Electrosynthesis of Amino Acids from Biomass-Derivable Acids on Titanium Dioxide

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Electrosynthesis of Amino Acids from Biomass-Derivable Acids on Titanium Dioxide

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Seven amino acids were electrochemically synthesized from biomass-derivable α-keto acids and NH_2OH with Faradaic efficiencies (FEs) of 77 – 99% using earth-abundant TiO_2 catalyst. Furthermore, we newly constructed a flow-type electrochemical reactor, named "polymer electrolyte amino acid electrosynthesis cell", and achieved continuous production of alanine with an FE of 77%.

Amino acids are key building blocks in nature and thus have many potential uses, e.g., animal feed additives, flavor enhancers, pharmaceuticals and cosmetics, and therefore the global demand for amino acids has spurred both academic and industrial researches to develop more environmentally-friendly and lower-cost processes for its production. Currently, amino acids are mainly produced through the microbial fermentation process from sugar- or starch-based feedstock.[1-3] Although the process has made continuous advances over several decades for the production of 20 proteinogenic amino acids, the production of some amino acids still suffers from low efficiency. Besides, the fermentation process has critical drawbacks such as high energy and time consumption for microbial culturing and complicated processes for product isolation and purification. Chemical synthesis is a simple and efficient way to produce amino acids, and the most conventional approach is the Strecker reaction,[4-6] in which aldehydes, ammonia and hydrogen cyanide react to produce an aminonitriles that can easily undergo hydrolyzation to form amino acids. However, the use of hazardous cyanide and nonrenewable aldehydes does not match the scope of this powerful reaction. Meanwhile, the reductive amination of α-keto acids is a potential candidate as a next-generation synthetic process for amino acid production without any consumption of toxic reagents (Fig. 1).[7-9] This process consists of two steps, namely, (i) condensation between a carbonyl compound and a nitrogen source, e.g., NH_3 and NH_2OH, to afford a nitrogenated intermediate, e.g., imines and oximes, and (ii) subsequent reduction of the intermediate. Noted that recent thorough studies advanced the production of several α-keto acid derivatives from lignocellulosic biomass as a non-food-competing chemical feedstock.[10, 11] For example, α-hydroxyl acids, which can be easily converted into the corresponding α-keto acids by simple two-electron oxidation,[12, 13] such as lactic acid,[14-21] glycolic acid,[22, 23] and α-hydroxyglutaric acid,[24] are obtainable via the hydrothermal degradation of cellulose. Possible routes for the preparation of α-keto acids are summarized in the supplementary materials (Fig. S2). We then envisage that electrochemically driven reductive amination of α-keto acids would offer a simple method for amino acid synthesis. Another merit of the electrochemical process is the availability of water and electricity produced from renewable energies as hydrogen and energy sources, respectively. A limited number of works dealing with the electrosynthesis of amino acids from α-keto acids have already been reported.[25-29] Most of these works, however, employ toxic metals such as Pb and Hg or precious Pt as electrocatalysts.

On another front, nontoxic, earth-abundant and durable TiO_2, which exhibits highly selective electroreduction of carbonyl groups due to its relatively large overpotential for hydrogen production, is a candidate as an electrocatalyst for synthesizing organic compounds in aqueous media.[30-34] Recently, we reported that a Ti mesh electrode covered with anatase TiO_2 (TiO_2/Ti mesh), which is prepared through a hydrothermal treatment of Ti mesh,[35-37] catalyses the electrochemical reduction of α-keto acids to produce the corresponding α-hydroxyl acids with a remarkably high selectivity under highly acidic conditions where hydrogen is usually a major product.[37] We, herein, report an efficient electrocatalytic system for the reductive amination of α-keto acids on the TiO_2/Ti mesh electrode in the presence of NH_3 or NH_2OH as the nitrogen source to produce the corresponding amino acids. We demonstrate the electrochemical synthesis of 7 types of amino acids with considerably high FE values. Electrochemical syntheses of aspartic acid, phenylalanine, and

