**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 ($\Delta G = -499$ kJ mol$^{-1}$) followed by CH$_4$ and CO$_2$ ($\Delta G = -430$ 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.

**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, 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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Δ\(G^\text{m}\) (formation) and Δ\(G^\text{m}\) (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 Δ\(G^\text{f}\) shows the selections that can be made in this way. Some cases of isomers where predicted Δ\(G^\text{f}\) 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\(^{19-21}\)).

**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 ethanol 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-COOH), glyoxylate (OH-COOH), hydroxycetate (also named glycolate), acetate, ethanol, ethane, oxomalonate (HOOC-CO-COOH), hydroxymalonate (HOOC-COOH), malonate, malonate semialdehyde (OHCH\(_2\)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\(_4\)H\(_8\)O\(_4\)) 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\(_5\)H\(_4\)O\(_2\)) would also be an interesting possibility, but eQuilibrator does not offer a Δ\(G^\text{f}\) 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 Δ\(G^\text{f}\) is still only −67 kJ mol\(^{-1}\). Production of glyoxylate always gives a less negative (worse) Δ\(G^\text{f}\) than that of hydroxycetate 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 lower.
Table 2. Selection of Compounds Related by (De)hydration

| Favored compound (with $\Delta G_{\text{r}}^{\text{fm}}$ in kJ mol$^{-1}$) | Higher Gibbs energy combinations (with $\Delta G_{\text{r}}^{\text{fm}}$ in kJ mol$^{-1}$) |
|-------------------------------------------------|-------------------------------------------------|
| C(s), graphite (0) | CH$_2$O (formaldehyde) + H$_2$O (98) |
| CH$_3$O, formate (−325) | CO + H$_2$O (−295) |
| C$_2$H$_4$O, ethanol (47) | C$_4$H$_6$ (ethene) + H$_2$O (69) |
| C$_2$H$_4$O, ethanal (5) | C$_2$H$_4$O$_2$ (ethylene glycol) + H$_2$O (47) |
| C$_2$H$_4$O, hydroxymethylacrylate (−429) | C$_2$H$_4$O$_2$ (glyoxal) + H$_2$O (−351) |
| C$_2$H$_4$, 2-propanol (123) | C$_6$H$_6$ (propene) + H$_2$O (134) |
| C$_2$H$_4$, acetone (66) | C$_3$H$_4$O (acrolein) + H$_2$O (−250) |
| C$_3$H$_6$O, propanoate (−178) | C$_4$H$_8$O$_3$ (glycerol) − H$_2$O (129) |
| C$_3$H$_6$O$_2$, 3-hydroxypropanoate (−333) | C$_3$H$_6$O$_2$ (acrylate) + H$_2$O (−323) |
| C$_2$H$_4$O$_3$, malonate semialdehyde (−374) | C$_2$H$_4$O$_3$ (glycerol) − H$_2$O (−325) |

Some other possible (de)hydration reactions where eQuilibrator does not offer values of $\Delta G_{\text{r}}^{\text{fm}}$ are discussed in the Methods.

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

| Overall process | Overall $\Delta G_{\text{r}}^{\text{fm}}$ |
|-----------------|-----------------|
| C$_2$H$_5$OH → 6C(s) + 6H$_2$O | −499 |
| C$_2$H$_5$O$_2$ → 3 acetate | −345 |
| C$_3$H$_5$O$_2$ → 2.3-hydroxypropanoate | −220 |
| C$_3$H$_5$O$_3$ → 1.2 glutarate + 1.2H$_2$O | −279 |

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$_2$O, 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

| Values are $\Delta G_{\text{r}}^{\text{fm}}$ in kJ mol$^{-1}$ | Oxidized product |
|-------------------------------------------------|-----------------|
| CO$_2$ | hydroxycacetate | oxalate | malonate | succinate |
| H$_2$ | −47 | −122 | 47 | −159 | −235 |
| CH$_4$ | −430 | −260 | −322 | −323 | −299 |
| methanol | −165 | −156 | −7 | −156 | −232 |
| methanol | −367 | −243 | −280 | −297 | −288 |
| ethane | −265 | −206 | −206 | −245 | −262 |
| propene | −350 | −239 | −270 | −290 | −285 |
| 2-propanol | −301 | −224 | −238 | −267 | −273 |
| acetone | −295 | −232 | −247 | −269 | −274 |
| propanoate | −319 | −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 |

Values are $\Delta G_{\text{r}}^{\text{fm}}$ in kJ mol$^{-1}$. Oxidized product changes for $\Delta G_{\text{r}}^{\text{fm}}$.

8/11 benzene and 6/11 oxomalonal, 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 $\Delta G_{\text{r}}^{\text{fm}}$ 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 ($\Delta G_{\text{r}}^{\text{fm}}$) will be less favorable where more than one molecule of products (other than H$_2$O) are formed from one molecule of glucose. Therefore, for the conversion reaction glucose $\rightarrow$ 0.5 benzene + 0.5 citrate + 2.5H$_2$O, $\Delta G_{\text{r}}^{\text{fm}}$ and $\Delta G_{\text{r}}^{\text{fm}_0}$ are exactly equal at $-267$ kJ mol$^{-1}$. However, for glucose $\rightarrow$ 3CH$_4$ + 3CO$_2$, $\Delta G_{\text{r}}^{\text{fm}}$ is $-430$ kJ mol$^{-1}$, but $\Delta G_{\text{r}}^{\text{fm}_0}$ is only $-345$ kJ mol$^{-1}$.
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 \(\text{H}_2\), 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). \(\text{CO}_2\) 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 \(\text{CO}_2\) would become favored at atmospheric pressure. If the accumulation to 34 mM before the formation of bubbles of pure \(\text{CO}_2\) becomes favored at atmospheric pressure. If the accumulation to 34 mM before the formation of bubbles of pure \(\text{CO}_2\) would become favored at atmospheric pressure.

