Journal of Bioprocessing & Biotechniques

Martin, et al. J Bioproces Biotechniq 2011, 1:4
DOI: 10.4172/2155-9821.1000107

Research Article Open Access

Characterization of a High Activity (S)-Aminotransferase for Substituted (S)-Aminotetralin Production: Properties and Kinetics

Abraham R. Martin[1]*, David Shonnard[1], Sachin Pannuri[1, 2] and Sanjay Kamat[2]

[1] *Dept. of Chemical Engineering, Michigan Technological University, Houghton, MI 49931, USA*
[2] *Cambrex North Brunswick, 661 Highway One, North Brunswick, NJ 08902, USA*

Abstract

The production of substituted (S)-Aminotetralins requires biocatalyst that have high activities at high temperatures and considerable tolerance to amine donors to shift the reaction towards products. The biocatalyst used in this process are (S)-Aminotransferases. An (S)-aminotransferase of a high activity derived from Athrobacter citreous by directed evolution for the production of substituted (S)-aminotetralin was characterized in the form of whole cells. Its optimum conditions were pH 7 and 55 °C. Maximum activity was 0.21 mM/min per gram of whole cells. Substrate affinities were 750 mM for isopropylamine and 10.4 mM for substituted tetralone. A kinetic study to describe the production of substituted (S)-aminotetralin showed that two major reactions were involved: one enzyme catalyzed –by (S)-aminotransferase- that is the production of substituted (S)-aminotetralin from isopropylamine and substituted tetralone; the other a non-enzyme catalyzed reaction that forms a byproduct consisting in the imine formed by substituted tetralone and substituted (S)-aminotetralin.

Keywords: (S)-aminotransferase; Substituted (S)-aminotetralin

Introduction

The production of substituted (S)-Aminotetralins requires biocatalyst that have high activities at high temperatures and considerable tolerance to amine donors to shift the reaction towards products. The biocatalyst used in this process are (S)-Aminotransferases. When the correspondent parochial amine and an amine acceptor are mixed with the biocatalyst at the proper conditions of pH and temperature, among other factors, it is desired that the majority of the prochiral ketone be converted to the corresponding amine. From the economical point of view, the prochiral ketone is the most expensive, meanwhile the amine donor is the cheapest. Thermodynamically, it is possible to achieve in a reversible reaction high conversion of one of the reactants if another is added in excess; but in order to do that, the biocatalyst must tolerate such conditions. Especially, in this study was selected the biocatalyst that allows the production of Subtituted (S)-Aminotetralins from (S)-Aminotetralone. Substituted (S)-aminotetralins are substances with potential for the treatment of Parkinson's disease [1-2], depression [3], and cardiovascular problems [4-5]. Due to this several synthesis procedures have been developed. These procedures consist in a sequence of organic reactions [6-10], use of catalysts [10-12], and use of biocatalyst [13-18]. In this study a characterization of the properties, and kinetics of an (S)-aminotransferase (S-AT) developed for production of substituted (S)-aminotetralins (SS-AT) was done.

Materials and Methods

S-AT

The S-AT used in this study was derived from Athrobacter citreous after five rounds of mutagenesis by directed evolution (Mutant CNB05-01, International Patent WO 2006063336 [18]. It was produced by the microorganism Escherichia coli MG1655 (Cambrex) at an expression level of about 65 mg per gram of whole cells. Its molecular weight is about 65 kDa. S-AT for production of SS-AT was used in the form of whole cells, since the S-AT in this form does not requires purification neither it is required by the specifications of the desired product.

Source: The aminotransferase used in this study was obtained from a naturally occurring S-AT found in Athrobacter citreus in the fifth round of mutagenesis and selection. This S-AT as well as its predecessors was coded by a gene of 1.5 kbp, its molecular weight was 65 kDa and was composed by 480 amino acids. The corresponding genes were isolated, and then sub cloned into pSE420 and transformed into Escherichia coli MG1655. Once the selected gene was expressed in the host cell, it was subjected to random mutagenesis, and selected to improve the properties of the enzyme.

