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Thermodynamic Favorability of End Products of Anaerobic Glucose Metabolism

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ABSTRACT: The eQuilibrator component contribution method allows calculation of the overall Gibbs energy changes for conversion of glucose to a wide range of final products in the absence of other oxidants. Values are presented for all possible combinations of products with up to three carbons and selected others. The most negative Gibbs energy change is for the formation of graphite and water (−499 kJ mol^{-1}) followed by CH_4 and CO_2 (−430 kJ mol^{-1}), the observed final products of anaerobic digestion. Other favored products (with various combinations having Gibbs energy changes between −300 and −367 kJ mol^{-1}) are short-chain alkanes, fatty acids, dicarboxylic acids, and even hexane and benzene. The most familiar products, lactate and ethanol + CO_2, are less favored (Gibbs energy changes of −206 and −265 kJ mol^{-1} respectively). The values presented offer an interesting perspective on observed metabolism and its evolutionary origins as well as on cells engineered for biotechnological purposes.

	$\Delta G_r'^m$/ kJ mol^{-1}
Glucose → 6 C + 6 H_2O	-499
Glucose → 3 CH_4 + 3 CO_2	-430
Glucose → 0.63 C_6H_{14} + 2.21 CO_2 + 1.58 H_2O	-342
Glucose → 2 lactate	-206

INTRODUCTION

There is a long history of using thermodynamic analysis to aid our understanding of metabolism. Biochemistry textbooks give standard Gibbs energy changes for reactions in core metabolic pathways based on measurements of equilibrium constants. The NIST Thermodynamics of Enzyme-Catalyzed Reactions database[1] (https://randr.nist.gov/enzyme/ DOI: 10.18434/T40W22) collects measured equilibrium constants for biochemical reactions. From these, it is possible to calculate Gibbs energies of formation for metabolites,[2] which can in turn be used to calculate Gibbs energy changes for other reactions for which there are no measurements. Methods have also been developed to give fairly accurate predictions of Gibbs energies of formation of a wide range of compounds of biochemical relevance with their chemical structure as the only input required.[3−9] These methods have been exploited for thermodynamic analysis of a variety of actual and hypothetical metabolic pathways and networks.[10−15] For example, it can be shown that the classic EMP glycolysis pathway is optimal for the conversion of glucose to lactate.[16]

Gibbs energies of formation can also be used to analyze the overall thermodynamics of transformation of nutrients into end products. The overall Gibbs energy change for this process can be calculated, independent of whatever might be the exact metabolic pathway involved. This approach has been used to assess the feasibility of formation of various end products by rumen microbes.[17] It has also been applied to the possible formation of various industrial chemicals by engineered organisms.[18] It is interesting to use these prediction methods to consider the choice of overall end products of sugar fermentation under anaerobic conditions (and in the absence of other oxidizing species such as nitrate or sulfate). This paper presents the overall Gibbs energy changes for conversion of glucose into all possible combinations of compounds having up to three carbon atoms and some selected larger molecules. These values have been calculated because they should be useful in analyzing the extent to which the overall Gibbs energy change has been a driver in the evolution of anaerobic metabolism. They also provide an interesting perspective on products of metabolism observed in current biochemistry. The values also highlight some options for metabolic engineering and synthetic biology, showing that some practically interesting end products are thermodynamically favored.

RESULTS

Gibbs Energies of Formation. The aim is to tabulate the overall Gibbs energy changes for the metabolism of glucose to a wide range of possible end products in the absence of additional oxidants. These changes can be calculated from estimates of the Gibbs energy of formation for glucose, water, and each possible end product. These Gibbs energies of formation were obtained using the online eQuilibrator service,[3,4] which has been shown to give generally good estimates.[7] There is a good argument in that basing the Gibbs energies of formation and reaction on a standard state of 1 mM in solution is more biochemically relevant than the 1 M choice usual in chemistry.[3,4] This has been adopted in the present work, so the values presented are for

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$\Delta G_f'^m$ (formation) and $\Delta G_r'^m$ (reaction) using the symbols of Noor et al.[3,4] for pH 7 except where otherwise stated.

