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Thermodynamic characterisation of carbohydrate-active enzymes

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Abstract

Many carbohydrate-active enzymes catalyse a specific reaction pattern instead of one particular reaction. For example, glucanotransferases and glucosyltransferases recognise the reducing ends of glucans irrespective of their degree of polymerisation. Thus, in principle, they are capable of catalysing an infinite number of reactions. Here we show how concepts from statistical thermodynamics can be employed to characterise the action patterns of polymer-active enzymes and determine their equilibrium distributions. For selected enzymes, we provide experimental evidence that our theory provides accurate predictions. We further outline how the thermodynamic description can be employed to experimentally determine bond energies from equilibrium distributions of polymer-active enzymes.

INTRODUCTION

The classical thermodynamic characterization of enzymes is based on the thermodynamic equilibrium K_{eq}. However, this equilibrium constant is only defined if the enzyme under consideration catalyses exactly one reaction of the general form

$$\sum_i \nu_i A_i = 0, \tag{1}$$

in which A_i are the involved chemical species and the ν_i are the stoichiometric coefficients, denoting how many molecules are consumed (negative ν_i) or produced (positive ν_i) per reaction.

In carbohydrate metabolism many enzymes important in the synthesis and degradation of biopolymers do not follow this rule. A prominent example are glucanotransferases [1] specifically binding the non-reducing end of a glucan, however irrespective of its exact length, or degree of polymerisation (DP). This leads to the problem that glucanotransferases mediate an infinite number of theoretically possible transfer reactions. For example disproportionating enzyme 1 (DPE1), which plays an important role in the starch breakdown pathway by metabolising maltotriose, transfers $q = 1,2,3$ glucosyl residues from one glucan to another, resulting in the chemical reactions

$$G_n + G_m \rightleftharpoons G_{n-q} + G_{m+q}. \tag{2}$$
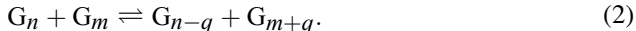
In this particular example, every reaction (2) is even energetically neutral [2], leading to $K_{eq} = 1$. Since the enzyme acts on a polydisperse mixture containing a large number of specific reactants, the characterisation of the thermodynamic equilibrium by the classical equilibrium constant is clearly insuffient.

An approach leading to a satisfying enzymatic characterisation is based on a description of polydisperse mixtures as statistical ensembles, which we recently proposed [3]. A glucan of DP n is described as a particle with energy state E_{n-1}, reflecting the energy stored in the interglucosidic bonds. In this framework, an enzyme such as DPE1 mediates a transition of particles between energy states, where the possible transitions reflect the enzymatic mechanism. For example, DPE1 will simultaneously shift one particle one, two or three energy levels up and one particle the same number of levels down.

In this paper, we will exploit this analogy and derive a consistent thermodynamic characterisation of the polydisperse equilibrium. We show how this understanding can be used to determine bond energies from experimentally observed equilibrium distributions.

THEORY

A mixture of dissolved substances can be explicitly characterised by its Gibbs energy which is conveniently written as

$$G = G^f - T\tilde{S}_{\text{mix}}. \tag{3}$$

Here, G^f is the total energy of formation and $\tilde{S}_{\text{mix}}$ is the mixing entropy. Numbering the dissolved substances by i and denoting their respective concentrations with c_i, the total energy of formation is

$$G^f = \sum_i c_i \Delta_f G_i^0, \tag{4}$$

where $\Delta_f G_i^0$ denotes the standard Gibbs energy of formation of substance i, and the mixing entropy of the solution can be written as [3, Supplementary information]

$$\tilde{S}_{\text{mix}} = -R \sum_i c_i (\ln c_i - 1). \tag{5}$$

Any reaction system acting on such a mixture of dissolved reactants changes the composition of the solution and therefore its formation energy and mixing entropy in such a way that the Gibbs energy decreases [4]. To identify the equilibrium, the minimum of the Gibbs energy has to be determined, taking into account that the concentrations of the dissolved substances cannot change completely arbitrarily but are constrained through conservation rules imposed by the reaction network. If $\mathbf{N}$ is the stoichiometric matrix of the reaction network, the conservation rules are defined by the left-sided kernel [5]

$$\mathbf{L} \cdot \mathbf{N} = 0. \tag{6}$$

There exist $k = \text{rank } \mathbf{L}$ independent conserved quantitites, which in their most general form can be written as

$$\sum_j l_{ij} c_j = b_i, \quad \text{with} \quad i = 1 \ldots k, \tag{7}$$

where the values l_{ij} are stoichiometric coefficients and b_i are constants that are defined by the initial conditions and thus are experimentally controllable. The equilibrium of any reaction system can consequently be calculated by minimising the Gibbs energy (3) under the constraints (7).