Production: The S-AT used in this study (CNB05-01, gene sequence given on International Patent WO 2006063336 [18] was produced by Escherichia coli MG1655 as follows. To a 15-liter fermenter (New Brunswick®) was added 8 liters of water, 55 grams of KH2PO4 (Fisher Scientific), 18 grams of MgSO4·7H2O (Fisher Scientific), 2 ml of Antifoam (Sigma), and 80 ml of a solution whose composition per liter was 6 grams of Fe (III) citrate hydrate (Aldrich), 1.6 grams of EDTA free acid (Aldrich), 1.5 grams of MnCl2·2H2O (Aldrich), 0.8 grams of Zn(CH3COO)2·2H2O (Aldrich), 0.3 grams of H3BO3 (Aldrich), 0.25 grams of Na2MoO4·2H2O (Aldrich), 0.25 grams of CoCl2·6H2O (Aldrich), and 0.118 grams CuCl2 (Aldrich). After that, the fermenter was sterilized at 121 °C for 30 minutes. The system was cooled down to 30 °C and then a sterile solution of 300 ml with 150 grams of glucose was added and the pH was adjusted to 7 by addition of NH4OH (Sigma). After 18 hours of growth, the glucose supplied at inoculation time had been consumed. Then a sterile solution of glucose 50% w/w and MgSO4·7H2O 2% w/w (Fisher Scientific), was supplied by a peristaltic pump -equipped with sterilized tubes sterilized controlled by the fermenter concentration of dissolved oxygen. Ten hours later 0.6 grams

***Corresponding author:** Abraham R. Mártin, Dept. of Chemical Engineering, Michigan Technological University, Houghton, MI 49931, USA; E-mail:armartin@mtu.edu

Received October 17, 2011; **Accepted** November 14, 2011; **Published** November 17, 2011

Citation: Martin AR, Shonnard D, Pannuri S, Kamat S (2011) Characterization of a High Activity (S)-Aminotransferase for Substituted (S)-Aminotetralin Production: Properties and Kinetics. J Bioprocess Biotechniq 1:107 doi: 10.4172/2155-9821.1000107

of isopropyl-β-D-thiogalactopyranoside (IPTG) from Sigma was added to the fermenter, and then 12 hours later the cells were harvested by spray drying. Typical yields were 250-350 grams of whole cells per 8 liters of fermentation. S-AT expression level was about 65 mg per gram of whole cells.

Synthesis of SS-AT

The synthesis of SS-AT occurs upon reaction of isopropylamine (Sigma) and substituted tetralone (Cambrex) catalyzed by S-AT in the presence of pyridoxal-5-phosphate (PLP) as the enzyme cofactor (Schweizer Hall) and a buffer (Description of starting material and product available in International Patent WO 2006063336 [18]. The synthesis reaction is shown in Figure 1.

In addition to the reaction shown in Figure 1, during production of the desired product there is a side reaction. This undesirable process consists on the reaction of SS-AT and substituted tetralone to form an imine (Figure 2). Its formation was important later on the system, once that there was a considerable amount of the desired product, being negligible early on the reaction.

Analysis of SS-AT by HPLC

Enzyme properties and enzyme kinetics were determined by monitoring the concentration of SS-AT in the reaction system. Periodically samples were withdrawn from the system. Samples were diluted, centrifuged and then analyzed using an HPLC (Hewlett-Packard Model 1100 Series). Absorbance readings were taken at 254 nm after injecting a volume of 15-25 µl. Flow rate was set to 1.0-1.5 ml/min for 20 minutes through a Nova-Pak Phenyl (Waters) column. The mobile phase was comprised of isopropanol: aqueous buffer at 30%:70% (v/v), where the aqueous buffer was comprised of 0.24% (w/v) 1-Octanesulfonic acid (Janssen Chimica) and 0.15% (v/v) H3PO4 (Sigma).

Enzyme properties

Optimum pH: The effect of pH on initial rate was investigated incubating 50 ml of reaction mixture at specific pH and 55°C, at a concentration of free whole cells of S-AT of 5 grams per liter. The composition of the corresponding reaction mixture was 130 mM substituted tetralone (Cambrex), 750 mM isopropylamine (Sigma), 2 mM PLP (Schweizer Hall), and 200 mM

sodium acetate as a buffer (Sigma). Samples of 0.5 ml were withdrawn from the system and mixed with 9.5 ml of HCl 0.1 N (Sigma); at 20, 40, 60, 80, and 100 min.