Compounds Considered. The analysis attempts to include all possible C_1, C_2, and C_3 compounds containing also hydrogen and/or oxygen. Some classes were not considered in detail because they were expected to be always higher in energy: ethers, esters, cyclopropanes, oxetane, cumulenes, enols, ynols, and gem-diols (see Methods). The number of compounds that have to be considered in the final analysis can be reduced further by making some pre-selections.

Isomeric Compounds. First, some compounds are isomers of each other. By comparing the formation Gibbs energies of each isomer, the lowest energy can be selected; this is the one that has the best chance of being a favored end product. Table 1 shows the selections that can be made in this way. Some cases of isomers where predicted $\Delta G_f'^m$ values are not available from eQuilibrator are discussed in Methods. It is interesting to note that, even though lactate is a classic product of anaerobic glycolysis, its formation is slightly less favored than 3-hydroxypropanoate (whose production in engineered organisms has been studied as a possible biotechnological process[19−21]).

Table 1. Selection of Favored Isomers

empirical formula	favored isomer (with $\Delta G_f'^m$ in kJ mol^{-1})	higher Gibbs energy isomers (with $\Delta G_f'^m$ in kJ mol^{-1})
C_2H_4O	ethanal (5)	ethylene oxide (29)
$C_2H_4O_2$	acetate (−264)	glycolaldehyde ($CH_2OH.CHO$) (−148)
C_3H_8O	2-propanol (123)	1-propanol (131)
$C_3H_8O_2$	propylene glycol (−25)	1,3-propanediol (−23)
C_3H_6O	acetone (66)	propanal (93), epoxypropane (111), allyl alcohol (135)
$C_3H_6O_2$	propanoate (−178)	hydroxyacetone (−68), 2-hydroxypropanal (−66), 3-hydroxypropanal (−64), glycidol (−46)
$C_3H_6O_3$	3-hydroxypropanoate (−333)	lactate (−326), dihydroxyacetone (−226), glyceraldehyde (−223)
$C_3H_4O_2$	acrylate (−165)	propiolactone (−124), pyruvaldehyde (−112), malondialdehyde (−106), glycidaldehyde (−87)
$C_3H_4O_3$	malonate semialdehyde (−374)	pyruvate (−363)[a]
$C_3H_4O_4$	malonate (−624)	hydroxypyruvate (−514), 2-hydroxy-3-oxopropanoate (−513)

[a]There are several other isomers here for which eQuilibrator offers no predictions. Hydroxypyruvaldehyde ($CH_2OH{\cdot}CO{\cdot}CHO$) and hydroxymalondialdehyde ($OHC{\cdot}CHOH{\cdot}CHO$) are expected to be higher in energy than pyruvate from comparisons of isomers having a carboxyl group as opposed to separate hydroxyls and carbonyls. (Compare acetate and glycoladehyde, propanoate with hydroxypropanals, and malonate with hydroxypyruvate. This general pattern was noted by Weber[22]). Epoxypropanoate is expected to be of higher Gibbs energy than that of pyruvate based on comparisons of other epoxy versus carbonyl compounds: epoxypropane versus acetone and glycidol versus hydroxyacetone. Finally, the 4-membered ring in the lactone of glyceric acid can be thought of being opened by cleavage of the CH_2−O bond giving the enol of pyruvate. The equivalent cleavage in propiolactone gives acrylate with a Gibbs energy of 41 kJ mol^{-1} lower.

Compounds Inter-related by Hydration. Other compounds (or groups of isomers) have carbon in the same formal oxidation state and are interrelated by the addition or removal of elements of water. There may not be a simple hydration reaction, but in calculating the overall thermodynamics, just one compound in that oxidation state will be the lowest-Gibbs energy form. This is because we assume that the overall conversion is taking place in aqueous solution and water may be a net reactant or product in the process. Table 2 shows how this can be used to reduce the number of products to be considered in identifying the most thermodynamically favorable options. The systematically favorable dehydration of diols to give carbonyl compounds (see ethanal and acetone) was previously noted by Weber.[22]