Glucanotransferases mediate bimolecular reactions of the type (2), converting two substrate molecules in two product molecules by transferring a number of residues from one to the other. Consequently, a glucanotransferase in isolation catalyses a reaction network in which the number of molecules is conserved,

$$c^{\text{tot}} = \sum c_i = \text{const.,} \tag{8}$$

and it is convenient to introduce the molar fractions

$$x_i = \frac{c_i}{c^{\text{tot}}}, \tag{9}$$

leading to

$$\sum c_i(\ln c_i - 1) = \sum c_i \ln c_i - c^{\text{tot}} = c^{\text{tot}}\left[\sum x_i \ln x_i + \ln c^{\text{tot}} - 1\right]. \tag{10}$$

Since $\ln(c^{\text{tot}}) - 1$ is constant, it is irrelevant for the determination of the minimum of the Gibbs free energy. Defining the molar energies of formation by $G^f = c^{\text{tot}} g^f$ allows to define the molar Gibbs free energy

$$g = g^f - RT \sum x_i \ln x_i = g^f - TS, \tag{11}$$

where the mixing entropy

$$S = -R \sum x_i \ln x_i, \tag{12}$$

now assumes its well-known form. Notably, this definition is independent on the choice of the units in which concentrations are measured. In contrast, $\tilde{S}_{\text{mix}}$ as defined in Eq. (5) is dependent on the concentration units.

CHARACTERISATION OF THE EQUILIBRIUM OF VARIOUS TRANSFERASES

Disproportionating enzyme 1 (DPE1)

Disproportionating enzyme 1 (DPE1) catalyses reactions according to formula (2) mentioned in the Introduction, where $q = 1,2,3$ is the number of transferred glucosyl residues. Obviously, this reaction conserves the total number of molecules,

$$\sum x_i = 1. \tag{13}$$

This reaction is energetically neutral in the sense that the bond enthalpies of the cleaved α-1,4 glucosidic linkage and the formed α-1,4 glucosidic linkage are identical [2]. Thus, the catalytic activity of DPE1 does not change g^f and minimising g in Eq. (11) is equivalent to maximising

$$\frac{S}{R} = -\sum x_i \ln x_i. \tag{14}$$

Since the enzyme is only attacking glucosidic linkages between the glucosyl residues, we identify a glucan of DP n with an energy state E_{n-1} containing the bond enthalpies of $n-1$ α-1,4 glucosidic linkages.

Thermodynamic characterisation of carbohydrate-active enzymes
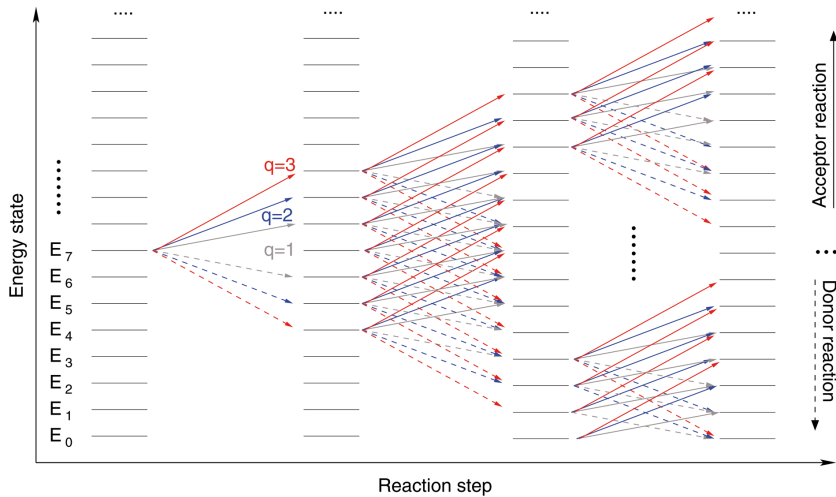
Figure 1. Scheme of the DPE1 mediated reaction system. DPE1 mediates transfers of glucose, maltose and maltotriose units, i. e. $q = 1,2,3$. In each reaction step the system follows an arbitrary dashed and solid arrow of the same colour simultaneously. This leads to a combinatorial explosion of the reaction system. The lower limit of DP leads to a reflecting boundary condition for G_1 which causes the Boltzmann distribution.

As depicted in Figure 1, the catalytic action of DPE1 corresponds to the simultaneous downward shift of one molecule from an energy state E_n to E_{n-q} (donor reaction) and an upward shift of another molecule from energy state E_m to E_{m+q} (acceptor reaction), where $q = 1,2,3$. We denote by x_i the molar fraction of molecule in energy state E_i. Thus, the molar fraction of glucose molecules is x_0 and that of a glucan of DP n is x_{n-1}.

Apart from the conservation of the total number of molecules, Eq. (13), the enzyme action also conserves the total number of interglucose bonds. Thus

$$\sum k \cdot x_k = b, \tag{15}$$

where the constant b describes the average number of bonds per glucan. This number is determined by the initial condition with which the reaction is initiated and is therefore an experimentally controllable parameter.

To obtain the molar fractions $\{x_i\}$ which maximise the entropy defined by Eq. (14), we define the Lagrange function

$$L(x_k; \alpha, \beta) = -\sum_k x_k \ln x_k - \alpha \left(\sum_k x_k - 1 \right) - \beta \left(\sum_k k \cdot x_k - b \right) \tag{16}$$

and set the partial derivatives to zero,

$$0 = \frac{\partial L}{\partial x_0} = -(\ln x_0 + 1) - \alpha, \tag{17}$$

$$0 = \frac{\partial L}{\partial x_k} = -(\ln x_k + 1) - \alpha - k \cdot \beta. \tag{18}$$