Optimum temperature: The effect of temperature on initial rate was investigated incubating 50 of ml reaction mixture at specific temperature and pH 7, at a concentration of free whole cells of S-AT of 5 grams per liter. The composition of the corresponding reaction mixture was 130 mM substituted tetralone (Cambrex), 750 mM isopropylamine (Sigma), 2 mM PLP (Schweizer Hall), and 200 mM sodium acetate as a buffer (Sigma). Samples of 0.5 ml were withdrawn from the system and mixed with 9.5 ml of HCl 0.1 N (Sigma); at 20, 40, 60, 80, and 100 min.

Substrate affinity: The affinity of the enzyme for isopropylamine was investigated incubating 50 of ml reaction mixture of specific isopropylamine concentration at 55°C and pH 7, at a concentration of free whole cells of S-AT of 5 grams per liter. The composition of the corresponding reaction mixture was 130 mM substituted tetralone (Cambrex), 2 mM PLP (Schweizer Hall), and 200 mM sodium acetate as a buffer (Sigma). Samples of 0.5 ml were withdrawn from the system and mixed with 9.5 ml of HCl 0.1 N (Sigma); at 20, 40, 60, 80, and 100 min.

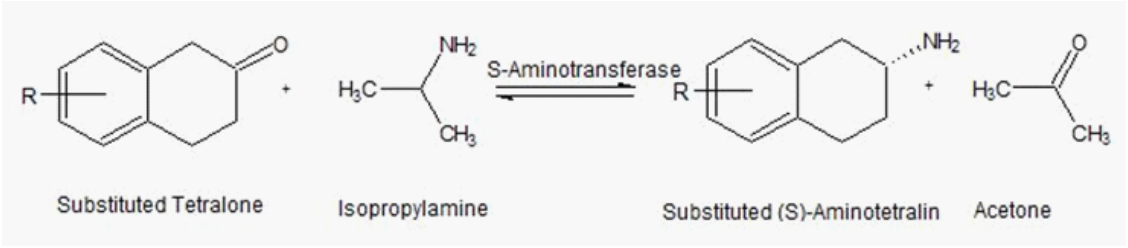

Figure 1: Reaction of substituted tetralone and isopropylamine catalyzed by S-AT to produce a SS-AT.

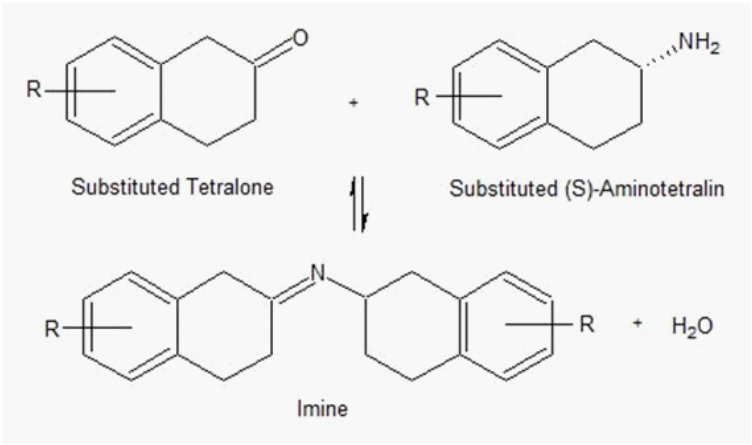

Figure 2: Reaction of imine formation.

Following the same methodology, the affinity for substituted tetralone was determined, withdrawing samples at 5, 10, 15, and 20 min.

Kinetics

Enzyme kinetic: Enzyme kinetic was studied at 55 °C, optimum pH, 2 mM concentration of PLP and at one gram of whole cells per liter. The kinetic studies consisted on the effect of substituted tetralone and isopropylamine in the concentration ranges from 2 to 30 mM and 750 to 2000 mM, respectively; the rate of reverse reaction (reaction between SS-AT and acetone); and the effect of product concentration (SS-AT and acetone). The reaction systems described previously for studying of enzyme kinetic were analyzed following concentration of SS-AT withdrawing 0.5 ml samples at 5, 10, 15, and 20 minutes. After withdrawing the sample it was mixed with 2 ml of water and centrifuged. Under the range of time chose to sample the system imine formation was considered to be negligible.