Possible Overall Transformations. Along with the favored products listed in Tables 1 and 2, a number of other compounds were included that have no plausible isomers or (de)hydration forms. This led to the following complete list: CO_2, formate, elemental carbon, methanol, methane, oxalate ($HOOC{\cdot}COOH$), glyoxylate ($OHC{\cdot}COOH$), hydroxyacetate (also named glycolate), acetate, ethanal, ethanol, ethane, oxomalonate ($HOOC{\cdot}CO{\cdot}COOH$), hydroxymalonate ($HOOC{\cdot}CHOH{\cdot}COOH$), malonate, malonate semialdehyde ($OHC{\cdot}CH_2{\cdot}COOH$), 3-hydroxypropanoate, propanoate, acetone, 2-propanol, and propane. To this list were added molecular hydrogen H_2 and a selection of compounds with more than three carbon atoms. Butane, butanoic acid, and succinic acid were found to be local minimum energy forms in a study of redox potentials of all possible oxidation states of a four-carbon chain.[23] Glutarate ($C_5H_8O_4$) is an interesting case of a dicarboxylic acid that has the same formal oxidation state as that of glucose. Benzene was added to explore the effect of aromatic stabilization, and hexane gives an aliphatic six-carbon comparison. Heterocyclic aromatic furan (C_4H_4O) would also be an interesting possibility, but eQuilibrator does not offer a $\Delta G_f'^m$ prediction. Finally, citrate was added as an example tricarboxylic acid.

Some possible products have carbon in the same formal oxidation state (0) as in glucose. Hence, they can be formed as a single carbon-containing product from glucose, perhaps with water as an additional reactant or product (Table 3).

Other possible transformations make two products, one more oxidized and one more reduced than glucose. The overall Gibbs energy change was calculated for every possible combination of such products from the list above. This used the unique overall stoichiometric equation that produces the two products, which may also involve water as an additional reactant or product.

It is of course possible for metabolism to produce more than two end products. However, the overall equation for such transformations can be written as the linear sum of two or more conversions that each makes just one or two carbon-containing products. Hence, the overall Gibbs energy change will be intermediate between those for the individual summed conversions. Therefore, there is no need to include these as separate possibilities to be tabulated.

Certain possible products have been removed from Table 4 presented here to make it easier to follow (a table with all possible oxidized and reduced products is available in the Supporting Information). Production of formate is somewhat worse than CO_2 formation in combination with all reduced products except H_2 where the overall $\Delta G_r'^m$ is still only −67 kJ mol^{-1}. Production of glyoxylate always gives a less negative (worse) $\Delta G_r'^m$ than that of hydroxyacetate or oxalate in combination with the same reduced product. Similarly, production of malonate semialdehyde, hydroxymalonate, or oxomalonate is always worse than malonate in combination with the same reduced product and production of citrate is always

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Table 2. Selection of Compounds Related by (De)hydration[a]

favored compound (with $\Delta G_f'^m$ in kJ mol^{-1})	higher Gibbs energy combinations (with $\Delta G_f'^m$ in kJ mol^{-1})
C (s), graphite (0)	CH_2O (formaldehyde) − H_2O (98)
CH_2O_2, formate (−325)	CO + H_2O (−295)
C_2H_6O, ethanol (47)	C_2H_4 (ethene) + H_2O (69)
C_2H_4O, ethanal (5)	$C_2H_6O_2$ (ethylene glycol) − H_2O (47)
$C_2H_4O_3$, hydroxyacetate (−429)	$C_2H_2O_2$ (glyoxal) + H_2O (−351)
C_3H_8O, 2-propanol (123)	C_3H_6 (propene) + H_2O (134)
C_3H_6O, acetone (66)	$C_3H_8O_2$ (propylene glycol) − H_2O (133)
$C_3H_6O_2$, propanoate (−178)	C_3H_4O (acrolein) + H_2O (−64); $C_3H_8O_3$ (glycerol) − H_2O (129)
$C_3H_6O_3$, 3-hydroxypropanoate (−333)	$C_3H_4O_2$ (acrylate) + H_2O (−323)
$C_3H_4O_3$, malonate semialdehyde (−374)	$C_3H_6O_4$ (glycerate) − H_2O (−325)

[a]Some other possible (de)hydration reactions where eQuilibrator does not offer values of $\Delta G_f'^m$ are discussed in the Methods.

