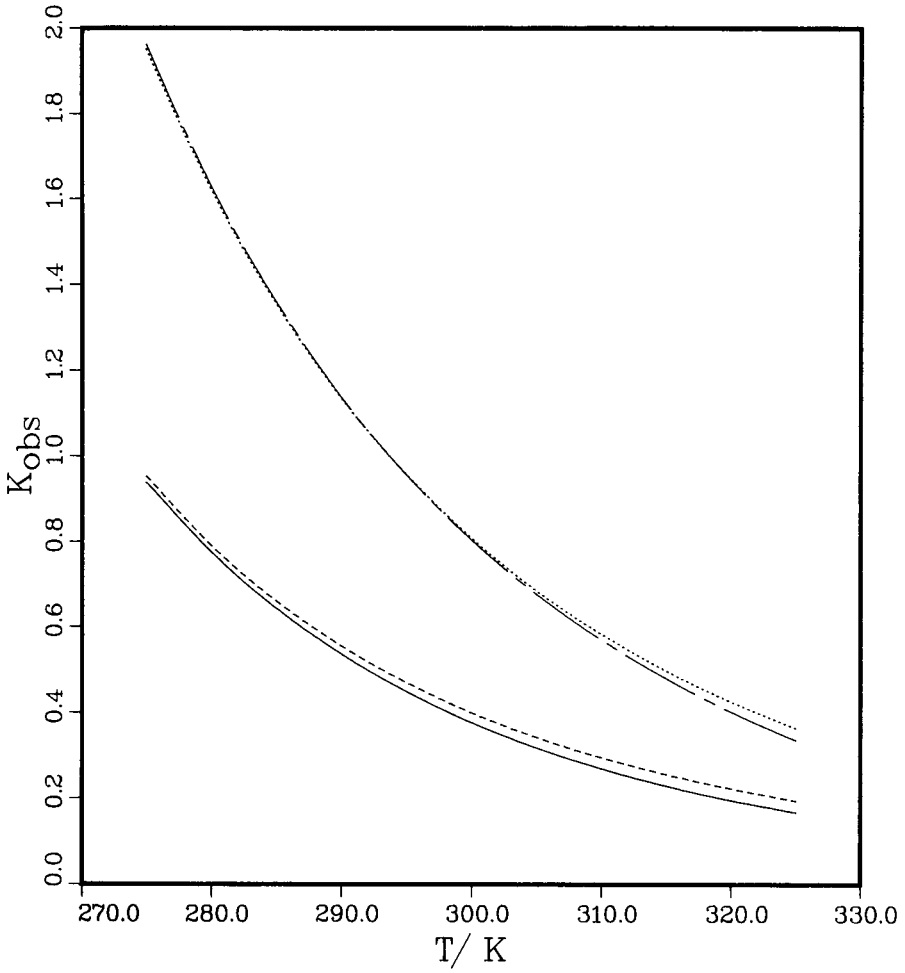


Fig. 5. The equilibrium constant (K_{obs}) for the conversion of transcinnamic acid and ammonia to phenylalanine as a function of temperature under the following conditions: (———) pH 7.0, [ΣPhAla]+[ΣAmm]=0.0025 mol/kg, [NH_4Cl]=1.0 mol/kg, and [Tris]=0.10 mol/kg; (----) pH 8.0, [ΣPhAla]+[Amm] =0.0025 mol/kg, [NH_4Cl]=1.0 mol/kg, and [Tris]=0.10 mol/kg; (•••••••) pH 7.0 and I=0.0 mol/kg; (——) pH 8.0 and I=0.0 mol/kg. K_{obs} is equal to [ΣPhAla]/{[ΣAmm][ΣTca]}.

stant. A plot of the overall equilibrium constant (K_{obs}) as a function of temperature, and at different values of pH and ionic strength, is given in Fig. 5.

L-Histidine Ammonia Lyase

Urocanic acid is used in the pharmaceutical and cosmetic industries and has been produced (*23*) from L-histidine by the action of L-histidine ammonia lyase (EC 4.3.1.3)

$$\underset{\text{Hist}}{\text{L-Histidine}} \xrightarrow[\text{Ammonia-lyase}]{\text{Histidine}} \underset{\text{Uroc}}{\text{Urocanic acid}} + \underset{\text{Amm}}{\text{Ammonia}} \qquad \text{(G)}$$

The equilibrium constant for the process is

$$K_{obs} = [\Sigma Uroc][\Sigma Amm]/[\Sigma Hist] \qquad (14)$$

The equilibrium constant (K_{obs}) for this process has been reported (*24*) to be approximately 3 at 25°C and pH 8. However, Chibata et. al. (*25*) have reported that, when an aqueous solution of histidine (0.25*M*, pH 9.0) containing 1 m*M* Mg^{2+} is passed through a column packed with an immobilized enzyme, the L-histidine was completely converted to urocanic acid. Further experiments are needed to resolve this apparent discrepancy.