This yields

$$x_k = x_0 \cdot e^{-k\beta} = x_0 \, y^k, \tag{19}$$

where $y = e^{-\beta}$ is introduced for convenience. This result demonstrates that in equilibrium the degrees of polymerisation are exponentially distributed. The specific values for x_0 and y are determined from the constraints, where it is convenient to exploit the analogy to the formalism in statistical physics and introduce the partition function $Z = \sum y^k$, such that

$$x_0 = \frac{1}{Z} \quad \text{and} \quad b = \frac{y}{Z} \frac{\partial Z}{\partial y}. \tag{20}$$

These expressions fully characterise the equilibrium distribution. The entropy in equilibrium, S_{eq}, is given by

$$S_{eq}/R = -\sum_k x_0 y^k \ln(x_0 y^k) = -\ln x_0 - b \ln y = \ln Z - \ln y \cdot \frac{\partial \ln Z}{\partial \ln y}. \tag{21}$$

These expressions are valid regardless of the precise range over which the sums in Eqs. (13), (15) and (16) have to be extended. Because DPE1 recognises the non-reducing end of a glucan, there is no *a priori* reason to assume any limit for the DP. If the sums are extended over all numbers $k \in \{0, 1, 2, \ldots\}$, the resulting expressions have a particularly simple form. The partition sum reads

$$Z = \sum_{k=0}^{\infty} y^k = \frac{1}{1-y}. \tag{22}$$

It follows that

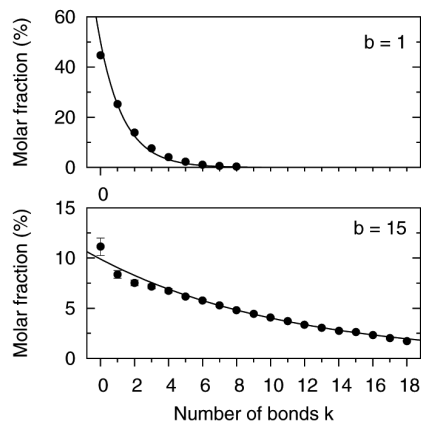$$b = \frac{y}{1-y} \Leftrightarrow y = \frac{b}{b+1} \tag{23}$$

and

$$x_0 = \frac{1}{b+1}. \tag{24}$$

Thus, the equilibrium distribution is given by

$$x_k = (1 - y) y^k = (1 - e^{-\beta}) e^{-\beta k}. \tag{25}$$

We have experimentally tested our predictions for various initial degrees of polymerisation, defining the parameter b.

Figure 2. Predicted and measured equilibrium distributions for two different initial conditions. In the upper panel, the reaction was initiated with maltose, corresponding to $b = 1$. In the lower panel, the initial distribution had an average degree of polymerisation of 16, corresponding to $b = 15$. Solid lines indicate the predicted equilibrium distribution, symbols represent experimentally determined values. Error bars indicate the standard deviation determined from three independent measurements.

In Figure 2, predicted and observed equilibrium distributions are shown for two initial conditions. We have tested the case for extremely short DPs by incubating the reaction with maltose ($b = 1$, upper panel) and for long DPs by incubating the reaction with a mixture of long glucans ($b = 15$, lower panel).
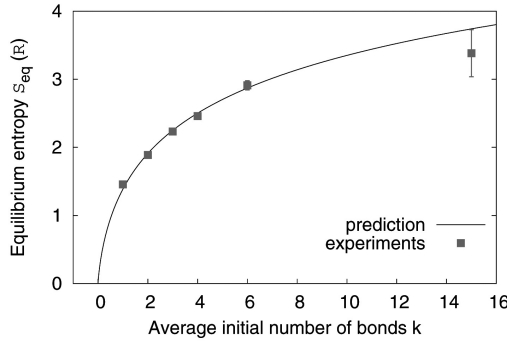
Clearly, $0 \leq y \leq 1$ holds for all positive values of b, implying that β is always positive. In the limit of very long initial DPs,

$$\lim_{b \to \infty} y = 1. \tag{26}$$

The entropy in equilibrium equals

$$S_{eq}/R = -\sum_{k=0}^{\infty} x_k \ln x_k = (b + 1) \ln(b + 1) - b \ln b. \tag{27}$$

In Figure 3, experimentally determined values for the equilibrium entropy are depicted together with the theoretical predictions according to Eq. (27).

Figure 3. Predicted and measured values for the entropy in equilibrium for different initial conditions. The solid line represents the theoretical prediction of the maximal entropy and the squares represent experimentally determined equilibrium entropies. Error bars are standard deviations of three independent experiments.

Various experimental studies suggested that DPE1 cannot utilise maltose as glucosyl donor and cannot form maltose as product [6, 7, 8]. We could show [3] that this is only approximately true, but that in fact maltose serves as a poor substrate with an approximately 800-fold reduced binding affinity when compared to glucans of other DPs. It is, however, possible to analytically find the equilibrium under the assumption that maltose strictly does not participate in the DPE1-mediated reactions. In this case, maltose (x_1) has to be excluded from the sums and the partition sum is

$$Z = \sum_{\substack{k=0 \\ k \neq 1}}^{\infty} y^k = \frac{1-y+y^2}{1-y} = \frac{1}{x_0} \tag{28}$$

and relations (20) allow to find the implicit equation determining y from b,

$$b = \frac{y}{1-y} \cdot \frac{2y-y^2}{1-y+y^2} = \frac{y}{1-y} + \frac{2y^2-y}{1-y+y^2}. \tag{29}$$

