Environmentally Compatible Synthesis of Adipic Acid from D-Glucose

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Industrial production of adipic acid is a premier example of synthetic methodology used on a massive scale that will likely be incompatible with public environmental expectations and environmental regulatory policies of the 21st century. Primarily used in production of nylon-6,6[1,2] the annual global demand for adipic acid exceeds 1.9×10^9 kg.[1] An environmentally compatible synthesis (Scheme 1) has now been established using a microbial catalyst to enzymatically convert D-glucose into *cis,cis*-muconic acid. Subsequent catalytic hydrogenation of the *cis,cis*-muconic acid affords adipic acid.

Benzene, a carcinogen[3] derived from nonrenewable fossil fuels, is the principal starting material from which adipic acid is currently synthesized (Scheme 1).[1,2] Hydrogenation of benzene to produce cyclohexane is followed by air oxidation to yield a mixture of cyclohexanol and cyclohexanone. Nitric acid oxidation (Scheme 1) then yields adipic acid and a byproduct, nitrous oxide,[4] which is involved in both ozone depletion and the greenhouse effect.[5] Adipic acid production may account for some 10% of the annual increase in atmospheric nitrous oxide levels.[4] Forcing reaction conditions, including temperatures as high as 250 °C and pressures reaching 800 psi, are also required by current methods of adipic acid manufacture.[1,2]

Catalytic conversion of D-glucose into *cis,cis*-muconic acid required creation of a biosynthetic pathway (Figure 1) not known to exist naturally. Increased in vivo synthesis of 3-dehydroshikimic acid (DHS) by AB2834, an *Escherichia coli* mutant lacking shikimate dehydrogenase, followed from elevated activities of plasmid pKD136-encoded transketolase, DAHP synthase, and DHQ synthase.[6] Dehydration of DHS to afford protocatechuic acid followed by subsequent decarboxylation to yield catechol resulted from expression of *aroZ* (encoding DHS dehydratase[7]) and *aroY* (encoding protocatechuate decarboxylase[8]) genes carried on plasmid pKD8.243A. Conversion of catechol into *cis,cis*-muconic acid was catalyzed by catechol 1,2-dioxygenase encoded by the *catA* gene of plasmid pKD8.292.[9]

Of the foreign genes introduced into AB2834, DHS dehydratase enables hydroaromatics such as shikimic acid to be catabolized via the *p*-hydroxybenzoate branch of the β-ketoadipate pathway[7,9] while the role of protocatechuate decarboxylase in microbial metabolism is unknown.[8,10] Both *aroZ* and *aroY* were isolated from a library of *Klebsiella pneumoniae* strain A170-40[11] genomic DNA. Catechol 1,2-dioxygenase is part of the benzoate branch