Table 3. Single Products that Can be Produced Non-oxidatively from Glucose[a]

overall process	overall $\Delta G_r'^m$
$C_6H_{12}O_6 \rightarrow 6C\ (s) + 6H_2O$	−499
$C_6H_{12}O_6 \rightarrow 3$ acetate	−345
$C_6H_{12}O_6 \rightarrow 2$ 3-hydroxypropanoate	−220
$C_6H_{12}O_6 \rightarrow 1.2$ glutarate $+1.2H_2O$	−279

[a]Note that three of these have been pre-selected as favored products compared with possible isomers (Table 1) or hydration/dehydration linked compounds (Table 2). Examples are C(s) over CH_2O, acetate over glycolaldehyde, and 3-hydroxypropanoate over lactate, dihydroxyacetone, and acrylate.

Table 4. Overall Gibbs Energy Changes for Metabolism of Glucose to One More Reduced and One More Oxidized Product[a]

Values are $\Delta G_r'^m$ in kJ mol^{-1}.		Oxidized product				
		CO_2	hydroxyacetate	oxalate	malonate	succinate
Reduced product	H_2	-47	-122	47	-159	-235
	CH_4	-430	-260	-322	-323	-299
	methanol	-165	-156	-116	-185	-232
	ethane	-367	-243	-280	-297	-288
	ethanol	-265	-206	-206	-245	-262
	propane	-350	-239	-270	-290	-285
	2-propanol	-301	-224	-238	-267	-273
	acetone	-295	-232	-247	-269	-274
	propanoate	-339	-281	-308	-311	-300
	butanoate	-330	-261	-289	-297	-290
	butane	-343	-238	-267	-288	-284
	benzene	-329	-260	-288	-296	-289
	hexane	-342	-240	-269	-288	-284

[a]Progressively darker shading highlights values in the ranges of −250 to −300 kJ mol^{-1}, −300 to −350 kJ mol^{-1}, −350 to −400 kJ mol^{-1}, and values more negative than −400 kJ mol^{-1}.

worse than succinate in combination with the same reduced product. Finally, the formation of ethanal is always less favorable than producing ethanol in combination with the same oxidized product.

Effect of pH. The overall Gibbs energy changes presented so far are for pH 7, but most will depend significantly on the pH. Most of the overall transformations produce one or more acidic products, so the $\Delta G_r'^m$ values will become more favorable (negative) as the pH rises. The size of the effect depends on the number of H^+ released in the overall reaction equation. The biggest dependencies would be for the formation of six formate or three oxalate compounds in combination with H_2, although neither of these are very favored conversions at pH 7. At the other extremes, there are product combinations like 0.5 benzene and 1.5 glyoxal that have no acidic products at all, although again, with a $\Delta G_r'^m$ of only −143 kJ mol^{-1}, this is not particularly favored. For some intermediate cases, differences in pH dependency can change the ordering of possible product combinations. Figure 1 shows examples of the formation of

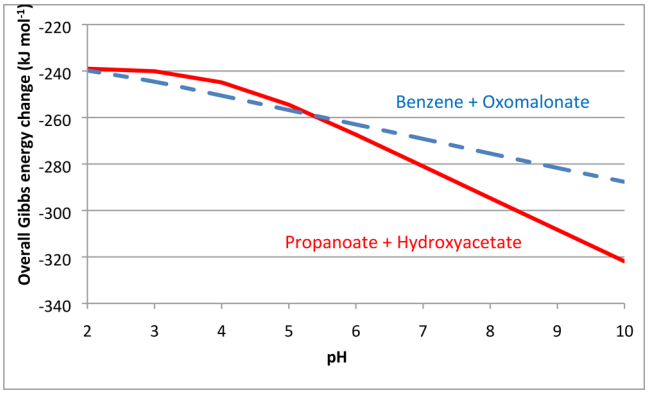

Figure 1. Examples of the pH effect on overall Gibbs energy change. These are overall $\Delta G_r'^m$ values calculated by eQuilibrator for the conversions $C_6H_{12}O_6$ = 8/11 C_6H_6 + 6/11 $C_3H_2O_5$ (oxomalonate) + 36/11 H_2O and $C_6H_{12}O_6$ = 1.2 $C_3H_6O_2$ (propanoate) + 1.2 $C_2H_4O_3$ (hydroxyacetate).