The corresponding value of the equilibrium parameter β describes the quasi-equilibrium which is observed in experiments when the incubation time is long enough for the equilibriation of all glucans except maltose but still too short for the detection of maltose. The effect of excluding maltose from the sums is depicted in Figure 4.
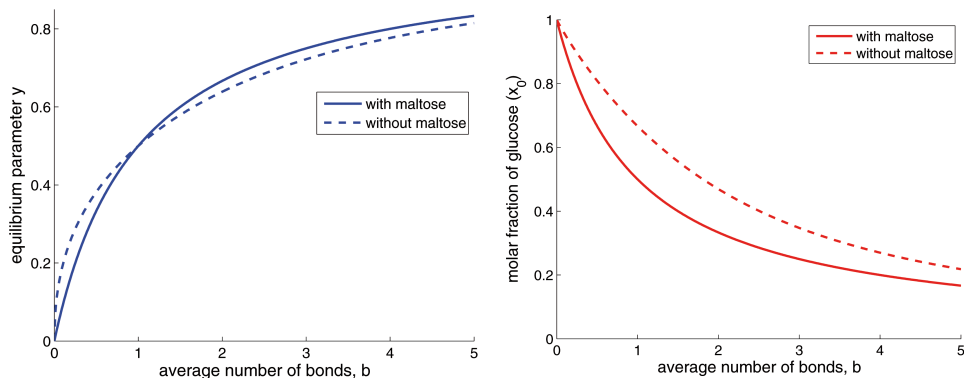
Figure 4. The equilibrium paramter y and the equilibrium concentration of glucose (x_0) in dependence on the initial conditions reflected by average number of bonds (b) for the case in which all DPs are included (solid lines) and the case in which maltose (x_1) is excluded from the sums (dashed lines).

Disproportionating enzyme 2 (DPE2)

DPE2 catalyses the transfer of a single glucose residue from one α-1,4-linked glucan to another. It therefore also belongs to the class of energetically neutral enzymes and obeys the constraints of conserved number of molecules (13) and conserved total number of bonds (15). However, as our and other [9] experimental findings suggest, maltose never acts as an acceptor of glucosyl residues and maltotriose never acts as a donor. DPE2 therefore catalyses reactions according to the formula

$$G_n + G_m \rightleftharpoons G_{n-1} + G_{m+1}, \quad \text{where } n \neq 3 \text{ and } m \neq 2. \tag{30}$$
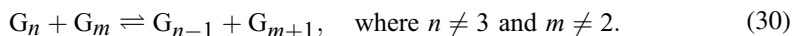
Like for DPE1, we again associate a glucan with DP n with an energy state E_{n-1} and denote the corresponding molar fraction by x_{n-1}. Similar to DPE1, the enzymatic action of DPE2 corresponds to a simultaneous down-shift of one molecule from energy state E_n to E_{n-1} and an up-shift of another molecule from energy state E_m to E_{m+1}.

The additional enzymatic constraint that maltose cannot accept glucosyl residues and maltotriose cannot act as donor implies an additional constraint, namely the conservation of the sum of glucose and maltose molecules,

$$x_0 + x_1 = p, \tag{31}$$

where p is determined by the initially applied amount of glucose and maltose. The DPE2 mediated reaction scheme is shown in Figure 5 where the separation of the glucose-maltose pool from the pool of larger DPs is shown by the red dashed line which is not crossed by any possible reaction path. In each DPE2 reaction step, one arbitrary donor reaction (dashed

arrows) occurs simultaneously with one arbitrary acceptor reaction (solid arrows). Starting from an initial substrate mixture of maltohexaose (E_5) and maltose (E_1), the 5 first possible reactions are shown in Figure 5, where in each step the reaction system follows a dashed and a solid line simultaneously.
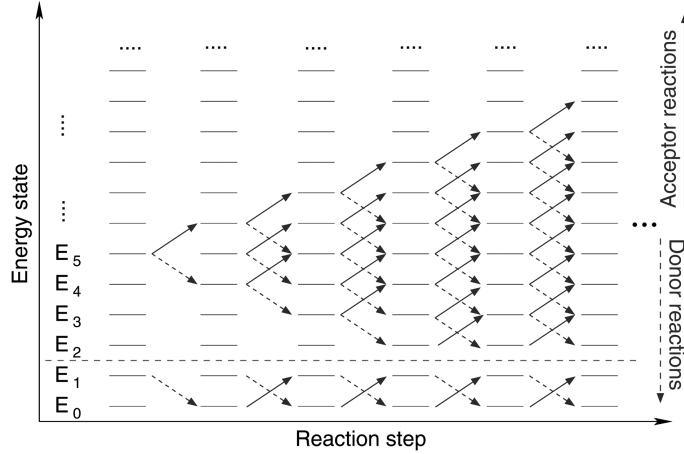
Figure 5. Scheme of the DPE2 mediated reaction system. Each DPE2 reaction step consists of one donor and one acceptor reaction depicted by a dashed and a solid arrow, respectively. Due to the restriction that maltose is never an acceptor and maltotriose is never a donor, the maltose and glucose pool is separated from the other DPs as shown by the red dashed line. The scheme exhibits all possible reaction pathways starting from the two indicated initial substrates maltohexaose and maltose, where in each step one arbitrary solid and one arbitrary dashed path is taken.