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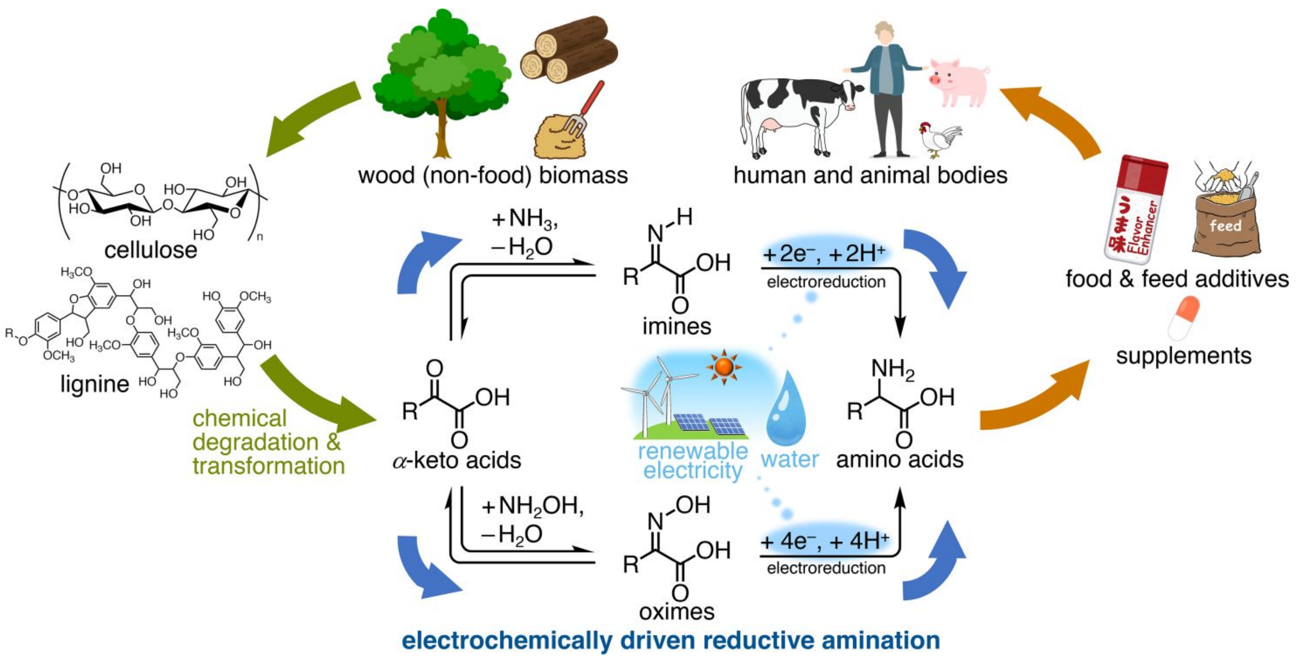

Fig. 1 Possible role of reductive amination in sustainable society. Reductive amination can convert biomass-derived α-keto acids into amino acids, which are building blocks of human and animal bodies, in the presence of nitrogen sources, i.e., NH_3 and NH_2OH.

tyrosine are realized in this study for the first time. Furthermore, we perform continuous synthesis of alanine using a flow-type reactor, named “polymer electrolyte amino acid electrosynthesis cell (AAEC)”.

We firstly examined the TiO_2/Ti mesh catalyzed electrochemical reduction of pyruvic acid in the presence of NH_3 and NH_2OH to produce alanine. Cyclic voltammetric (CV) curves of the TiO_2/Ti mesh electrode recorded in 1.5 M $NH_3/(NH_4)_2SO_4$ buffer with 30 mM pyruvic acid (pH 10) and in 0.20 M H_2SO_4 (aq.) with 160 mM pyruvic acid and 80 mM $(NH_2OH)_2 \cdot H_2SO_4$ (pH 0.53) are shown in Figs. 2b and 2c, respectively. Compared to that in the absence of pyruvic acid, the current density in the presence of pyruvic acid increased at potentials lower than –0.10 and –0.20 V vs RHE in Figs. 2b and 2c, respectively, clearly indicating the reduction of pyruvic acid or its nitrogenated species, i.e., the imine or oxime. Accordingly, we performed the electroreduction of pyruvic acid at applied potentials of –0.32 and –0.40 V in the presence of NH_3 and NH_2OH, respectively, using a two-compartment electrochemical cell separated by a Nafion membrane (Fig. 2a). After the 2-hour electroreduction, the electrolyte solution in the cathode chamber was analyzed by high-performance liquid chromatography (HPLC) and ^1H NMR. When we used NH_2OH as the nitrogen source, alanine was produced with a 78% FE, whereas the corresponding reduction conducted in NH_3 buffer resulted in less selective production of alanine, i.e., 28 and 24% of FEs for the production of alanine and lactic acid, respectively (Fig. 2d). As is evident from ^1H NMR measurements for pyruvic acid in D_2O solution containing NH_2OH (Fig. S3) and NH_3 (Fig. S4), pyruvic acid almost quantitatively reacted with NH_2OH to form pyruvic oxime, whereas pyruvate imine could not be observed even with a large excess amount of NH_3 in the sample solution. These results clearly explain the higher FEs for the production of alanine in NH_2OH and the production of lactic acid in NH_3 solution.

