Anal. Chem. **2006,** *78,* 901−904

High-Throughput Screening for the Asymmetric Transformation Reaction of L-Histidine to D-Histidine by Capillary Array Electrophoresis

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Asymmetric transformation reaction of L-histidine to D-histidine was studied by homemade capillary array electrophoresis for the first time. The enantiomeric excess value of asymmetric histidine products can be directly determined from the electrophoretogram of capillary array electrophoresis. The experiment results showed that the optimized asymmetric transformation reaction condition was in the presence of salicylaldehyde as catalyst and acetic acid as solvent.

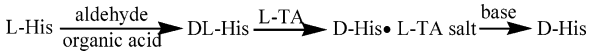
Combinatorial asymmetric catalysis involves time-saving parallel synthesis and the testing of large numbers of chiral catalysts.[1] The challenges in this interesting new area of asymmetric catalysis are twofold, which center around strategies for the modular synthesis of chiral ligands and on developing high-throughput assays for determining the enantiomeric excess (ee).[2] Several catalysts have already been identified by parallel synthesis and high-throughput screening (HTS) techniques.[3] However, the factor limiting an efficient extension of this research to asymmetric catalysis remains the lack of efficient methods for rapid screening of the enantioselective reaction.[4] To overcome this major obstacle, the first approach consists of screening the catalyst library for activity using an HTS procedure and then testing each lead for enantioselectivity by conventional methods,[5] but this is only amenable if few catalysts from the broad library are active. The first high-throughput ee assay was a rather crude UV/visible-based screening system for the lipase-catalyzed kinetic resolution of chiral *p*-nitrophenol esters.[6] Nevertheless, it forms the basis of other tests as well, for example, the recent development using coupled enzymatic transformations.[7] Several more general screening systems have been devised, such as methods based on the mass spectrometric analysis,[8] the use of capillary array electrophoresis (CAE)[9] and HPLC/circular dichroism (CD) spectroscopy.[10]

There are also some disadvantages for the application of HTS; for example, the bottleneck in such an application was the data analysis and processing.[11] In the case of slow analytical methods such as GC and HPLC, it became crucial to perform measurements at the correct sampling time.[12] The possible way to solve such a problem would be the application of time-resolved HTS.[12] To broaden this scope and avoid the problem of such incorrect sampling time, we developed a multichannel CAE system, which could serve as a real-time measurement, to optimize the reaction conditions of crystallization-induced asymmetric transformations of L-histidine to D-histidine.

CAE, which was initially designed for DNA analysis and sequencing, had been applied in screening of enantioselective catalysts of chiral amines first by Reetz et al.[9] Their initial elegant investigation indicated that CAE could be used for high-throughput determination of enantiomeric purity. Evidently, a high-throughput technique allowing quantification of both activity and enantioselectivity would accelerate the rate at which asymmetric catalysts are discovered. Continued efforts in this fascinating area are necessary, since no single assay is universal.

D-Histidine (His) can be prepared from inexpensive L-His by asymmetric transformation,[13] described by Scheme 1. Despite great advances in biocatalysis and asymmetric synthesis, the resolution of racemates is an important approach in the industrial synthesis of enantiomeric pure compounds. It is often the most economical and convenient way to prepare enantiomeric pure

Scheme 1



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10.1021/ac051377w CCC: $33.50
Published on Web 12/15/2005

compounds. Traditional resolutions through selective crystallization of diastereomeric salts imply the introduction of in situ racemization of the unwanted isomer resulting in crystallization-induced asymmetric transformations.[14] For amino acids, this is a well-known process whereby in situ racemization is achieved under mild conditions through catalytic amounts of Schiff bases.

When L-His was heated with 1 equiv of L-tartaric acid (TA) in the presence of 0.1 equiv of aldehyde in organic acid, the salt of L-TA and D-His crystallized, from which D-His can be obtained in the form of optical purity. The crystallization-induced asymmetric transformation reaction can be divided into three steps. First, DL-His can be prepared by racemization method with aldehyde catalysts and organic acid solvents; then, D-His and optically active TA continuously formed precipitable salt along with the continuous racemization of L-His in solution; finally, the salt was alkalified and D-His was separated from the mixture.