8/11 benzene and 6/11 oxomalonate, hence just over 1 H^+ per glucose, compared with 1.2 propanoate and 1.2 hydroxyacetate, so approximately 2.4 H^+ per glucose. The latter has a more negative $\Delta G_r'^m$ at pH 7, but in acid, the former becomes more favored because of its lower pH dependence.

Effect of Product Concentrations and Ease of Product Removal. The calculations so far have been made on the basis that all products accumulate to a concentration of 1 mM. If we consider instead the conversion of 1 M glucose to 1 M products, the Gibbs energy change ($\Delta G_r'^0$) will be less favorable where more than one molecule of products (other than H_2O) are formed from one molecule of glucose. Therefore, for the conversion glucose → 0.5 benzene + 0.5 citrate +2.5H_2O, $\Delta G_r'^m$ and $\Delta G_r'^0$ are exactly equal at −267 kJ mol^{-1}. However, for glucose →3CH_4 + 3CO_2, $\Delta G_r'^m$ is −430 kJ mol^{-1}, but $\Delta G_r'^0$ is only −345 kJ mol^{-1}.

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However, in discussing the effect of product concentrations that accumulate, it is important to consider means by which they can be removed from the environment of cells producing them. The hydrocarbons methane, ethane, propane, and butane may be removed in a gas phase. Henry's law constants[24] for 25 °C show that 1 mM aqueous dissolved concentrations correspond to gas partial pressures between 0.5 and 1 bar. Hence, they should be readily removed by evaporation into the atmosphere with dissolved concentrations remaining low. For H_2, the Henry's law constant is even lower, so a 1 mM dissolved concentration is already supersaturated at atmospheric pressure (0.78 mM is saturated at 1 bar and 25 °C). CO_2 is more soluble with 34 mM saturation at 1 bar and 25 °C. Hence, it would need to accumulate to 34 mM before the formation of bubbles of pure CO_2 would become favored at atmospheric pressure. If the environment of a cell could easily equilibrate with the atmosphere, the low CO_2 partial pressure of 0.0004 bar would make removal favorable for any free-CO_2 concentration above 0.014 mM. For organisms living at much higher pressures, for example, in the deep sea, removal to a gas phase will of course become more difficult or impossible, and these compounds will accumulate like any other solute.

In the case of hexane and benzene (and other very hydrophobic products), removal is also possible by separation of a second liquid phase. The solubility of hexane at 25 °C is about 0.12 mM,[25] so it is already supersaturated at 1 mM. For benzene, the solubility is considerably higher at 22.7 mM, but separation as a liquid is still possible if it tends to accumulate above this concentration. Under high-pressure conditions, shorter chain alkanes like butane could also separate as a liquid phase, although the database offers no solubility value for this.

DISCUSSION

It must be acknowledged at the outset that the overall Gibbs energy change is only one factor that may influence the selection of metabolic end products, both in natural biochemistry and engineered organisms. The availability of pathways using possible enzyme reactions and the thermodynamics of individual steps are clearly important.[10−16] Pathways must also be compatible with suitable metabolite concentrations and cofactor use[10−13,16] and avoid excessive enzyme protein requirements.[15,16,26] However, comparison of the overall most thermodynamically favored products with those observed naturally can show where other factors have been dominant. Where thermodynamically strongly favored products are observed naturally, it can be seen as an experimental validation of the idea that the overall thermodynamics has had important influence.

Among all the possibilities in Tables 3 and 4, the thermodynamically most favorable (most negative $\Delta G_r'^m$) is the formation of elemental C (graphite) and H_2O. To the best of my knowledge, no organism producing carbon as an end product of metabolism has been described. Possibly handling a product that would tend to precipitate as soon as it formed is too much of a challenge (if it did not precipitate, then the overall Gibbs energy change to a metastable state would be lower). The next most favorable option is conversion to CH_4 and CO_2. These are, of course, the main final products of anaerobic biological decomposition of organic matter, although I am unaware of any single organism that carries out the entire transformation from glucose. Methane formation is restricted to special organisms that can carry out the necessary biochemistry. One of the limitations in biochemical CH_4 production is considered to be the difficulties of handling reduced C_1 species. Tables 3 and 4 highlight the existence of C_2 and C_3 alternatives that are not a lot worse than making CH_4. Acetate, propanoate, and butanoate are there, and the formation of such acids is a standard intermediate stage in anaerobic decomposition of organic matter.[27]

