Study on the Metabolic Mechanism of Chiral Inversion of *S*-Mandelic Acid *In vitro*

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ABSTRACT Mandelic acid (MA) is generally used as a biological indicator of occupational exposure to styrene, which is classified as a class of hazardous environmental pollutants. It was found to undergo one-directional chiral inversion (*S*-MA to *R*-MA) in Wistar and Sprague-Dawley rats *in vivo*. This study was aimed to explore the metabolic mechanism of chiral inversion of *S*-MA *in vitro*. *S*-MA was converted to *R*-MA in rat hepatocytes, whereas MA enantiomers remained unchanged in acidic and neutral phosphate buffers, HepG2 cells, and intestinal flora. In addition, the synthesized *S*-MA-CoA thioester was rapidly racemized and hydrolyzed to *R*-MA by rat liver homogenate and S9, cytosolic and mitochondrial fractions. The data suggest that chiral inversion of *S*-MA may involve the hydrolysis of *S*-MA-CoA, and its metabolic mechanism could be the same as that of 2-arylpropionic acid (2-APA) drugs. *Chirality 24:86–95, 2012.*

KEY WORDS: mandelic acid; chiral inversion; intestinal bacteria; chemical synthesis; ibuprofen

INTRODUCTION

Styrene is widely used in plastic industry and has been implicated as reproductive toxicant, neurotoxicant, or possible carcinogen.[1,2] Occupational and environmental exposure to styrene occurs predominately via inhalation.[3] Mandelic acid (MA, structure of its two enantiomers see Fig. 1) is one of the major urinary metabolites of styrene in both rodents and humans.[4,5] It can be further metabolized to phenylglyoxylic acid (PGA). Therefore, MA and PGA are generally used as biological indicators of occupational exposure to styrene.[6] Though MA was deemed low toxic in the past, a recent study indicated that it may contribute to the depletion of dopamine in rabbit and the adverse effects on the peripheral nervous system in rats.[7,8] In recent years, there has been an increased awareness of the effects of stereochemistry on drug metabolism and toxicity. Thus the investigation of mechanism of the chiral inversion of MA would be meaningful to evaluate toxicity and stereo-metabolism of styrene and also helpful to assess the hazard of styrene to organisms.

Besides monitoring workers exposed to styrene vapor, MA is also used as an intermediate for the synthesis of target molecules, such as urinary tract bactericide hexamine mandelate, peripheral vasodilator hacosan, eyedrops hydrobenzole, and spasmolytic agent. It has also been used effectively as a urinary antiseptic, particularly as a bladder irrigant during urological procedures.[9] Lately, MA has gained popularity as a topical skin care treatment for adult acne.

Early investigation on MA metabolism showed that it can partly dehydrogenate to PGA in human and dogs. In 1990s, besides stereoselectively metabolized to PGA, MA was also found could undergo one-directional chiral inversion (*S*-MA to *R*-MA) in Wistar rats by Drummond et al for the first time.[10] Our laboratory study also indicated that an obvious phenomenon of *S*-MA inverted to *R*-MA had occurred in SD rats after a single oral administration of 100 mg/kg *S*-MA.[5]

The first information regarding the *bi-directional* chiral inversion of MA enantiomers in some bacterias was published by Kenyon and Hegeman in 1970.[11] Later, it was found in some types of pseudomonas bacteria, a stereospecific particulate *S*-Mandelic acid dehydrogenase is responsible for the *S*-MA metabolism, whereas *R*-MA is metabolized only after its isomerization to *S*-MA by mandelate racemase.[12,13]

To date, the mechanism of one-directional chiral inversion of MA in mammals remains unknown. Our present study was undertaken to further characterize the potential metabolic mechanism of chiral inversion of *S*-MA by several *in vitro* models.

MATERIALS AND METHODS

Chemicals and Reagents

S-MA and *R*-MA (purity >99.5%) were kindly presented by Yiming Fine Chemicals Ltd. (Taixing, China). (*S*)-(−)-alpha-(1-naphthyl) ethylamine (*S*-NEA) was purchased from Sigma Chemical Company (St. Louis). 1-Hydroxy-benzotriazole (HOBT) and 1-(3-dimetthylaminopropyl)-3-ethylcarbodiimide HCl (EDC) were purchased from Acros Organics. (NJ). Phenylglyoxal was purchased from *Alfa Aesar*. 2, 6-dimethyl pyridine, magnesium acetate tetrahydrate, magnesium chloride, hydroquinone, calcium chloride, epsom salt, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride, dimethylformamide, and acetic ether were supplied by Sinopharm Chemical Reagent (Beijing, China). Tryptone, yeast extract, cysteine hydrochlorate were obtained from BBI (Canada). Coenzyme A was kindly presented by Biochemical Pharmaceutical. Jinan Weier