In this paper, the asymmetric transformation reactions, L-His to D-His,[13] were successfully investigated for the first time by our homemade 16-channel CAE setup, which solved the problem of capillary orientation of previous CAE systems and recorded the D-His signal in real-time scale.

EXPERIMENTAL SECTION

Reagents and Chemicals. Fluorescein isothiocyanate, L-histidine (L-His), and β-cyclodextrin (β-CD) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemical compounds are analytical pure grade.

Capillary Array Electrophoresis. Details about our rotary CAE setup can be found elsewhere.[15] Herein, we just gave a brief description. Racemate separations of optically active amino acids were completed on a CAE using a confocal fluorescent rotary scanner. A total of 16 capillaries (50-μm inner diameter) of 26-cm total length and 23-cm effective length were symmetrically distributed on a round capillary holder. The inlet ends of 16 capillaries were spatially dipped into 16-well microtiter plates, in which 16 platina electrodes were placed to make independent injections possible into each capillary. The excitation laser, 488-nm output of a 20-mW argon ion laser, passed through a dichroic mirror (T <500 nm, R >500 nm), was focused, and reflected to the capillaries by a rotary reflection mirror, which was seated in the center of the round capillary holder. Fluorescent signal from the irradiated capillary was confocally reflected by the same rotary reflection mirror and the dichroic mirror to the photomultiplier tube (PMT). The rotary reflection mirror and a rotary encoder (Omron, model E6CP, absolute position readout) were coaxally combined to a high-speed direct current motor. By reading the output data of the encoder, the absolute position information of each capillary could be oriented directly by the control computer. The main advantage of this design was that both the accuracy and the repeatability of capillary position were independent of the speed and stability of the dc motor rotation. While rotating the motor, the surrounding capillaries were scanned by the focused excitation laser beam one by one, and the corresponding fluorescent signal output, detected by PMT, was recorded together with the capillary position readout. Currently, the motor was set at ~1000 rpm due to the speed limitation of the rotary encoder. For the given speed, each capillary was addressed at the interval of 60 ms. A homemade data acquiring card was employed to transfer data to a computer. The data were analyzed and converted into electrophoretogram by in-house C Program. By using this setup, 16 racemate products of the racemization reaction could be studied in the same run. Before experiment, the capillary array was rinsed with the corresponding running Borax buffer (20 mmol/L, pH 10) containing 2.5 mmol/L β-CD. The running buffer was the optimal separation condition of DL-His, which was optimized with different buffer concentrations or different additives by this CAE. Samples were loading by 20 s of gravity flow. Separations were performed at 6000 V (230 V/cm).

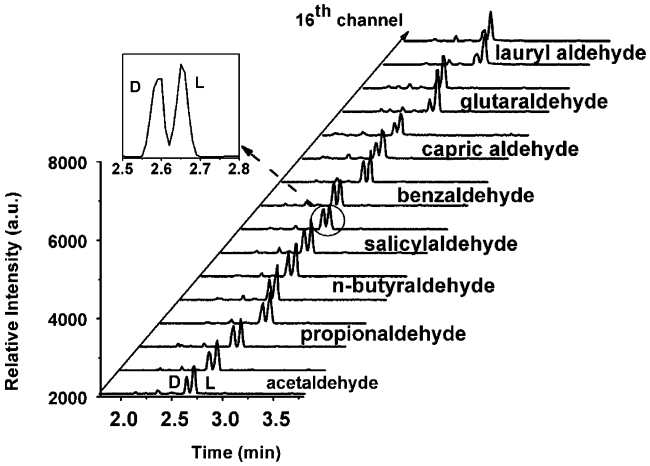

Figure 1. Electrophoretogram of racemic products of L-His by eight aldehydes as catalyst in the presence of acetic acid.