We next optimized reaction conditions, such as applied potential, temperature, pH, concentration of pyruvic acid, and amount of the nitrogen source, for alanine electrosynthesis (Figs. 2e, S5, and S6, Tables S1 and S2). When NH_3 was introduced as the nitrogen source, the reaction conditions did not greatly affect the product selectivity, and the maximum FE for alanine production was 29% (Fig. S5 and Table S1). By contrast, the product selectivity in the presence of NH_2OH was strongly affected by NH_2OH amount, pH, and temperature (Figs. 2e and S6, Table S2). When the NH_2OH amount was higher than 1.2 equiv. relative to pyruvic acid, the FE for alanine production decreased with increasing NH_2OH amount (Fig. 2e-i), which is possibly attributable to the electroreduction of residual NH_2OH. At pH values lower than 3, protonation of the of the nitrogen atom on the pyruvic oxime molecule occurs, which possibly accelerates alanine production (Fig. 2e-ii); cf. the pK_a for the oxime protonation is 1.3.[38] Indeed, it is well known for proton-coupled reduction reactions such the oxime reduction that protonation of the substrates increases their redox potentials and facilities the progress of the reduction.[39, 40] Considering that oximes undergo hydrolysis upon heating in acidic media and afford the original ketone and NH_2OH,[41, 42] a temperature increase can induce lactic acid formation (Fig. 2e-iii). Finally, we achieved an FE of 99% for alanine production under the optimum conditions, i.e., applied potential of –0.50 V, pH 0.19, temperature of 0 ºC, pyruvic acid concentration of 160 mM, NH_2OH amount of 1.2 equiv. relative to pyruvic acid.

We further examined the electrosynthesis of other amino acids having various types of functional groups in their residues. Glycine, aspartic acid, glutamic acid, and leucine were synthesized from corresponding α-keto acids and NH_2OH (Fig. 3) with FE values