Abbreviations: 2-APA, 2-Arylpropionic acid; CoA, coenzyme A; DMF, dimethyl formamide; EDC, 1-(3-dimetthylaminopropyl)-3-ethylcarbodiimide; ESI, Electrospray ionization; HOBT, 1-Hydroxy-benzotriazole; HPLC, high-performance liquid chromatography; MA, mandelic acid; MeCN, methyl cyanide; PGA, phenylglyoxylic acid; *S*-NEA, (*S*)-(−)-alpha-(1-naphthyl) ethylamine.

Contract grant sponsor: National Program on Key Basic Research Project; Contract grant number: 2011CB710800.
Contract grant sponsor: Natural Science Foundation of China; Contract grant number: 81072691.

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Received for publication 21 August 2010; Accepted 10 August 2011
DOI: 10.1002/chir.21031
Published online 2 December 2011 in Wiley Online Library (wileyonlinelibrary.com).

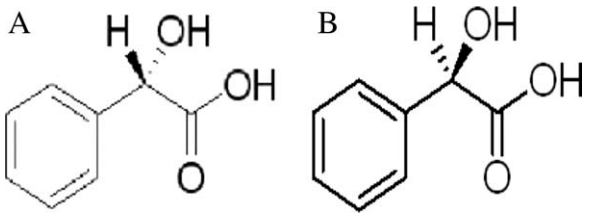

Fig. 1. Structure of *S*- and *R*-MA. (**A**) S-MA and (**B**) R-MA.

Kang. Deionized water was purified using a Milli-Q water system (Millipore, Bedford, MA). HPLC grade methanol and ethanol were obtained from Merck (Darmstadt, Germany), other chemicals used were of analytical grade.

Chiral Derivatization and HPLC Analysis

The enantiomers *R*- and *S*-MA were determined by HPLC using *S*-NEA as a chiral derivatization reagent. In brief, MA enantiomers were extracted with ethyl acetate from reaction mixture at acidic pH. After centrifugation at 10,000*g* for 5 min, the upper organic layer was removed

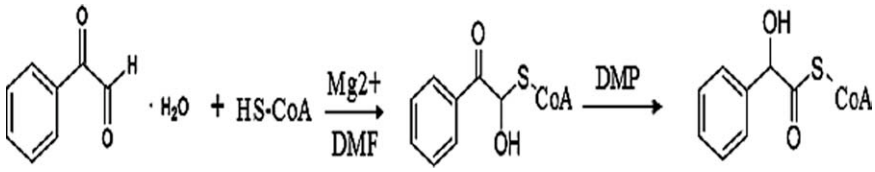

Fig. 2. The synthesis procedure from phenylglyoxal to *S*-MA-CoA thioester using $Mg(OAc)_2 \bullet 4H_2O$ and 2.6-dimethylpyridine as catalyst.