Again, the maximal entropy is determined using the method of Lagrangian multipliers. The Lagrangian

$$L(x_k; \alpha, \beta, \gamma) = -\sum_k x_k \ln x_k - \alpha\left(\sum_k x_k - 1\right) - \beta\left(\sum_k k \cdot x_k - b\right) - \gamma(x_0 + x_1 - p) \quad (32)$$

now contains an additional Lagrange multiplier γ reflecting the new contraint (31). Setting the partial derivatives to zero,

$$0 = \frac{\partial L}{\partial x_0} = -(\ln x_0 + 1) - \alpha - \gamma, \quad (33)$$

$$0 = \frac{\partial L}{\partial x_1} = -(\ln x_1 + 1) - \alpha - \beta - -\gamma, \quad (34)$$

$$0 = \frac{\partial L}{\partial x_k} = -(\ln x_k + 1) - \alpha - k \cdot \beta \text{ for } k \leq 2, \quad (35)$$

and defining $y = e^{-\beta}$ it follows that

$$\frac{x_1}{x_0} = y \quad \text{and} \quad x_k = x_2 \cdot y^{k-2}, \tag{36}$$

showing that the DPs again follow an exponential distribution. The difference to DPE1 is that the ratio $x_{k+1}/x_k = y$ is not observed for the ratio x_2/x_1. Constraints (13) and (31) imply

$$x_0 = \frac{p}{1+y} \quad \text{and} \quad x_2 = (1-p)(1-y). \tag{37}$$

Constraint (15) allows to derive the formula

$$b - 2(1-p) = p \cdot \frac{y}{1+y} + (1-p) \cdot \frac{y}{1-y}, \tag{38}$$

from which y can be determined from the initial conditions b (average number of bonds) and p (initially applied molar fraction of glucose and maltose).

For the special case $p = 0$ (in which no glucose or maltose is initially applied), Eq. (38) assumes the same form as for DPE1, with the exception that the left hand side reads $b - 2$. This is not surprising because under these conditions DPE2 will only mediate chemical transformations of glucans with at least two bonds (maltotriose). The dependency of the equilibrium parameter y on the average number of bonds, b and the initially applied amount of glucose and maltose, p is depicted in Figure 6.
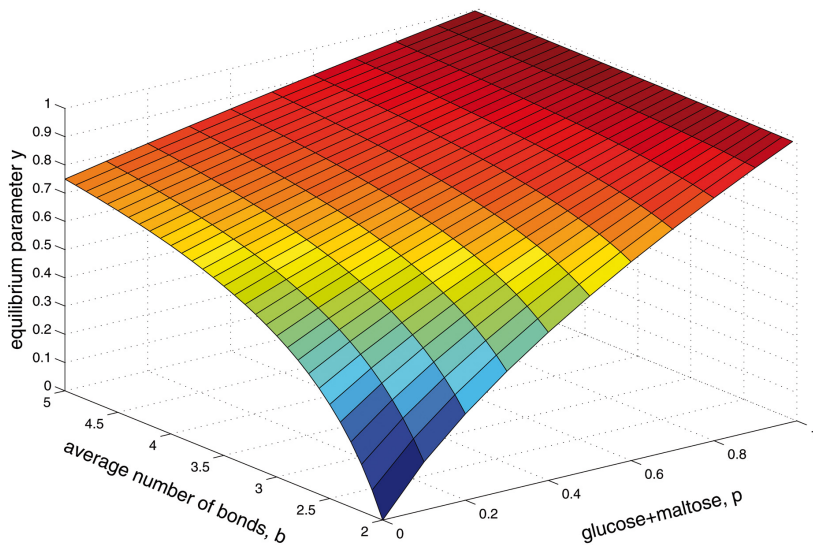
Figure 6. Predicted equilibrium parameter of DPE2 for varying average number of bonds (b) and for different amounts of glucose and maltose (p).

We have tested these predictions experimentally by incubating DPE2 with an initial mixture containing 40% maltose and 60% maltoheptaose [3]. These conditions correspond to the parameters $p = 0.4$ and $b = 4$. The experimentally observed equilibrium distribution and the theoretically predicted distribution for this initial condition is plotted in Figure 7.
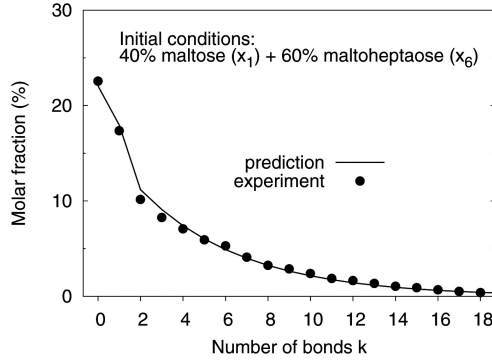
Figure 7. Predicted equilibrium distribution of the degree of polymerisation (solid line) and experimental validation (symbols) for DPE2. The reaction was initiated with a mixture of 40% (molar fraction) of maltose and 60% maltoheptaose, resulting in $p = 0.4$ and $b = 0.4 \times 1 + 0.6 \times 6 = 4$.