Kinetic of Imine formation: Imine formation was monitored during SS-AT production at 55°C, and pH 6 and 7. The reaction system was composed of 130 mM substituted tetralone, 750 mM isopropylamine, 2 mM PLP, 200 mM sodium acetate at an enzyme concentration of 5 grams of whole cells per liter; in a reaction volume of 500 ml. At the desired sampling time two samples of 0.5 ml were withdrawn. One of these samples was added to 9.5 ml of distilled water centrifuged and analyzed; the other was added to 9.5 ml 0.1N HCl. The sample added to HCl solution was incubated at 80°C for 30 minutes, then brought to room temperature, centrifuged and analyzed by HPLC. This sample would provide information about the total SS-AT in the system –since HCl and heat hydrolyzes the imine formed-, whereas the sample added water would provide the amount of free SS-AT in the system; then by difference imine concentration was obtained.

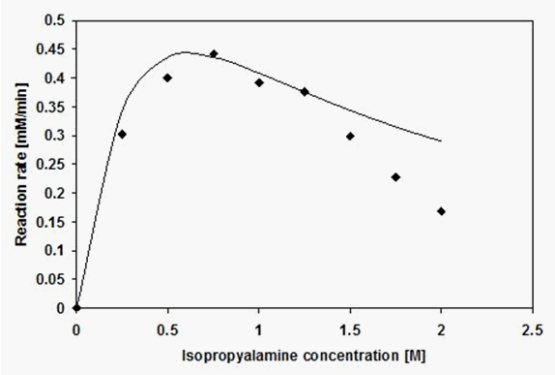

Figure 3: Isopropylamine affinity of S-AT for SS-AT production at 55°C, pH 7, and 5 grams per liter of whole cells. The continuous trend correspond to Equation (4) evaluated at V_m = 1.50 mM/min, K_m = 0.83 M, and K_S = 0.45 M.

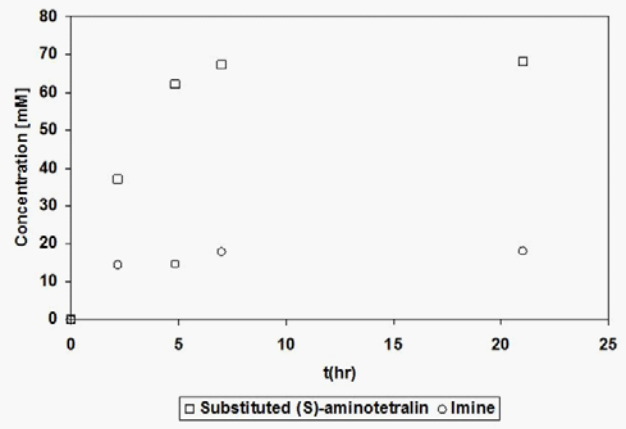

Figure 4: Profile concentration of SS-AT and Imine at 55°C, pH 7, at a concentration of 5 grams per liter of whole cells of S-AT.

S-AT model

The data collected during the experiments were fitted to a modified ping-pong mechanism [19-21] that included substrate and product inhibition by isopropylamine and SS-AT, respectively. Enzyme inhibition by substituted tetralone was not present in the range of solubility of this substance at the temperatures of experimentation. Acetone inhibition was not considered since its boiling point is 56.53°C, and the temperature of the system was 55°C, therefore acetone evaporation rate was high, which did not allow acetone accumulation in the system to cause strong enzyme inhibition. The model used is presented below.

$$v=\frac{v^f_{\max}v^r_{\max}\left((A)(B)-\frac{(P)(Q)}{Keq}\right)}{v^r_{\max}K_{mB}(A)\left(1+\frac{(A)}{K_{iA}}\right)+v^r_{\max}K_{mA}(B)+v^f_{\max}\frac{K_{mQ}}{Keq}(P)+v^f_{\max}\frac{K_{mP}}{Keq}(Q)\left(1+\frac{(Q)}{K_{iQ}}\right)+v^r_{\max}(A)(B)+\frac{v^f_{\max}K_{mQ}}{KeqK_{iA}}(A)(P)+\frac{v^f_{\max}}{Keq}(P)(Q)+\frac{v^r_{\max}K_{mA}}{K_{iQ}}(B)(Q)} \quad (1)$$

Where $v^f_{\max}$ is the forward rate of reaction, $v^r_{\max}$ is the reverse rate of reaction, Keq is the equilibrium constant, K_{iA} is inhibition constant of isopropylamine [A], K_{iQ} is the inhibition constant of SS-AT, and K_{mA}, K_{mB}, K_{mP} and K_{mQ} are the affinities for isopropylamine [A], substituted tetralone [B], acetone [P] and SS-AT [Q], respectively.