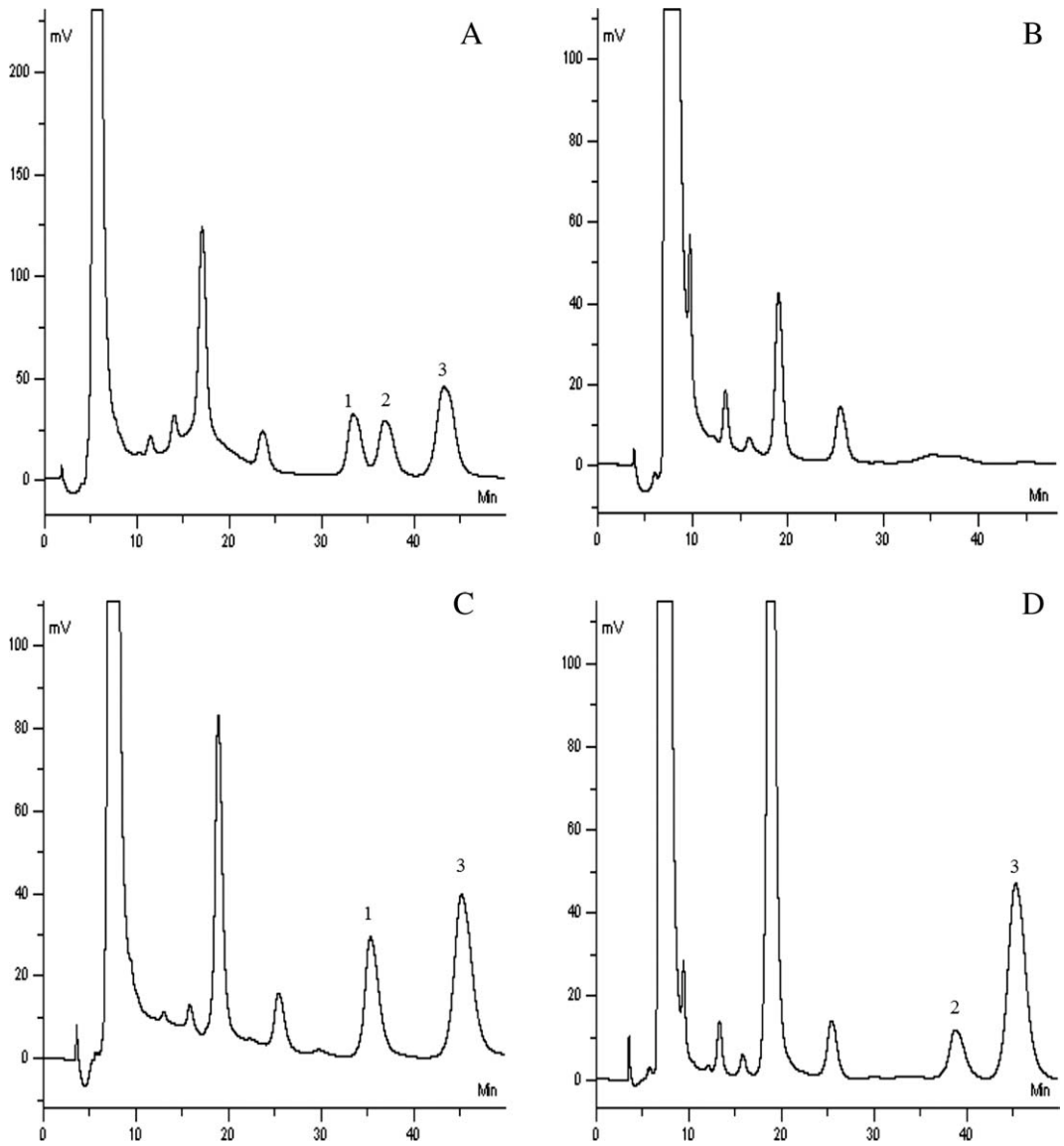

Fig. 3. Chromatograms of MA enantiomers coincubated with intestinal bacteria. (**A**) Blank intestinal bacteria incubate spiked with MA enantiomers and internal standard; (**B**) Blank intestinal bacteria incubate for 24 h; (**C**) *S*-MA coincubated with intestinal bacteria for 24 h; (**D**) *R*-MA coincubated with intestinal bacteria for 24 h. 1: *S*-MA-naphthylethylamide derivative; 2: *R*-MA-naphthylethylamide derivative; 3: I.S-naphthylethylamide derivative.