Phosphorylase

The enzyme α-glucan phosphorylase catalyses the transfer of a single glucose residue from the non-reducing end of a glucan onto orthophosphate to form glucose-1-phosphate. The general reaction is

$$P_i + G_n \leftrightarrow G1P + G_{n-1},\qquad(39)$$

Apparently, this enzyme also conserves the total number of molecules. However, since the bond enthalpies of the α-1,4 glucosidic linkages in polyglucans and the phosphoester bond in glucose-1-phosphate are different, the total energy of formation is not a conserved quantity. As a consequence, the equilibrium distribution will be determined by a combined effect of minimising the Gibbs energy of reaction and maximising the entropy. We denote by Δ_g the change in Gibbs energy when breaking one mole of α-1,4 glucosidic linkages and simultaneously forming one mole of phosphoester bonds. The molar fractions of orthophosphate (P_i) and of glucose-1-phosphate (G1P) are denoted by u and v, respectively. We assume that phosphorylase can be active on glucans with a minimal number of bonds, denoted m. As above, we denote with x_k the molar fraction of the glucan with k bonds. The total energy of formation of the reaction mixture (per mole) is thus

$$g^f = const. + v \cdot \Delta g\qquad(40)$$

and the mixing entropy reads

$$S_{\text{mix}} = -R\left[u\ln u + v\ln v + \sum_{k\geq m} x_k \ln x_k\right]. \tag{41}$$

The equilibrium distribution is determined by identifying the minimum of the Gibbs free energy Eq. (11) under the constraints

$$\text{total number of molecules}: \quad u + v + \sum_{k\geq m} x_k = 1, \tag{42}$$

$$\text{conservation of bonds}: \quad v + \sum_{k\geq m} kx_k = b, \tag{43}$$

$$\text{conservation of phosphate groups}: \quad u + v = p. \tag{44}$$

These constraints are analogous to the three constraints (13), (15) and (31) which apply to DPE2. Indeed, they formally become identical if u is identified with x_0, v with x_1 and $m = 2$. The main difference is that here the Lagrange function

$$L(u,v,x_k;\alpha,\beta,\gamma) = v\cdot\Delta g + RT\left[u\ln u + v\ln v + \sum_k x_k \ln x_k\right] \tag{45}$$

$$+\alpha\left(u+v+\sum_k x_k - 1\right) + \beta\left(v+\sum_k kx_k - b\right) + \gamma(u+v-p)$$

contains the molar change in Gibbs energy Δ_g. Here, introducing

$$y = e^{-\frac{\beta}{RT}} \quad\text{and}\quad k_0 = e^{-\frac{\Delta g}{RT}} \tag{46}$$

and setting the partial derivatives to zero yields

$$\frac{v}{u} = y\cdot k_0 \quad\text{and}\quad \frac{x_{k+1}}{x_k} = y \quad\text{for}\quad k\geq m. \tag{47}$$

An analogous calculation to that performed for DPE2 yields

$$u = \frac{p}{1+yk_0} \quad\text{and}\quad x_m = (1-p)(1-y) \tag{48}$$

and y is determined by solving the equation

$$b - m\cdot(1-p) = p\frac{yk_0}{1+yk_0} + (1-p)\frac{y}{1-y}. \tag{49}$$

The implicit formula (38) for DPE2 represents a special case of Eq. (49) when $k_0 = 1$, which corresponds to identical bond energies ($\Delta g = 0$). The analogous structure of the solutions is not surprising considering the parallels in the constraints that the respective enzymes observe. In both, the number of molecules as well as the number of bonds is conserved and

both obey an additional, third, constraint. Whereas DPE2 conserves the sum of the glucose and maltose moieties, phosphorylase conserves the sum of the moieties of orthophosphate and glucose-1-phosphate.

The experimental validation of our predictions is illustrated in Figure 8. We have experimentally tested our predictions for two different initial conditions. We have initiated the reactions with a 1:4 mixture of glucans and glucose-1-phosphate, leading to $p = 0.8$. In one case, we chose maltoheptaose as the glucan, resulting in $b = 2.0$ (upper panel), whereas in the other case the glucan was maltotetraose, resulting in $b = 1.4$ (lower panel).
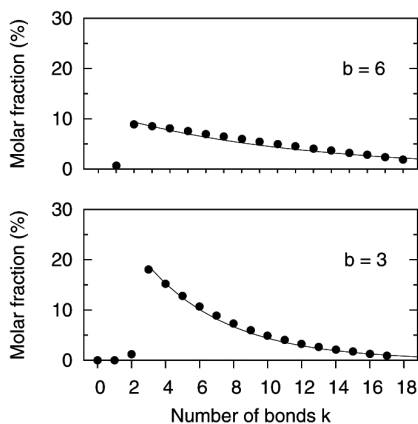
Figure 8. Predicted equilibrium distribution of the degree of polymerisation (solid line) and experimental validation (symbols) for phosphorylase. The reaction was initiated with a mixture of 20% (molar fraction) of glucan and 80% glucose-1-phosphate, resulting in $p = 0.8$. In the upper panel, maltoheptaose was chosen as glucan, leading to $b = 0.2 \times 6 + 0.8 \times 1 = 2.0$, for the lower panel the shorter glucan maltotetraose was used, resulting in $b = 0.2 \times 3 + 0.8 \times 1 = 1.4$.