Racemization Reaction of L-His. L-His was heated in the presence of 0.1 equiv of aldehyde as catalyst in 5 mL of acetic acid for 7 h. A 100-μL aliquot of the solution was pipetted out, rapidly cooled, and diluted to 10^{-3}mol/L, fluorescently labeled, and finally diluted to 10^{-5}mol/L for sample injection.

Asymmetric Transformation Reaction of L-His. L-His was heated and stirred for 10 h with 1 equiv of L-TA in the presence of salicylaldehyde or *n*-butyraldehyde in 3 mL of organic acid. The enantiomeric His was fluorescently labeled, and the ee value could be measured after the synthetic D-His salt was filtered, dried, and dissolved with Borax buffer (10 mM, pH 10). The additional purification step as reported in previous methods,[13] the third step in Scheme 1, was not necessary in our experiments.

RESULTS AND DISCUSSION

CAE for the Racemization of L-His. The racemic products of L-His with eight kinds of aldehydes were respectively hydrodynamically injected into 16 capillaries. To estimate the reliability of the separation and detection, two adjacent capillaries were used for same conditions; for example, both the first channel and the second channel were for samples in the presence of acetaldehyde as catalyst. Figure 1 showed the racemization results of L-His in the presence of acetic acid by eight aldehydes: acetaldehyde, propionaldehyde, *n*-butyraldehyde, salicylaldehyde, benzaldehyde, capric aldehyde, glutaraldehyde, and lauryl aldehyde. The inset figure in Figure 1 illustrated the details of the eighth capillary, containing salicylaldehyde as catalyst in the presence of acetic acid. D-His and L-His were baseline separated. The sampling interval of 60 ms, compared with the full width of D-His or L-His,

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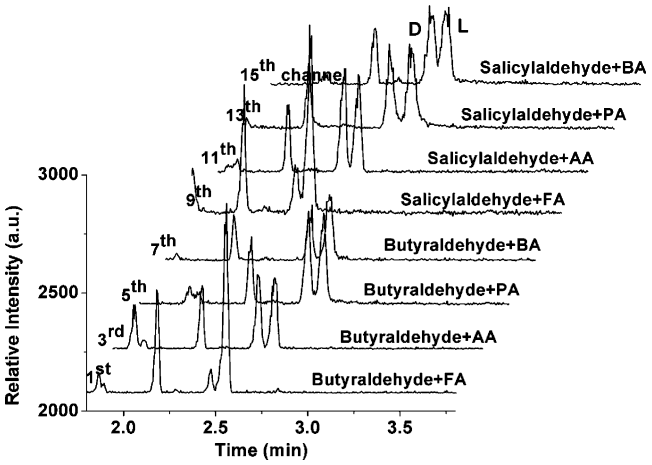

Figure 2. Organic acid solvent effects on racemization of L-His in the presence of *n*-butyraldehyde and salicylaldehyde.

~0.1 min, could provide almost real-time measurement without peak profile distortion. In fact, during illumination of each capillary by the excitation laser beam, the fluorescent signal from the capillary was sampled at the rate of 100 kHz. Each data point in Figure 1 was the average result of ~10 real sampling points. Although this sampling rate resulted in large numbers of data, these data were automatically divided into different arrays according to the encoder readout and corresponded to each capillary by software.

As illustrated in Figure 1, it was obvious that salicylaldehyde was the best choice for the racemization of L-His among the eight aldehydes. Glutaraldehyde and lauryl aldehyde were the second choices as catalysts for the racemization reaction of L-His.

The racemates of L-His in the presence of salicylaldehyde or *n*-butyraldehyde with four organic acids as solvents were respectively hydrodynamically injected into different capillaries. To verify the reproducibility of the setup, two adjacent channels were used for the same asymmetric transformation products; for example, both the first to second channels were for the reaction of L-His in the presence of formic acid (FA) as solvent. Three kinds of organic acids were investigated, i.e., acetic aid (AA), propionic acid (PA) and butanoic acid (BA). To clearly demonstrate the racemization results, eight channels were selected in Figure 2.