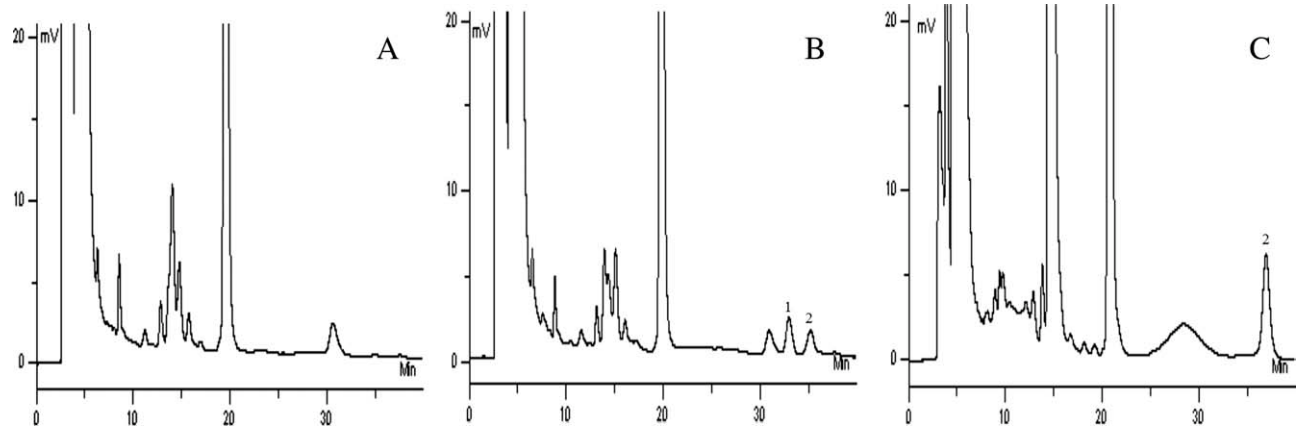

Fig. 4. Chromatograms of MA incubated with rat hepatocytes for 2 h. (**A**) blank, (**B**) *S*-MA incubate, (**C**) *R*-MA incubate. 1: *S*-MA-naphthylethylamide derivative; 2: *R*-MA-naphthylethylamide derivative.

and evaporated to dryness under a gentle stream of nitrogen and then derivatized with *S*-NEA. EDC and HOBT were used as coupling agents. The chiral derivatization was performed as described previously.[5]

Chromatography was performed on a ZORBAX SB-C_{18} column (250 × 4.6 mm^2, I.D., 5 μm, Agilent) coupled with a C_{18} guard column (20 × 4.6 mm^2, I. D., 5 μm). The mobile phase was composed of methanol-10 m*M* phosphate buffer (pH 2.5) [65:35, (v/v) at a flow-rate of 0.8 ml/min (flow-rate of 1.0 ml/min for S-MA-CoA thioester incubation]. Detection was set at UV wavelength of 254 nm. The column temperature was maintained at room temperature. The injected volume was 20 μl.

Nonenzyme Metabolism of MA Enantiomers In Vitro

MA enantiomers (20 μg/ml) were incubated with 0.1*M* phosphate buffer (pH 1.5 or 7.4) at 37°C in triplicate, respectively. After 0, 1, 2, 4, 8, 12, and 24 h of incubation, samples were treated and analyzed as Chiral derivatization and HPLC analysis. Phenylacetic acid was used as internal standard.

Incubation of MA Enantiomers with Intestinal Bacteria

Preparation of intestinal bacteria in vitro. Male Sprague-Dawley rats (150–180 g) obtained from Laboratory Animal Center of Zhejiang University (Hangzhou, China), were raised under laboratory condition to collect fresh feces. Two volumes of sterile physiological saline were added to the feces, filtered. Then the filtrate was added with nine volumes of anaerobic medium, cultivated overnight in anaerobic incubator.

The anaerobic medium was modified PY medium contained 20 mg/ml tryptone, 10 mg/ml yeast extract, 0.5 mg/ml cysteine hydrochlorate, 40 ml/l VPI salt solution, pH 7.2. The VPI salt solution was consisted of 0.2 mg/ml calcium chloride, 0.2 mg/ml epsom salt, 1 mg/ml dipotassium hydrogen phosphate, 1 mg/ml potassium dihydrogen phosphate, 10 mg/ml sodium bicarbonate, 2 mg/ml sodium chloride.

Incubation of MA enantiomers in SD rats intestinal bacteria. MA enantiomers (final concentration 50 μg/ml) were added to the overnight cultivated intestinal bacteria, misce bene, cultivated in anaerobic incubator for 0, 2, 4, 8, 12, and 24 h. Incubation in the absence of intestinal bacteria was used as negative control. Samples were treated and analyzed as Chiral derivatization and HPLC analysis. Phenylacetic acid was used as internal standard.

Chiral Inversion of MA Enantiomers in Hepatocyte

Rat hepatocytes were isolated by the two-step collagenase perfusion method with a minor modification. The isolated cells were resuspended in pH 7.4 Krebs-Henseleit buffer (containing 12.5 m*M* HEPES and 0.1% bovine serum albumin) and seeded in six-well plates (Costar Corning) at a density of 1×10^6 cells/well. After seeded for 4 h, the medium was discarded, and new culture medium containing 20 μg/ml MA enantiomers was added and incubated for 0, 0.5, 1, and 2 h to analyze the chiral inversion of MA.