Other glucosyltransferases

DPE2 and phosphorylase catalyse the reversible transfer of a terminal glucosyl residue onto an acceptor molecule. For DPE2, the acceptor is glucose, for phosphorylase, the acceptor is orthophosphate. Various other enzymes catalyse reactions according to this pattern. Most notably, starch synthases catalyse the elongation of glucans through the activated compound ADP-glucose. The general reaction scheme is

$$ADPglc + G_n \rightleftharpoons ADP + G_{n+1}. \tag{50}$$
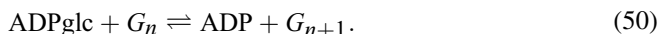
Formally, ADP functions as the acceptor molecule even though here the equilibrium is far on the side of elongated chains and therefore transfer of glucosyl residues onto ADP are thermodynamically unfavourable and can be expected to occur with a slow rate. However,

this reaction is structurally similar to reaction Eq. (39) and consequently the mathematical expression describing the equilibrium is also equivalent to Eq. (49). The only difference is the quantity of Δ_g, which for starch synthase describes the change in Gibbs free energy if one mole ADP-glucoses are split and one mole α-1,4 glucosidic linkages are formed. Similarly, glycogen synthase catalyses the elongation of glucans utilising UDP-glucose as donor molecule and the thermodynamic equilibrium distribution is expected to be highly similar to that of starch synthase.

The enzyme amylosucrase parallels DPE2 in that it utilises fructose (Fru) as an acceptor molecule, forming sucrose (Suc) according to the scheme

$$G_n + \text{Fru} \rightleftharpoons G_{n-1} + \text{Suc}. \tag{51}$$

Again, the reaction is structurally similar to reaction Eq. (39) and the mathematical formalism to determine the equilibrium is identical.

Experimental determination of bond energies

The analytic calculations of equilibrium distributions for different glucosyltransferases have revealed interesting parallels. All reaction systems can be written in the form

$$u + x_k \rightleftharpoons v + x_{k-1} \quad \text{with } k \geq m. \tag{52}$$

In the forward direction (left to right), a glucan (x_k) is shortened by one glucosyl residue, which is transferred to an acceptor molecule u. In the opposite direction, the bound form v acts as a glucosyl donor, transferring the glucosyl residue back to a glucan (x_{k-1}), thereby elongating it. The minimal number of bonds in a glucan in order to act as a substrate is denoted by m. For reaction systems of this type, the sum of u and v is a conserved quantity, $u + v = p$.

For DPE2, u and v are identified with glucose (x_0) and maltose (x_1), respectively, and $m = 2$, reflecting the separation of the glucose/maltose pool from glucans with higher DP. In the case of amylosucrase, u is to be identified with fructose and v with sucrose. For phosphorylase, u and v are identified with orthophosphate (P_i) and glucose-1-phosphate (G1P), respectively. For starch synthase u stands for ADP and v for ADP-glucose. While the mechanisms of all three enzymes are quite different, their catalytic activity can formally be written in the same way (see Eq. 52) and the only difference is in their Gibbs free energy of reaction.

It is therefore worthwhile studying Eq. (49) to investigate the effect of Δ_g on the equilibrium distribution to develop a method by which Δ_g can be determined from experimentally observed equilibria. First, it is important to note that the left-hand side of Eq. (49) denotes

the average number of *active* bonds in the reactant mixture, because b is the total average number of bonds and $m(1 - p)$ is the average number of *inactive* bonds ($1 - p$ is the molar fraction of all glucans with m or more bonds, and in every such glucan these m bonds are never subject to enzymatic hydrolysis). We therefore introduce the quantity b' reflecting the number of active bonds by letting

$$b' = b - m \cdot (1 - p). \tag{53}$$

We now consider the extreme case of an irreversible reaction (52) running in the direction right to left. The equilibrium is completely on the left side of Eq. (52), thus no v is present ($v = 0$ and $u = p$). Under these conditions, $\Delta g \to \infty$, implying $k_0 \to 0$ and Eq. (49) becomes

$$\frac{b'}{1-p} = \frac{y}{1-y}. \tag{54}$$

This expression is exactly analogous to Eq. (23), determining the equilibrium parameter y for DPE1 for an average number of bonds $b'/(1 - p)$, which corresponds to the average number of active bonds in the glucans.

The other extreme situation of irreversibility in the direction left to right in Eq. (52) is described by $\Delta g \to -\infty$, implying $k_0 \to \infty$. Under these circumstances, glucan chains will be maximally shortened and the glucosyl residues will be transferred to the donor u to form v. Here, we need to distinguish two cases: If there exist more active bonds in the system than acceptor molecules, the equilibrium will be characterized by an absence of acceptor molecules ($u = 0$ and $v = p$). If, however, there are fewer transferrable glucosyl residues than acceptor molecules, some unbound acceptor molecules remain in the system ($u > 0$). The first scenario is observed when $b' > p$, because then there exist more active bonds than acceptor molecules. In this case, the equation (49) determining the equilibrium parameter y becomes

$$\frac{b'-p}{1-p} = \frac{y}{1-y}. \tag{55}$$

Again, this expression is exactly analogous to Eq. (23) for DPE1, now with an average number of bonds $(b' - p)/(1 - p)$. The difference to Eq. (54) results from the fact that here p bonds are present in the molecules v.