SS-AT production model

Equation 1 describes the main reaction occurring in the system early on, when imine formation is negligible. A complete description of the reaction system considering imine formation was done. Imine [I] formation occurs parallel to synthesis of SS-AT [Q]. Substituted tetralone [B] reacts with isopropylamine [A] in the presence of S-AT, rendering as products SS-AT [Q] and acetone [P]. SS-AT [Q] reacts further with substituted tetralone [B], in a reversible fashion, given as product imine [I]. The models that describe imine formation [I] and SS-AT [Q] are shown below.

$$A+B \underset{\text{Enzyme catalyzed process}}{\leftrightarrow} Q+P$$

$$B+Q \underset{k_2}{\overset{k_1}{\rightleftarrows}} I$$

$$\frac{dQ}{dt}=\frac{v^f_{\max}v^r_{\max}\left((A)(B)-\frac{(P)(Q)}{Keq}\right)}{\left[\begin{array}{l}v^r_{\max}K_{mB}(A)\left(1+\frac{(A)}{K_{iA}}\right)+v^r_{\max}K_{mA}(B)+\\ v^f_{\max}\frac{K_{mQ}}{Keq}(P)+v^f_{\max}\frac{K_{mP}}{Keq}(Q)\left(1+\frac{(Q)}{K_{iQ}}\right)\\ +v^r_{\max}(A)(B)+\frac{v^f_{\max}K_{mQ}}{KeqK_{iA}}(A)(P)+\\ \frac{v^f_{\max}}{Keq}(P)(Q)+\frac{v^r_{\max}K_{mA}}{K_{iQ}}(B)(Q)\end{array}\right]}+k_2(I)-k_1(Q)(B) \quad (2)$$

$$\frac{dI}{dt}=k_1(Q)(B)-k_2(I) \quad (3)$$

In these equations the relation between substrates and products is done by mass balance, monitoring the concentration of SS-AT [Q] and imine [I], A=[Ao]-([Q]+[I]), P=([Q]+[I]), and B=[Bo]-[Q]-2[I]. If total concentration of B is higher than the solubility of it at 55°C (33 mM), then B concentration is considered as its solubility.

Effect of PLP

The effect of PLP concentration, the enzyme cofactor, on initial rate of S-AT used in this study was investigated at 55 °C. It was done in systems of 50 ml of solution of composition 130 mM substituted tetralone, 750 mM isopropylamine, 200 mM sodium acetate, and variable concentration of the cofactor. Enzyme concentration was 1 gram per liter. Samples were withdrawn from the systems at 5, 10, 15 and 20 minutes and then analyzed by HPLC to measure concentration of SS-AT.

Results

Enzyme properties and kinetic

Enzyme properties of the S-AT used for production of SS-AT are shown in Table 1. During the experiments done to determine substrate affinity it was noticed that isopropylamine causes enzyme inactivation as shown in Figure 3, while substituted tetralone does not in its soluble range. Substituted tetralone is a compound that has limited solubility (around 33 mM at 55°C). Experimentally, optimum isopropylamine concentration was 750 mM, but Equation (1) predicts an optimum concentration of of 0.61 M, obtained as the square root of the product of *Km KS*. The values of the parameters shown Equation (4) are *Vm* = 1.50 mM/min, *Km* = 830 mM, and *KS* = 450 mM for the data shown in Figure 3.

$$v = \frac{V_m[S]}{K_m + [S] + \frac{[S]^2}{K_S}} \quad (4)$$

where v is reaction rate, *Vm* is maximum reaction rate, S is substrate concentration, *Km* is the Michaelis-Menten constant, and *KS* is the inhibition constant of the substrate [19].