Chemical Synthesis, Racemization and Hydrolysis of S-MAndelic Acid-CoA Thioester

Synthesis of S-MA-CoA thioester. *S*-MA-CoA thioester was synthesized in a flask by the method of Stan S. Hall[14] used for the synthesis of α-hydroxythiol esters, with some modifications (Fig. 2). The method involved the following:

To a solution of 0.38 g (0.5 m*M*) of phenylglyoxal and 0.03 g (0.25 m*M*) of $Mg(OAc)_2$ $4H_2O$ in 3 ml of DMF that had stirred for 10 min, a solution of 0.38 g (1 m*M*) of CoA and 0.009 g (0.016 m*M*) of hydroquinone in 6 ml of DMF was added. After reaction for 2 h at 25°C, 0.9 mg (0.25 m*M*) of 2, 6-dimethylpyridine was added to the reaction mixture. After an additional 4 h of stirring, some cold water was added into the reaction mixture, then extracted with acetic ether for three times.

Purification and identification of S-MA-CoA thioester. The reaction mixture was extracted with 3 × 10 ml ethyl acetate. Then the ethyl acetate layer was concentrated by vacuum centrifugal concentrator. *S*-MA-CoA thioester was purified from the mixture by preparative HPLC. It was purified on a semi-preparative column Hypersil ODS2 (5 μm, 10 × 250 mm^2) column with a flow of 3.0 ml/min using a gradient elution (100% A 20 min, 20.01–35.00 min 0–50% MeCN). The mobile phase A was composed of H_2O:MeCN:1*M* ammonium acetate 95:5:1 (v:v:v). Frac-

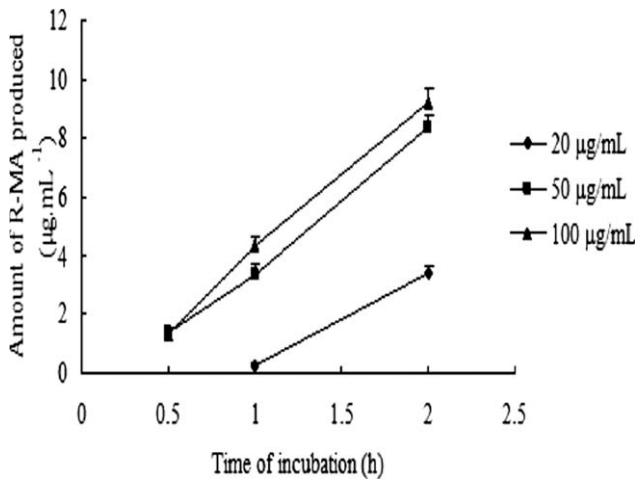

Fig. 5. Time profile of *R*-MA produced by different concentrations of *S*-MA incubated in hepatocytes suspension.