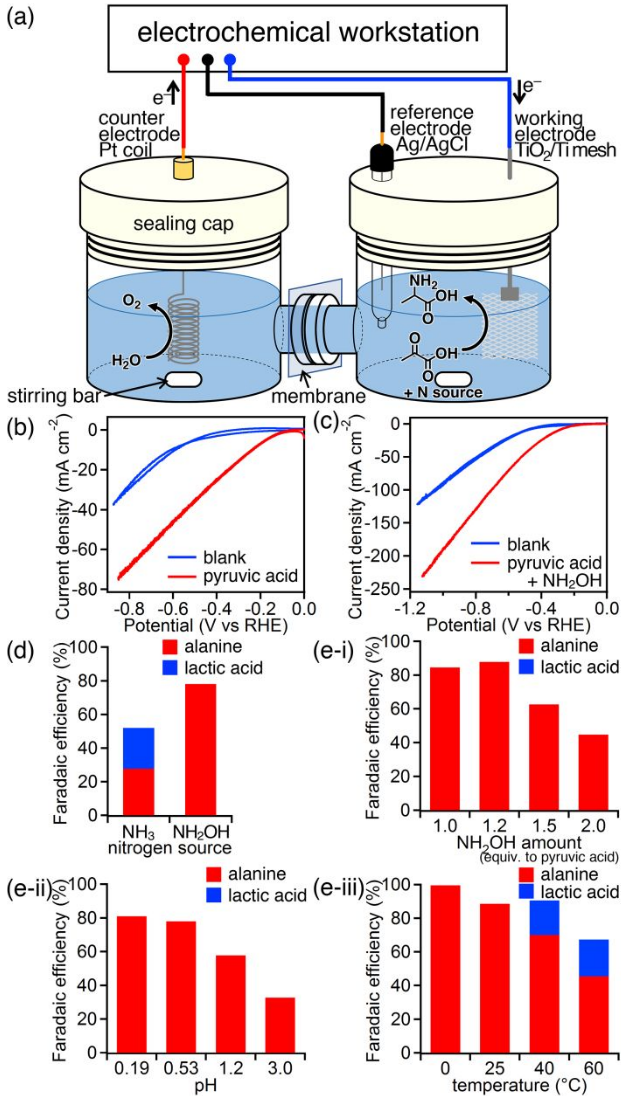

Fig. 2 Electrochemical reduction of pyruvic acid on a TiO_2/Ti mesh electrode in the presence of NH_3 and NH_2OH to produce alanine. (a) A schematic diagram of the electrochemical system for alanine electrosynthesis using two-compartment glass cell. (b) CV curves of the TiO_2/Ti mesh electrode in 1.5 M $NH_3/(NH_4)_2SO_4$ buffer (pH 10) at 40 ºC with (red line) and without (blue line) 30 mM pyruvic acid. (c) CV curves of the TiO_2/Ti mesh electrode in 0.2 M H_2SO_4 (aq.) at 25 ºC with (red line, pH 0.53) and without (blue line, pH 0.62) 160 mM pyruvic acid and 80 mM $(NH_2OH)_2{\cdot}H_2SO$. (d) FEs for alanine (red bar) and lactic acid (blue bar) production via pyruvic acid (160 mM) reduction at –0.32 V in 1.5 M $NH_3/(NH_4)_2SO_4$ buffer (40 ºC, pH 10) and at –0.4 V in 0.2 M H_2SO_4 aq. with 80 mM $(NH_2OH)_2{\cdot}H_2SO$ (25 ºC, pH 0.53). (e) FEs for alanine (red bar) and lactic acid (blue bar) production in the presence of NH_2OH under varying (i) NH_2OH amounts, (ii) pH values, and (iii) temperatures. Detailed conditions for each electrochemical run are summarized in Table S2 in the supplementary materials.

higher than 90%, whereas FE values for the production of phenylalanine and tyrosine were slightly lower, i.e., 87 and 77%, respectively, which is attributable to the low concentration of the starting α-keto acid due to their solubility limit. To the best of our knowledge, we demonstrated electrochemical synthesis of aspartic acid, phenylalanine, and tyrosine for the first time in this study, and achieved the highest FE values for the electrochemical synthesis of alanine and glutamic acid, i.e., 99 and 97%, as summarized in Table S4.

We finally investigated the continuous electrochemical production of alanine from pyruvic acid and NH_2OH using an electrolyser, called an AAEC. Fig. 4a shows the structure of the AAEC. We employed Ti felts covered with anatase TiO_2 (TiO_2/Ti felt) and nanoscale IrO_2 as cathode and anode, respectively, which we originally developed.[35, 36] The TiO_2/Ti felt (2×2 cm^2) and a membrane electrode assembly (MEA) prepared by hot pressing of a Nafion membrane bearing an IrO_2 deposited layer (2×2 cm^2) and porous Ti paper were sandwiched between the cathode and anode current collectors with sample flow channels and sealed with silicone gaskets. Alanine electrosynthesis with the AAEC was performed at various applied potentials in the range from –2.2 to –2.8 V with continuous flows of water (1 mL min^{-1}) and 0.2 M H_2SO_4 (aq.) containing 160 mM pyruvic acid and 96 mM $NH_2OH{\cdot}H_2SO_4$ (0.5 mL min^{-1}) in the anode and the cathode, respectively. The amperometric *i-t* curves acquired at a series of potentials reveal the constant nature of the current densities during the AAEC operation, suggesting the good stability of the catalysts (Fig. 4b). The conversion of pyruvic acid increased as the applied potential increased and ultimately reached 89% at an applied potential of –2.8 V (Fig. 4c). Reasonably high FEs of 73 – 77% for alanine production were obtained at a potential range of –2.2 – –2.8 V (Fig. 4d).

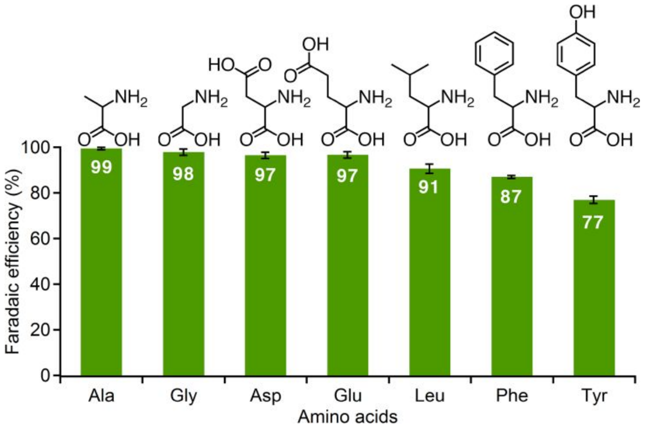

Fig. 3 Electrochemical production of various amino acids from the corresponding α-keto acids and NH_2OH. FEs for amino acid production are presented. Detailed conditions for each electroreduction are summarized in Table S3 in the supplementary materials.