Imine formation

Imine formation in the system together with SS-AT in solution is presented in Figure 4 and 5. Imine formation is believed to be a reversible process where substituted tetralone and SS-AT combines to for the imine, at the same time imine decomposes to form substituted tetralone and SS-AT. Maximum imine and SS-AT was at about 7 hours, and remained practically constant towards the rest of the biotransformation. SS-AT reached a concentration about 70 mM, whereas imine concentration reached a maximum concentration of about 20 mM.

During the production of SS-AT at pH 6 and 7, at a 500 ml scale, the same overall conversion was achieved. Twenty-one percent of the SS-AT produced at pH 7 was obtained after hydrolysis of imine, meanwhile 3.5% of the SS-AT produced at pH 6 was originated after acid hydrolysis of imine. Therefore, low pH appears to decrease overall imine formation, but at the pH of 6 enzyme activity is lower than the activity of the enzyme at pH 7, therefore pH is selected as the optimum for this S-AT.

Effect of pyridoxal-5-phosphate

The data shown in Figure 6 were correlated to a Michaelis-Menten-like model of saturation kinetics.

$$r = \frac{V_m S}{K_m + S} \quad (5)$$

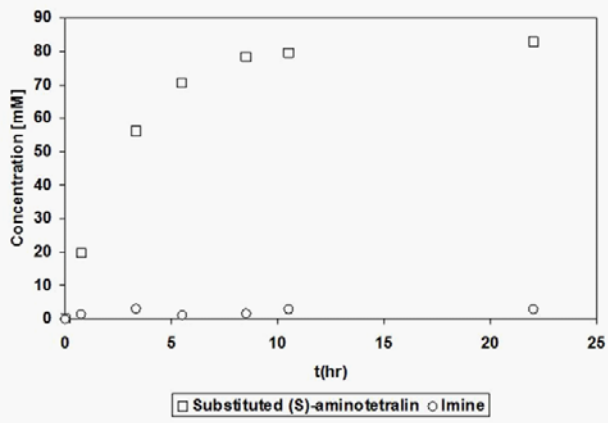

Figure 5: Profile concentration of SS-AT and Imine at 55°C, pH 6, at a concentration of 5 grams per liter of whole cells of S-AT.

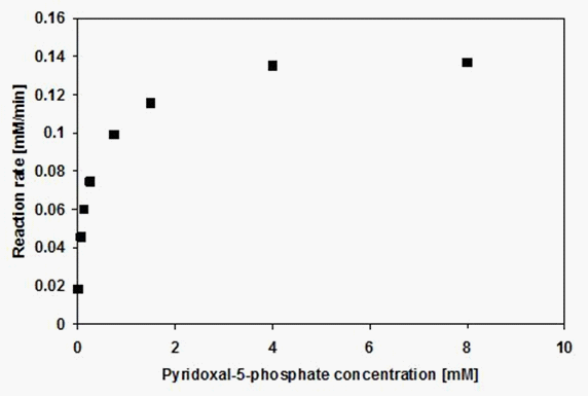

Figure 6: Effect of Pyridoxal-5-phosphate (PLP) concentration on reaction rate of free S-AT catalyzing the reaction system of substituted tetralone at 55 °C, pH 7.

Property	Value
Optimum pH	7
Optimum Temperature (°C)	55
Activation Energy (kcal/mol)	13.0
Deactivation Energy (kcal/mol)	43.9
Maximum activity per gram of whole cells per liter (mM/h)	12-18
Apparent Michaelis-Menten constant for Substituted Tetralone (mM)	10.4
Optimum Isopropylamine concentration (mM)	750

Table 1: S-AT properties.

The model gave as a result a maximum value of rate Vm of 0.14 mM/min, and a Km of 0.22 mM by means of non-linear regression using the solver tool from Microsoft® Office Excel 2003. Figure 6 also shows a reaction rate of 0.02 mM/min at a cofactor concentration of 0 mM, obtained when the enzyme was used as it was after obtaining the whole cells by spray drying. Adding cofactor up to a concentration of 4 mM increases the activity (reaction rate) by a factor of about 7 times. From these data the need is shown of adding the cofactor, PLP, to all the reactions mediated by S-AT expressed in *Escherichia coli* MG1655, since it appears that the amount of PLP inside of the cell is not enough

to form the complex enzyme-cofactor with all the S-AT produced.