The second scenario represents a situation with an insufficient number of bonds ($b' < p$). Eq. (55) cannot be used to determine the equilibrium distribution in this case because no positive solution for y exists. The only non-negative solution of Eq. (49) for $k_0 \to \infty$ is given by $y = 0$, where

$$\lim_{k_0 \to \infty} y k_0 = \kappa \tag{56}$$

remains finite. Then,

$$b' = p \cdot \frac{\kappa}{1+\kappa} = v, \tag{57}$$

and the equilibrium distribution is monodisperse with

$$x_m = 1 - p \text{ and } x_k = 0 \text{ for } k \geq m. \tag{58}$$

These theoretical deliberations allow the following conclusion: In a situation with a small average number of active bonds and a large excess of glucosyl acceptors u, the equilibrium parameter y will strongly depend on Δ_g, representing the change in Gibbs energy when converting α-1,4 glucosidic linkages in bonds linking glucosyl residues to form v. Let us, for example, assume a 1:50 mixture of glucans and glucosyl acceptors, where the glucans contain on average three active bonds. This means, that the total average number of bonds in a glucan is $m + 3$, where m is the number of inactive bonds. Then, $p = 50/51$ and $b' = 3/51 < p$. Together with Eq. (54) the limiting cases read

$$\lim_{\Delta g \to \infty} y = \frac{3}{4} \quad \text{and} \quad \lim_{\Delta g \to -\infty} y = 0. \tag{59}$$

These extreme values allow to experimentally determine Δ_g from the equilibrium distribution of the glucans. The equilibrium parameter can be directly read out from the equilibrium distribution since in a semi-logarithmic plot of the molar fractions of the glucans the slope amounts to $-\ln(y)$.
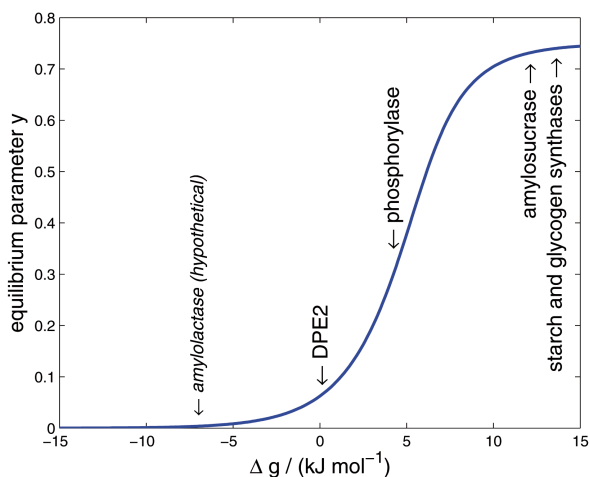
Figure 9. The equilibrium parameter y in dependence on the difference in Gibbs free energies. The chosen parameters are $b' = 3/51$ and $p = 50/51$. The values for the glucosyltransferases DPE2 ($\Delta g = 0$), phosphorylase ($\Delta g = 4.1$ kJ/mol), amylosucrase ($\Delta g = 12$ kJ/mol) and starch/glycogen synthase ($\Delta g = 13.5$ kJ/mol) are indicated. Further, a hypothetical enzyme, *amylolactase* with a negative value ($\Delta g = -7.1$ kJ/mol) is included for illustration.

Figure 9 depicts the dependence of the equilibrium parameter y on the change in Gibbs free energy Δ_g for these parameter values. The predicted equilibrium distributions for the glycosyltransferases discussed in this paper are denoted in the plot. For DPE2, $\Delta g = 0$. For phosphorylase, we previously predicted with this method a value of $k_0 = 0.19$ [3], corresponding to $\Delta g = +4.1$ kJ/mol. For amylosucrase, we estimated the equilibrium constant using the eQuilibrator web interface [10] as $\Delta g = +12$ kJ/mol. For starch synthase, this method estimated a value of $\Delta g = +13.5$ kJ/mol.

Additionally, a hypothetical enzyme with negative Δ_g is indicated. In analogy to amylosucrase, which transfers the terminal glucosyl residue from a glucan to fructose forming sucrose, a hypothetical amylolactase would catalyse the reaction

$$G_n + \text{galactose} \; G_{n-1} + \text{lactose} \tag{60}$$
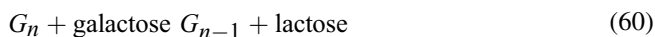
with an estimated value $\Delta g = -7.1$ kJ/mol.

Conclusions

We have presented a concise thermodynamic characterization of enzymatic reactions on carbohydrate polymers. The description of the action of these carbohydrate-active enzymes by concepts from statistical thermodynamics allows to precisely predict and characterise the corresponding equilibrium distributions. We introduced the equilibrium parameter β, or equivalently $y = \exp(-\beta/RT)$, which fully charaterizes the distributions of degrees of polymerisation in equilibrium and thus represents a suitable generalisation of the classical equilibrium constant K_{eq} for carbohydrate-active enzymes.

The theory further provides a method how differences in bond enthalpies can be experimentally determined through the observation of equilibrium distributions. For this, an excess of acceptor molecules and rather short chain lengths should be chosen as initial conditions, which leads to a strong dependence of the equilibrium parameter on the bond enthalpy difference Δ_g.

Due to the generality of our theory, our new concepts are not limited to the specific enzymes discussed in the text or even to carbohydrate-active enzymes. We expect that our thermodynamic approach is widely applicable to many polymer-active enzymes in general.

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