Lactase

Lactose, a disaccharide milk sugar is hydrolyzed by the enzyme lactase or β-galactosidase (EC 3.2.1.23) into glucose and galactose

$$\text{Lactose} \xrightarrow{\text{Lactase}} \text{Glucose} + \text{Galactose} \qquad \text{(H)}$$

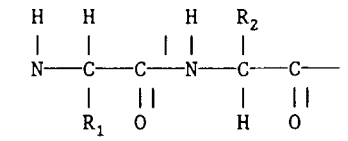
This hydrolysis is interesting (*26,27*) from several points of view. A large percentage of the world's population cannot tolerate lactose and, therefore, cannot drink normal (or untreated) milk and milk products. Lactose is the major byproduct of cheese manufacturing and makes up nearly 70% of whey solids. Very large quantities of whey, therefore, are discharged every year, causing disposal problems and the loss of valuable nutrients. Lactose is less soluble and not as sweet as sucrose. However, its hydrolyzed products have about 0.8 times the sweetening capacity of sucrose, and they are about three to four times more soluble (*2*). The glucose may also be isomerized to fructose (*see* Glucose Isomerase Section) to increase sweetening power of the product.

The hydrolysis of lactose has been studied recently (*28*). The recommended thermodynamic parameters for this process at 298.15 K are: $K = 14.7 \pm 1.3$, % conversion = 94 (at m = 1 mol/kg), $\Delta G^\circ = -6.66 \pm 0.21$ kJ/mol, $\Delta H^\circ = 0.44 \pm 0.11$ kJ/mol, and $\Delta C_p^\circ = 9 \pm 20$ J/(mol K).

L-Aminoacylase

Amino acids have extensive applications in the food industry, as feed additives, in medicines, and as starting materials in the chemical industries. In the food industry, amino acids are used as flavor enhancers, such as sodium glutamate. Sodium aspartate and alanine are added to fruit juices, and glycine is added to food containing sweeteners. As discussed earlier, the artificial sweetener "Nutrasweet" is a dipeptide of

Table 1
Specific Enzymatic Cleavages of Polypeptide Chains



amino acid 1 amino acid 2

Enzyme	Peptide Bonds Cleaved
Trypsin	amino acid 1 = Lys or Arg
Chymotrypsin	amino acid 1 = Phe, Trp, or Tyr
Pepsin	amino acid 1 = Phe, Trp, Tyr, and several others
Thermolysin	amino acid 2 = Leu, Ile, or Val

L-aspartaic acid and L-phenylalanine. One of the routes for the synthesis of amino acids is enzymatic conversion. Here, a class of enzymes, L-aminoacylases, have come into general use for the production of biologically-active amino acids. Several important amino acids, such as L-alanine, L-methionine, L-tryptophane, and L-valine, are currently manufactured using this technology (*29*). At present, thermodynamic data on most of these systems are not available.

Proteases

Measured by weight, proteases represent the greatest single item enzyme. The annual production is more than 500 tons (*30*). The bulk of these proteases are used as additives by the detergent industry. Alkaline proteases facilitate the hydrolytic degradation of proteins that cause stains that are hard to remove. Today, 80% of detergents offered in the market contain enzymes with active ingredients in the range of 0.015 to 0.025 mass percent. Acid proteases are used in the manufacture of cheese in the dairy industry (*30*) and are marketed as a substitute or supplement to renin. Proteases have also found application in the hydrolysis of soybean and in the baking industry. Proteases are generally used to hydrolyze the peptide bond selectively (*31*), such as trypsin, chymotrypsin, pepsin, and thermolysin. The specificities of enzymes for peptide bonds are shown in Table 1. Enthalpies of hydrolysis of several peptide bonds have been determined (*32*), and some equilibrium data exists for a few systems (*33*). However, a systematic investigation of both the equilibrium constants and the enthalpies of hydrolysis of peptide bonds would be interesting to industries and biochemists.

SUMMARY

Optimization of product yields, and the efficient utilization of energy in processes using enzyme-catalyzed reactions, is aided using thermodynamic information. Using the appropriate data in a thermodynamic model allows one to predict the effects of temperature, pH, and ionic strength on the directions of chemical reactions. Thermodynamic data on seven of the 10 enzyme-catalyzed reactions have been summarized. There are no thermodynamic data on the systems catalyzed by aminoacylase. A limited amount of data exists for L-histidine ammonialyase and the proteases. Systematic investigations of both the equilibrium constants and enthalpies of these reactions would be interesting to process engineers and chemists.

ACKNOWLEDGMENTS

I thank to Robert N. Goldberg and Eugene S. Domalski for their encouragement and helpful discussions.

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