Discussion

The experiments regarding to enzyme properties and kinetic showed clearly that S-AT presents substrate inhibition by isopropylamine, and suggested that SS-AT might cause product inhibition. The data collected during the experiments mentioned above allowed modeling the data by a modified ping-pong mechanism [20-22] that included substrate and product inhibition by isopropylamine and SS-AT, respectively. The parameters that best describe the experimental data are shown in Table 2. These parameters were estimated by non-linear regression, using as guessing parameters the values of substrate affinity constants and maximum activity shown in Table 1.

Individual parameter	Value
v^f_{max}	0.21 (mM/min) [12.6 mM/hr]
v^r_{max}	0.02 (mM/min)
Keq	0.33
K_{mB}	1.72 (mM)
K_{iA}	200.10 (mM)
K_{mA}	42.59 (mM)
K_{mQ}	0.45 (mM)
K_{mP}	14.95 (mM)
K_{iQ}	6.93 (mM)

Table 2: Apparent kinetic parameters of whole cells of S-AT.

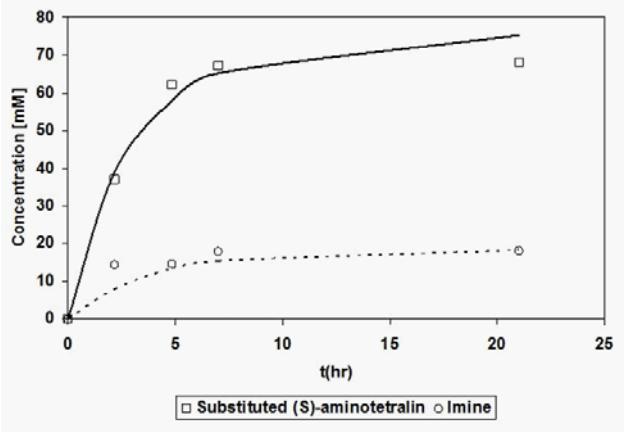

Figure 7: Description of profile concentration of SS-AT and Imine at 55°C, pH 7, at a concentration of 5 grams per liter of whole cells of S-AT by Equation 3 and 4 evaluated with the parameters shown in Table 3.

Parameters	Values
v^f_{max} (mM/hr)	59.29 [11.9 mM/hr/gram whole cells per liter]
v^r_{max} (mM/hr)	59.23
Keq	0.33
K_{mB} (mM)	1.71
K_{iA} (mM)	200.10
K_{mA}(mM)	42.59
K_{mQ} (mM)	0.45
K_{mP}(mM)	14.95
K_{iQ}(mM)	6.93
k_1 (mM*hr)$^{-1}$	0.02
k_2 (hr)$^{-1}$	2.04
k_{eq} for Q	0.33
k_{eq} for I	0.01

Equilibrium constant were estimated using the values of final system composition. Where, $k_{eq} = \frac{(P)(Q)}{(B)(A)}$ for Qand $k_{eq} = \frac{(I)}{(B)(Q)}$ for I.

Table 3: Apparent kinetic parameters of whole biotransformation at 55°C, pH 7, 5 grams of whole cells per liter

The whole process of SS-AT production is described by Equations 2 and 3 with the parameters shown in Table 2 were used to model both SS-AT and imine formation during the biotransformation. Equation 2 and 3 were solved numerically by Runge-Kutta method of fourth order and the imine formation parameters obtained by non-linear regression. The parameters that describe the whole biotransformation process, at 55°C, pH 7, 5 grams of whole cells per liter are shown in Table 3; and their prediction of the biotransformation is shown in Figure 7.

Figure 7 shows the biotransformation description by Equation 2 and 3 evaluated with the parameters shown in Table 3. Overall SS-AT formation is described fairly well, at least to the point where it reaches it maximum concentration, after that the model over predicts its concentration (by 17% more). Imine formation apparently is not well described early on the biotransformation, but its final concentration is predicted accurately.

Acknowledgments

Cambrex Corporation for financial support, laboratory facilities, and expertise, to Michigan Technological University, and to the National Council of Science and Technology of Mexico (Conacyt).