The production of alkanes, ethane, propane, butane, and even hexane are rather favorable, especially with \(\text{CO}_2\) 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.\(^{31−35}\) 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 \(\text{CO}_2\) 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, 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 (\(\Delta G^r_{\text{mol}} = -206 \text{ kJ mol}^{-1}\)) or ethanol + \(\text{CO}_2\) (\(\Delta G^r_{\text{mol}} = -265 \text{ kJ mol}^{-1}\)), 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.\(^{27,19−21}\) As a single product from glucose, glutarate is rather more favorable.

### 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.\(^{10−15,16}\) 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 the possibilities in Tables 3 and 4, the thermodynamically most favorable (most negative \(\Delta G^r_{\text{mol}}\)) is the formation of elemental C (graphite) and \(\text{H}_2\). 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 \(\text{CH}_4\) and \(\text{CO}_2\). 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 \(\text{CH}_4\) production is considered to be the difficulties of handling reduced C species. Tables 3 and 4 highlight the existence of \(\text{C}_2\) and \(\text{C}_3\) alternatives that are not a lot worse than making \(\text{CH}_4\). Acetate, propionate, and butyrate are there, and the formation of such acids is a standard intermediate stage in anaerobic decomposition of organic matter.\(^{27}\)
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.\textsuperscript{45−46}

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.

\section*{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.

\section*{METHODS}

\subsection*{Gibbs Energies of Formation.}

Gibbs energies of formation were obtained using the online eQuilibrator service\textsuperscript{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.\textsuperscript{5,6,8,9} For some compounds not available in eQuilibrator, arguments are based on neutral dissolved CO\textsubscript{2} values but are not as readily available for use.\textsuperscript{5,6,8,9} For some

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\textsubscript{2}, eQuilibrator offers values based on 1 mM of the neutral dissolved molecule CO\textsubscript{2} or a total concentration including hydrated species particularly HCO\textsubscript{3}\. The difference is only 5 kJ mol\textsuperscript{−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\textsubscript{2} produced. Values given here are based on neutral dissolved CO\textsubscript{2}, which would be appropriate if loss to or equilibration with a gas phase is relevant.

\subsection*{Compounds Considered.}

The analysis attempts to include all possible C\textsubscript{1}, C\textsubscript{2}, and C\textsubscript{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 $\Delta G^\text{f}^\text{mm}$ for isomerizations of dimethyl ether to ethanol and diethyl ether to 1-butanol are calculated as $-42$ and $-48$ kJ mol\textsuperscript{−1}, respectively. Similarly, for hydrolysis of methyl acetate to methanol and acetate $\Delta G^\text{r}^\text{mm} = -33$ kJ mol\textsuperscript{−1}, while for ethyl acetate to ethanol and acetate, $\Delta G^\text{r}^\text{mm} = -29$ kJ mol\textsuperscript{−1}.

(b) Compounds with a cyclopropane ring. In the gas phase, the Gibbs energy of cyclopropane is 42 kJ mol\textsuperscript{−1} higher than that of its isomer propane (Detherm). The four-membered heterocyclic ring of oxetane (C\textsubscript{3}H\textsubscript{6}O) is 144 kJ mol\textsuperscript{−1} higher in the gas phase than in its isomer acetone.

(c) Cumulene structures with adjacent double bonds, such as ketene (CH\textsubscript{2}=C\textsubscript{2}=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 $\Delta G^\text{f}^\text{mm}$. 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, $\Delta G^\text{r}^\text{m}$ of $-114$ kJ mol\textsuperscript{−1}, so ethanol is likely to be strongly favored also in solution.

(b) Propyne can be hydrated to acetone. For its hydration in the gas phase, $\Delta G^\text{m}$ values in Detherm give a $\Delta G^\text{r}^\text{m}$ of $-119$ kJ mol\textsuperscript{−1}, so acetone is likely to be strongly favored also in solution. The propyne isomer propadiene is marginally even less favored with a $\Delta G^\text{r}^\text{m}$ of 8 kJ mol\textsuperscript{−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\textsuperscript{−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).
(d) Propynal ($C_3H_2O$) can have two $H_2O$ molecules added to give $C_3H_4O_3$, 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 ($C_3H_2O_2$) may be formally dehydrated to $C_3H_2O_3$ (OHC-CO-CHO or the four-membered cyclic malonic anhydride) and $C_2O_2$ (carbon suboxide). From enthalpies of formation in Detherm, we calculate for the reaction $C_6H_12O_6 (g) + 2 H_2O (l)$ → $C_3H_2O_3$ (malonic acid, solid), $\Delta f^\circ H^\circ = -226 \text{ kJ mol}^{-1}$. Thus, it seems likely that this overall hydration is strongly favored in aqueous solution. For the intermediate tricarbonyl compound, the unfavorable dehydrogenation 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 $C_3H_2O_3$ (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

$$C_6H_{12}O_6 + 2H_2O = 2C_2H_4O (ethanal) + C_2H_2O_3 (glyoxylate, OHC-CHO).$$

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