The production of alkanes, ethane, propane, butane, and even hexane are rather favorable, especially with CO_2 as the oxidized product. The possibility of removal into a gas or separate liquid phase would also be attractive in preventing build-up of product concentrations. Alkanes with two to six carbons are not considered usual products of anaerobic metabolism, but there is evidence that they can be formed in some organisms and ecosystems.[17,28−33] Because of interest as fuel products, cells have been engineered to produce alkanes, especially propane.[33−35] In principle, it could be attractive for organisms to decarboxylate short-chain fatty acids to alkanes, even if only to avoid acid accumulation. However, it seems the enzymes required have not been described, and perhaps it is difficult to engineer an appropriate active site mechanism. Benzene also appears as a reasonably attractive reduced product, but there is no clear demonstration of its formation in anaerobic metabolism. Benzene or other aromatic hydrocarbons could be further interesting targets for metabolic engineering or synthetic biology.

Among alternative oxidized products, malonate is often almost as favorable as CO_2 and, in the case of methanol as the reduced product, slightly preferred (Table 4). In the biochemistry of most present organisms, malonate formation would be strongly discouraged because of its action as an inhibitor of succinate dehydrogenase, but for an archaic organism deprived of alternative oxidants, it might have been a useful option. I am not aware of any natural organism producing significant amounts of malonate, but a strain has been engineered for possible commercial manufacture.[36] Production of succinate is usually quite similar to malonate, rather more favorable in combination with some reduced products, rather less so with others. Succinate is a known end product of anaerobic metabolism in several organisms, and there has been extensive effort to engineer cells for use in biotechnological production.[37−40] Oxalate is always less favored than malonate, but with some reduced products, they are not very different (Table 4). Production of oxalate is known in many organisms,[41] and recent studies have engineered cells to produce higher amounts.[42,43] Production of hydroxyacetate (glycolate) as an oxidized product is usually less favorable than the other options in Table 4 but, sometimes, not by too much. Although it is sometimes found as a metabolite, it has not been described as a major end product from natural organisms, although strains have been engineered to produce it.[44]

It is notable that most of the options discussed so far are however more favorable than the formation of lactate ($\Delta G_r'^m$ = −206 kJ mol^{-1}) or ethanol + CO_2 ($\Delta G_r'^m$ = −265 kJ mol^{-1}), considered to be the canonical products of anaerobic metabolism. It may be that their prominence reflects in part a focus on facultatively anaerobic cells because of their ready formation via the glycolytic pathway that integrates with oxidative metabolism. As noted (Table 1), the formation of 3-hydroxypropanoate is actually slightly more thermodynamically favorable than lactate. Some natural microorganisms produce 3-hydroxypropanoate, and substantial work has been done to improve production for use as an industrial process.[19−21] As a single product from glucose, glutarate is rather more favorable

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ACS Omega XXXX, XXX, XXX−XXX

than 3-hydroxypropanoate or lactate (Table 3). The formation of glutarate from glucose has not been reported from natural organisms, just in cells engineered for possible biotechnological use.[45−48]

As noted above, the overall Gibbs energy change is not the only factor controlling the selection of metabolic end products. They have to be accessible by pathways of biochemically feasible reactions, and it might be difficult for natural evolution to generate a new pathway requiring a series of metabolites and enzymes very different from ones already present. The values presented here suggest that the overall thermodynamics has not been the dominant factor in the evolution of metabolism but it has had some influence on the selection of final products. These values should certainly provide an interesting perspective on natural metabolism. They also highlight some products of biotechnological interest whose formation is thermodynamically strongly favorable. Some of these have been described as targets of metabolic engineering, but others remain future challenges for this field.

CONCLUSIONS

Calculation of overall Gibbs energy changes for conversion of glucose to a wide range of possible final products offers an interesting perspective on metabolism. These values help inform our view of current natural biochemistry, its evolution, and possible constructs from synthetic biology. It appears that the overall thermodynamics has had some influence over evolutionary choices of metabolic end products but other factors are clearly also very important. A number of thermodynamically favored products are interesting targets for metabolic engineering; some are not yet explored.