References

1. Scheller D, Dressen F (2005) Use of substituted 2-aminotetralins for the preventative treatment of Parkinson's disease. PCT Int Appl 35 pp.
2. Warawa EJ, Migler B (1998) Preparation of aminotetralins for the treatment or prevention of movement disorders. US 16 pp.
3. Scheller D, Breidenbach A, Selve N (2005) Substituted 2-aminotetralins for the treatment of depression. PCT Int Appl 34 pp.
4. Montanari S, Cavalleri P, Santangelo F, Marchini F (1998) Preparation of 6-hydroxy-5-hydroxymethyl-2-aminotetralins as cardiovascular agents. PCT Int Appl 19 pp.
5. Chiesi P, Bongrani S., Razetti R, Civelli M, Umile A (1996) Aminotetralin derivative for the therapy of cardiovascular diseases. PCT Int Appl 18 pp.
6. Nair V, Balagopal L, Rajan R, Mathew J (2004) Recent Advances in Synthetic Transformations Mediated by Cerium(IV) Ammonium Nitrate. Acc Chem Res 37: 21-30.
7. Youngman MA, Willard NM, Dax SL, McNally JJ (2003)The synthesis of novel cis-a-substituted-b-aminotetralins. Synthetic Communications 33: 2215-2227.
8. Yamaura M (2002) Preparation of optically active 2-aminotetralins from racemates. Jpn Kokai Tokkyo Koho 5 pp.
9. Yamada M, Nagashima N (1999) Preparation of 7-methoxy-2-aminotetralin carbamates and improvement of optical purity of 7-methoxy-2-aminotetralin using them. Jpn Kokai Tokkyo Koho 8 pp.
10. Lennon IC (2001) Speeding up synthesis of chiral chemicals. Chemical Engineering 108: 70-72.
11. Dupau P, Hay AE, Bruneau C, Dixneuf PH (2001) Synthesis of optically active 2-aminotetralin derivatives via enantioselective ruthenium-catalyzed hydrogenation of ene carbamates. Tetrahedron: Asymmetry 12: 863-867.
12. Kanai Y, Saito K (2000) Preparation of optically active 2-amino-substituted tetralins. Jpn. Kokai Tokkyo Koho 8 pp.
13. Honda T, Fujii A, Inoue K, Yasohara Y, Itagaki Y, et al.(2003) Process for preparation of 2-aminotetralin derivatives and intermediates thereof. PCT Int Appl 47 pp.

14. Orsini F, Sello G, Travaini E, Di Gennaro P(2002) A chemoenzymic synthesis of (2R)-8-substituted 2-aminotetralins. Tetrahedron: Asymmetry 13: 253-259.

15. Khumtaveeporn K, Cosway TC, Yeh WL (2001) Enzymatic resolution of aminotetralins. U.S 5 pp.

16. Takashima H (2001) Manufacture of optically-active aminotetralins with Pichia. Jpn. Kokai Tokkyo Koho 7 pp.

17. Pannuri S, DiSanto R, Kamat S (2002) Encyclopedia of Catalysis. 1, JohnWiley 610 & Sons Inc 722–737.

18. Pannuri S, Kamat SV , Garcia ARM (2006)Methods for engineering Arthrobacter citreus w-transaminase variants with improved thermostability for use in enantiomeric enrichment and stereoselective synthesis. PCT Int Appl 54 pp.

19. Shuler ML, Kargi F (2002) Bioprocess Engineering Basic Concepts. Second Edition. Prentice Hall International Series in the Physical and Chemical Engineering Sciences. Upper Saddle River, New Jersey.

20. Cleland WW (1963) The kinetics of enzyme-catalyzed reactions with two or more substrates or products. I. Nomenclature and rate equations. Biochimica et biophysica acta 67: 104-137.

21. Cleland WW (1963) The kinetics of enzyme-catalyzed reactions with two or more substrates or products. II. Inhibition: nomenclature and theory. Biochimica et biophysica acta 67: 173-187.

22. Cleland WW (1963) The kinetics of enzyme-catalyzed reactions with two or more substrates or products. III. Prediction of initial velocity and inhibition patterns by inspection. Biochimica et biophysica acta 67: 188-96.