In summary, we have presented highly efficient electrochemical conversion of α-keto acids into amino acids, i.e., with 77 – 99% FE values, on TiO_2/Ti mesh electrodes using NH_2OH as a nitrogen source, which owes to the exceptionally high selectivity for the hydrogenation of preferentially-formed oximes on the TiO_2 catalyst even in aqueous media. The continuous electrosynthesis of alanine from pyruvic acid and NH_2OH using the AAEC equipped with a TiO_2/Ti felt was demonstrated, and 89% conversion of pyruvic acid and 77% FE for alanine production were achieved at applied potentials of –2.8 and –2.4 V of applied potentials, respectively.

Electrochemical processes have been believed to play a role in the origin of life. Recently, various studies have clarified that fundamental chemicals that make up living organisms are producible through abiological pathways; e.g., acetates and pyruvate can be generated via CO_2 reduction.[43, 44] Thus, our report on the electrochemical production of amino acids from α-keto acids may contribute to the elucidation of the

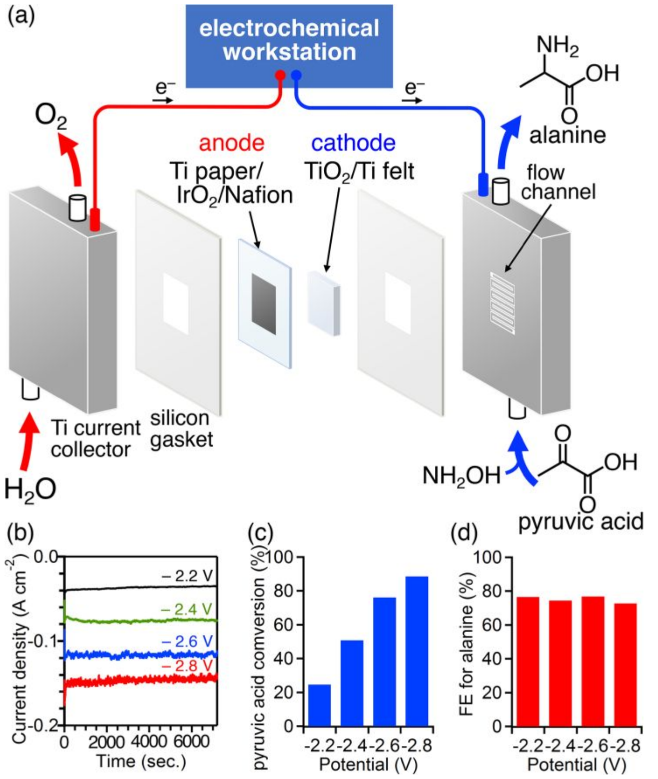

Fig. 4 Electrosynthesis of alanine from pyruvic acid and NH_2OH using the AAEC. (a) A schematic diagram of the AAEC. The presented building blocks are stacked to form the AAEC. (b) Current densities, (c) conversions of pyruvic acid, and (d) FEs for alanine production during the operations of the AAEC at various applied potentials in the range from –2.2 to –2.8 V with flows of water (1 mL min^{-1}) and 0.2 M H_2SO_4 (aq.) containing 160 mM pyruvic acid and 96 mM $NH_2OH{\cdot}H_2SO_4$ (0.5 mL min^{-1}) in the anode and the cathode, respectively.

mystery of the creation of life.

Assuming that future of human beings involves living away from Earth, it will be necessary to conveniently and efficiently synthesize essential nutrients such as amino acids for maintaining their life in space. In this context, electrochemical processes using solar electricity will play a significant role in material synthesis under space-limited and resource-restricted conditions. We hope that our approach will provide useful clues for the future construction of artificial carbon and nitrogen cycles in space.

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Conflicts of interest

There are no conflicts to declare.

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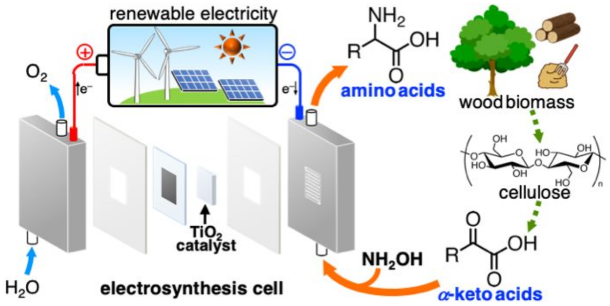

Amino acids were electrochemically synthesized from biomass-derivable α-keto acids and water with high Faradaic efficiencies using safe and earth-abundant TiO_2.