Figure 2 indicated that AA was the optimal solvent for the racemization reaction of L-His in the presence of *n*-butyraldehyde or salicylaldehyde. PA and BA were better than FA for the racemization reaction. For BA, the racemization result in salicylaldehyde was better than that of *n*-butyraldehyde.

From Figure 1 and Figure 2, we obtained the optimal conditions for the racemization reaction of L-His in the presence of salicylaldehyde as catalyst and AA as solvent. Since the racemization principle was also adapted for the asymmetric transformation of L-His, we used salicylaldehyde as catalyst and acetic acid as solvent for reaction conditions of the asymmetric transformation from L-His to D-His.

CAE for Asymmetric Transformation of L-Histidine. We investigated the asymmetric transformation of L-His in the presence of salicylaldehyde and L-TA with various organic acids as solvents. We also investigated the effects of *n*-butylaldehyde.

The asymmetric transformation products of L-His in the presence of salicylaldehyde or *n*-butylaldehyde with four kinds of organic acid as solvents were respectively hydrodynamically injected into different capillaries. To verify the reproducibility of the setup, four adjacent channels were used for the same asymmetric transformation products, i.e., 1st−4th channels for FA, 5th−8th for AA, 9th−12th for PA, and 13th−16th for BA. To clearly demonstrate the asymmetric transformation results, we just selected four channels to show in Figure 3. Figure 3a illustrated the electrophoretogram of asymmetric transformation products of L-His in the presence of butyraldehyde with the four organic acids. Figure 3b showed the results in the presence of *n*-butyraldehyde.

The ee values were calculated from the integrated height of peaks in the electrophoretogram, as illustrated in Figure 3,

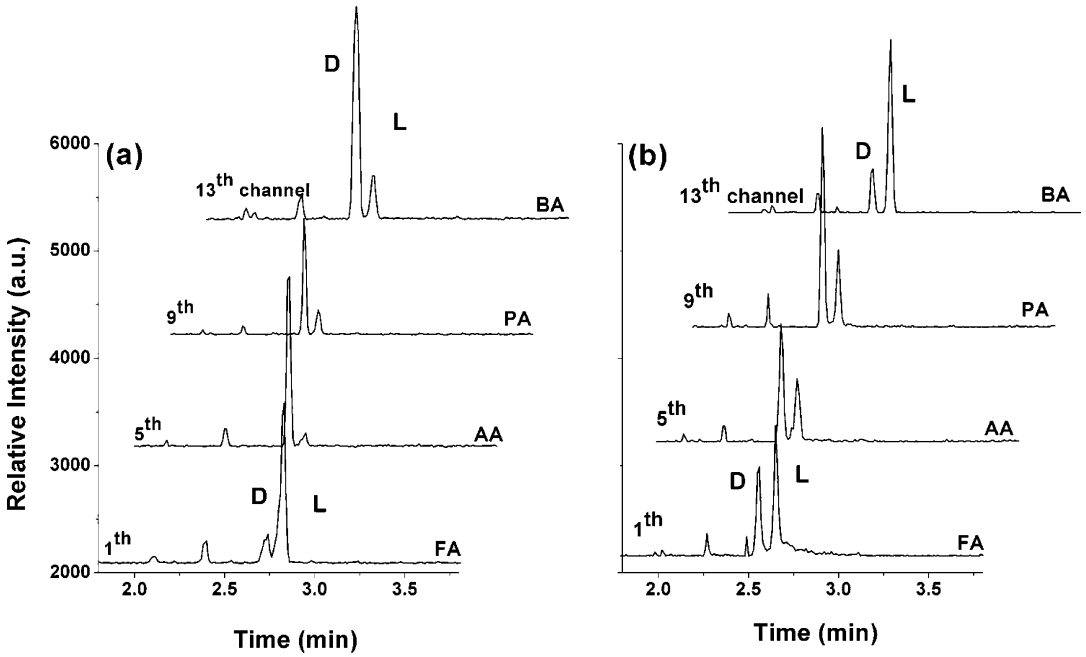

Figure 3. Organic acid solvent effects on asymmetric products of L-His in the presence of (a) salicylaldehyde and (b) *n*-butyraldehyde.