METHODS

Gibbs Energies of Formation. Gibbs energies of formation were obtained using the online eQuilibrator service[3,4] (http://equilibrator.weizmann.ac.il/; used October and November 2019). Some extended methods give slightly more accurate values but are not as readily available for use.[5,6,8,9] For some compounds not available in eQuilibrator, arguments are based on energies, usually in the gas phase, from the DECHEMA database of thermophysical properties (Detherm, https://dechema.de/en/detherm.html).

The values obtained from eQuilibrator are transformed Gibbs energies using the usual biochemical convention where pH is specified but H^+ ions are not included in the reaction equation. The mathematical transformation is from Alberty[2] where the number of hydrogen atoms in a species is multiplied by the chemical potential of H^+ ions then subtracted from the Gibbs energy of formation. As a result, the Gibbs energy of formation for any species containing hydrogen atoms becomes more positive (or less negative) as pH increases by approximately 5.7 kJ mol^{-1} (RT ln 10) for each pH unit and hydrogen atom. However, much of this pH dependence disappears when values are combined to give the Gibbs energy change for a reaction, leaving only the expected dependence when the reaction actually consumes or produces H^+ ions. The Alberty transformation has the effect of making Gibbs energy equal for all ionization states of acidic or basic compounds, so they can be treated as a single pseudo-species, as normal in biochemical equations. Many carboxylic acids are considered in the present analysis and named as the anions that predominate at neutral pH. However, they can also be treated as having the empirical formula of the undissociated species in balancing equations.

For the main analysis, Gibbs energies of formation and reaction were obtained for the 1 mM in solution standard state with the pH set at 7.0. The Gibbs energies are also somewhat dependent on ionic strength, which was left at the default value of 0.1 M.

In the case of CO_2, eQuilibrator offers values based on 1 mM of the neutral dissolved molecule CO_2 or a total concentration including hydrated species particularly HCO_3^-. The difference is only 5 kJ mol^{-1} at pH 7, negligible if more acidic but grows significantly if more alkaline. In terms of overall metabolic transformations, the interpretation depends on what is assumed to be the eventual fate of CO_2 produced. Values given here are based on neutral dissolved CO_2, which would be appropriate if loss to or equilibration with a gas phase is relevant.

Compounds Considered. The analysis attempts to include all possible C_1, C_2, and C_3 compounds containing also hydrogen and/or oxygen. However, certain classes were not considered in detail because they are generally higher in energy:

(a) Compounds in which the carbon atoms are not in a chain. The $\Delta G_r'^m$ for isomerizations of dimethyl ether to ethanol and diethyl ether to 1-butanol are calculated as −42 and −48 kJ mol^{-1}, respectively. Similarly, for hydrolysis of methyl acetate to methanol and acetate $\Delta G_r'^m$ = −33 kJ mol^{-1}, while for ethyl acetate to ethanol and acetate, $\Delta G_r'^m$ = −29 kJ mol^{-1}.

(b) Compounds with a cyclopropane ring. In the gas phase, the Gibbs energy of cyclopropane is 42 kJ mol^{-1} higher than that of its isomer propene (Detherm). The four-membered heterocyclic ring of oxetane (C_3H_6O) is 144 kJ mol^{-1} higher in the gas phase than in its isomer acetone.

(c) Cumulene structures with adjacent double bonds, such as ketene (CH_2═C═O), as they react rapidly with water by addition to give lower-energy products such as acetate.

(d) Enol and ynol structures being higher in Gibbs energy than the tautomer carbonyl compounds.

(e) Gem-diols were taken to be in hydration equilibrium with the corresponding carbonyl compounds with the latter being favored.

For some compounds not excluded on the above basis, eQuilibrator does not have an entry or does not offer a value of $\Delta G_f'^m$. However, they could be dismissed as being significantly higher in energy than other compounds that are related by hydration/dehydration:

(a) Ethyne (acetylene) can be hydrated to ethanal. For its hydration in the gas phase, ΔG_f^0 values in Detherm give a ΔG_r^0 of −114 kJ mol^{-1}, so ethanal is likely to be strongly favored also in solution.