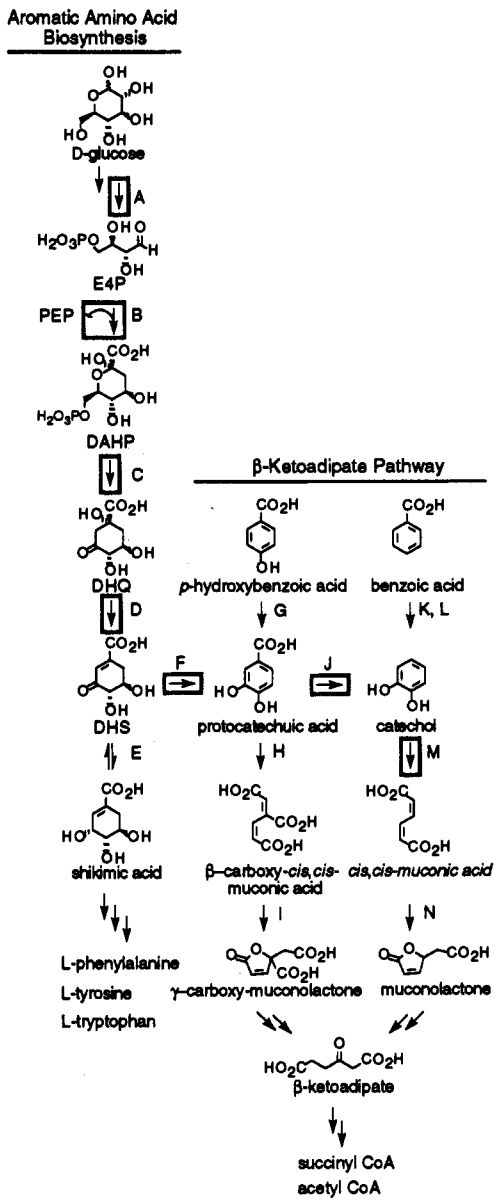

Figure 1. The biocatalytic pathway (boxed arrows) created for microbial conversion of D-glucose into *cis,cis*-muconate from the perspective of the biochemical pathways from which the enzymes were recruited. These pathways and enzymes include the pentose phosphate pathway (enzyme A), the common pathway of aromatic amino acid biosynthesis (enzymes B–E), hydroaromatic catabolism (enzyme F), the *p*-hydroxybenzoate branch (enzymes G–I) and the benzoate branch (enzymes K–N) of the β-ketoadipate pathway, and protocatechuate decarboxylase (enzyme J). Enzymes and the loci which encode the enzymes include (A) transketolase (*tkt*), (B) DAHP synthase (*aroF*, *aroG*, and *aroH*), (C) DHQ synthase (*aroB*), (D) DHQ dehydratase (*aroD*), (E) shikimate dehydrogenase (*aroE*), (F) DHS dehydratase (*aroZ*), (G) *p*-hydroxybenzoate hydroxylase (*pobA*), (H) protocatechuate 3,4-dioxygenase (*pcaG*, *pcaH*), (I) β-carboxy-*cis,cis*-muconate cycloisomerase (*pcaB*), (J) protocatechuate decarboxylase (*aroY*), (K) reductive benzoate dioxygenase (*benABC*), (L) 1,2-dihydro-1,2-dihydroxybenzoate dehydrogenase (*benD*), (M) catechol 1,2-dioxygenase (*catA*), and (N) *cis,cis*-muconate cycloisomerase (*catB*).

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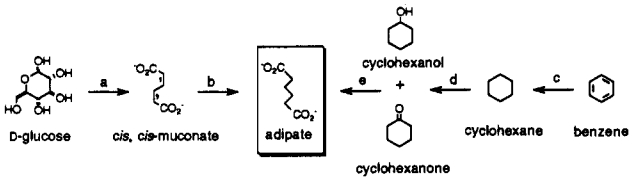
Scheme 1[a]



[a] (a) *E. coli* AB2834/pKD136/pKD8.243A/pKD8.292. (b) 10% Pt on carbon, H_2, 50 psi. (c) Ni–Al_2O_3, H_2, 370–800 psi, 150–250 °C. (d) Co, O_2, 120–140 psi, 150–160 °C. (e) Cu, NH_4VO_3, 60% HNO_3, 60–80 °C.

of the β-ketoadipate pathway.[9] The *catA* gene, obtained from Ornston and co-workers, was isolated from *Acinetobacter calcoaceticus*.[12]

Stable maintenance of plasmids by AB2834/pKD136/pKD8.243A/pKD8.292 followed from differing origins of replication and drug resistances for each plasmid. The measured specific activity of catechol 1,2-dioxygenase (0.25 units/mg) was significantly higher than the specific activities of DHS dehydratase (0.078 units/mg) and protocatechuate decarboxylase (0.028 units/mg). Evaluation of AB2834/pKD136/pKD8.243A/pKD292 by ^{1}H NMR analysis of the culture supernatant indicated that, on a 1-L scale in laboratory shake flasks, the catalyst converted 56 mM D-glucose into 16.8 mM ± 1.2 mM *cis,cis*-muconic acid (Figure 2A). In order to accumulate *cis,cis*-muconate in the absence of *cis,trans*-muconate, the culture supernatant was maintained above pH 6.3. After addition of catalytic 10% platinum on carbon to the unpurified culture supernatant, the heterogeneous solution was hydrogenated at 50 psi for 3 h at room temperature. Reduction of *cis,cis*-muconic acid to adipic acid proceeded in 90% yield (Figure 2B).

Of the more recently developed, novel syntheses of adipic acid,[1,13] none simultaneously address the problems of petroleum-based feedstocks, toxic starting materials or reagents, generation of environmentally incompatible byproducts, and the use of forcing reaction conditions. All of these problems are addressed by synthesis of adipic acid from D-glucose. Starting material D-glucose is nontoxic and can be derived from abundantly available, renewable resources such as plant starch and cellulose.[14]

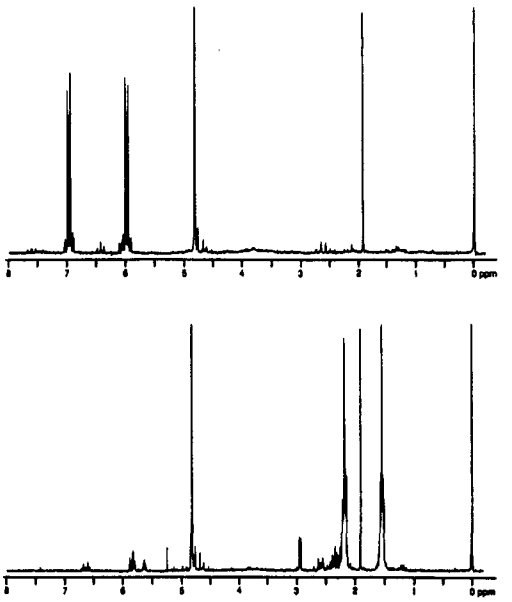


Figure 2. ^{1}H NMR (200 MHz) spectrum before (A, top) and after (B, bottom) hydrogenation of the unpurified, crude culture supernatant of *E. coli* AB2834/pKD136/pKD8.243A/pKD8.292. Relevant resonances include those for (A) *cis,cis*-muconic acid δ 6.0 (2H), δ 7.0 (2H), and (B) adipic acid δ 1.5 (4H), δ 2.2 (4H).

Ozone-depleting gases and greenhouse gases are not generated. Mild reaction temperatures and pressures are utilized. Therefore, while optimization and scaleup remain as daunting challenges, biocatalytic conversion of D-glucose into *cis,cis*-muconic acid and subsequent catalytic hydrogenation to afford adipic acid warrants consideration as a viable synthetic option as chemical manufacture moves into the next century.[15]

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Supplementary Material Available: Experimental procedures for biocatalytic synthesis of *cis,cis*-muconic acid from D-glucose (1 page). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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