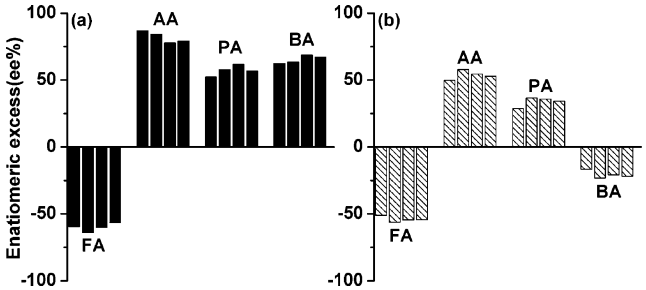

Figure 4. Organic acid effects on ee of asymmetric transformation of L-His with (a) salicylaldehyde and (b) *n*-butyraldehyde as catalyst.

Table 1. ee Values[a] (%) in Organic Acids with Salicylaldehyde and *n*-Butyraldehyde as Catalyst

acids	salicylaldehyde	*n*-butyraldehyde
FA (formic acid)	−60 ± 3	−54 ± 2
AA (acetic acid)	82 ± 4	54 ± 3
PA (propionic acid)	57 ± 3	34 ± 3
BA (butanoic acid)	65 ± 1	−21 ± 3

[a] Mean ± std dev ($N = 4$).

according to the following definition:

$$\text{ee}\% = (I_D - I_L)/(I_D + I_L) \times 100\%$$

where I_D and I_L were the integrated peak heights of D-His and L-His, respectively.

Figure 4a showed the ee values of asymmetric transformation of L-His in different organic acid solvents in the presence of salicylaldehyde. Figure 4b showed the results in the presence of *n*-butyraldehyde.

To estimate the reproducibility and systemic error of our CAE in the application of asymmetric transformation of L-His, we calculated the average ee and its standard deviation of each of the four capillaries with same conditions, as listed in Table 1. As one could find in the Table 1, AA with salicylaldehyde provided the highest enantiomeric excess, 82 ± 4%, for the asymmetric transformation reaction of L-His. This optimal condition for asymmetric transformation of L-His was also supported by Figure 1 and Figure 2, in which salicylaldehyde showed the best racemization results in different organic acids. The maximum standard deviation of these 16 capillaries, ±4%, was acceptable, considering the differences among each channel.

CONCLUSIONS

We applied a homemade high-throughput CAE system to optimize the reaction conditions of crystallization-induced asymmetric transformation of L-histidine to D-histidine and rapidly obtained the optimal reaction conditions, i.e., salicylaldehyde as the catalyst and acetic acid as solvent for the asymmetric transformation reaction. By using this setup, the operational step for determining enantiomeric excess was also reduced, compared with previous methods.[13,14] As shown in the present study, the sampling rate and sensitivity were all acceptable in achieving high throughput and high specificity with adequate sensitivity and without sampling distortion.[11,12] Our study indicated that the development of CAE for optimization of the asymmetric transformation reaction could be used in a truly high-throughput manner (16 ee determinations in less than 10 min). This high-throughput analysis of asymmetric transformation of L-histidine by CAE also demonstrated the capabilities of this method for quality assurance. Further development of our CAE system, up to 128 capillaries with higher electric field strength leading to the possibility of chiral separation within seconds, is in progress.

ACKNOWLEDGMENT

Funding support for the present studies was provided by the Chinese Academy of Sciences (KGCX2-201-3) and the National High Technology Research and Development Program of China (2002AA321020).

Received for review August 2, 2005. Accepted November 19, 2005.

AC051377W