(b) Propyne can be hydrated to acetone. For its hydration in the gas phase, ΔG_f^0 values in Detherm give a ΔG_r^0 of −119 kJ mol^{-1}, so acetone is likely to be strongly favored also in solution. The propyne isomer propadiene is marginally even less favored with a ΔG_f^0 8 kJ mol^{-1} higher.

(c) 2-Propynol is an isomer of acrolein (prop-2-enal). From data in Detherm, it can be calculated that the enthalpy of combustion of 2-propynol is approximately 80 kJ mol^{-1} more negative than that of acrolein, so it is clearly a less favored isomer. Hence, it must be much less favored than propanoate (see Table 2).

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ACS Omega XXXX, XXX, XXX−XXX

(d) Propynal (C_3H_2O) can have two H_2O molecules added to give $C_3H_6O_3$, the formula of 3-hydroxypropanoate. Addition of water to the triple bond of propynal, a probably favorable process, will give either pyruvaldehyde or malondialdehyde. As shown in Table 1, these products are considerably higher in energy than their isomer acrylate. Hence, we can assume that propynal is not a favored hydration form.

(e) Propynoate ($C_3H_2O_2$) can add water to give malonate semialdehyde or pyruvate. Addition of water to the triple bond is expected to be favorable, especially given the following enol to carbonyl isomerization. Hence, malonate semialdehyde is probably the favored hydration form here.

(f) Malonate ($C_3H_4O_4$) may be formally dehydrated to $C_3H_2O_3$ (OHC·CO·CHO or the four-membered cyclic malonic anhydride) and C_3O_2 (carbon suboxide). From enthalpies of formation in Detherm, we calculate for the reaction C_3O_2 (g) + 2 H_2O (l) → $C_3H_4O_4$ (malonic acid, solid), $\Delta H^0 = -226$ kJ mol^{-1}. Thus, it seems likely that this overall hydration is strongly favored in aqueous solution. For the intermediate tricarbonyl compound, the unfavorable dehydration of hydroxyacetate to glyoxal (Table 2) shows some analogy, and acid anhydride formation will not be favored in aqueous solution, even without taking into account the strain in the ring. On similar grounds, we can rule out dehydration of $C_3H_4O_5$ (hydroxymalonate) to $C_3H_2O_4$ (OHC·CO·COOH or a cyclic anhydride) or $C_3H_2O_5$ (oxomalonate, HOOC·CO·COOH) to a cyclic anhydride.

Overall Transformations. Glucose can be converted to two products, one more oxidized and one more reduced. In this case, there is only one overall stoichiometric equation that produces the two products, which may also involve water as an additional reactant or product. This equation has the form

$C_6H_{12}O_6 + wH_2O = a_1$ reduced product + a_2 oxidized product, where w, a_1, and a_2 are stoichiometric coefficients to be determined by balancing the three elements. These stoichiometric coefficients may be fractional, and w may be negative, meaning water is a product. From this equation, the overall Gibbs energy change of the transformation is calculated as

$\Delta G_r'^m = a_1\Delta G_f'^m$ (reduced product) + $a_2\Delta G_f'^m$ (oxidized product) − $w\Delta G_f'^m$ (H_2O) − $\Delta G_f'^m$ ($C_6H_{12}O_6$)

eQuilibrator gives $\Delta G_f'^m$ (H_2O) as −158 kJ mol^{-1} and $\Delta G_f'^m$ ($C_6H_{12}O_6$) as −447 kJ mol^{-1}.

Examples where w is negative, zero, and positive are

$C_6H_{12}O_6 = 2C_2H_4O$ (ethanal) + $C_2H_2O_3$ (glyoxylate, OHC·COOH) + H_2O; $\Delta G_r'^m = -149$ kJ mol^{-1}.

$C_6H_{12}O_6 = 2.4\ CH_4 + 1.2\ C_3H_2O_5$ (oxomalonate, HOOC·CO·COOH); $\Delta G_r'^m = -279$ kJ mol^{-1}.

$C_6H_{12}O_6 + 6/7H_2O = 6/7C_3H_8 + 24/7CH_2O_2$ (formate); $\Delta G_r'^m = -275$ kJ mol^{-1}.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00790.

Complete table of end products and all data and calculations (download address given)

(PDF)

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Notes

The author declares no competing financial interest.

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ACS Omega XXXX, XXX, XXX−XXX