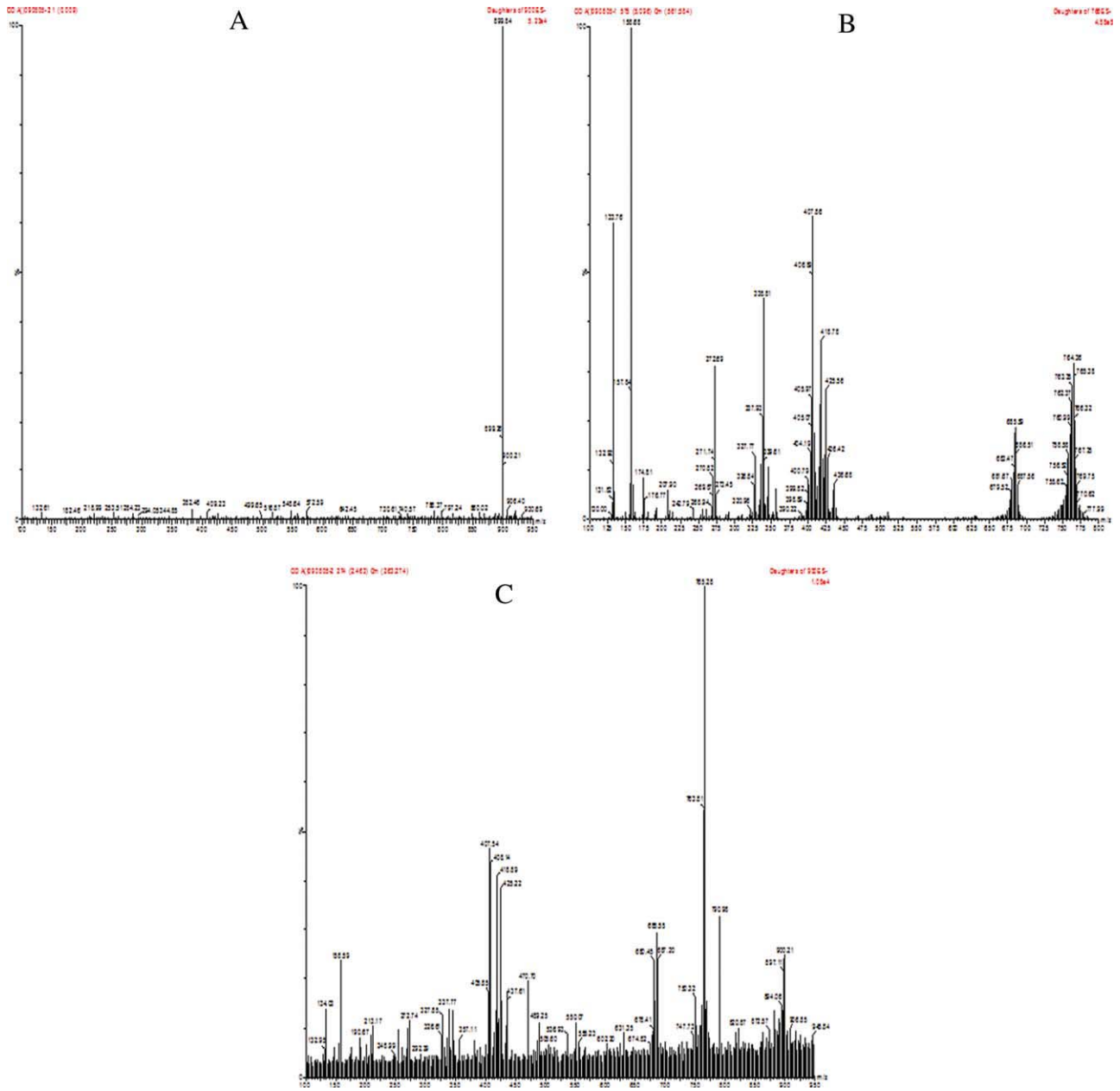

Fig. 6. Mass spectra of MA-CoA thioester and CoA. (**A**) The fragmentation of synthesized MA-CoA thioester; (**B**) Daughters of CoA; (**C**) Daughters of MA-CoA thioester. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

tions containing *S*-MA-CoA were collected, pooled, lyophilized to dryness, and stored at −20°C until use.

The identity of the synthesized and purified *S*-MA-CoA thioester was determined by LC-MS and LC-MS/MS with ESI using the infusion mode. The mass spectrometer (UPLC-TQD-MS, Waters Company) was operated in negative ion MS scan mode, with the following settings: dwell time, 0.5 s; source temperature, 100°C; desolvent gas temperature, 300°C; Capillary voltage, 3600 V. The collision gas was argon gas; collision energy was 20 eV. Masslynx V4.1 (Waters) was used for instrument control, data acquisition and data processing.

Preparation of rat liver homogenate, S9, cytosolic and mitochondria fractions. Male Sprague-Dawley rats obtained from Laboratory Animal Center of Zhejiang University (Hangzhou, China), weighing 180 ± 10 g, were fasted overnight before treatment. Tissue processing was performed at 4°C. Briefly, freshly obtained rat liver was cut into small pieces and washed with physiological saline. The samples were weighted and homogenized in four volumes of 20 m*M* Tris-HCl buffer (pH 7.4), containing 0.25 *M* sucrose. The homogenate was centrifuged for 15 min at 2000*g* and the supernatant, designed as the whole homogenate fraction. The S9, mitochondrial, cytosolic, and microsomal fractions were prepared by successive centrifugation at 9000*g* for 20 min, 19,000*g* for 20 min, and then at 105,000*g* for 60 min. The mitochondrial and microsomal fractions were washed twice with the same buffer. Protein concentration was determined using BCA Protein Assay Reagent with bovine serum albumin as the standard.

***In vitro* incubation of S-MA-CoA thioester with rat liver homogenate and subcellular fractions.** The reaction mixture was composed of 50 m*M* Tris-HCl buffer (pH 7.4, containing 150 m*M* KCl and 15 m*M* $MgCl_2$), *S*-MA-CoA thioester and either rat liver homogenate, S9, cytosolic fractions (final concentration of 5 mg/ml) or mitochondrial fraction (final concentration of 4 mg/ml). These samples were all placed in a shaking water bath maintained at 37°C. All incubates were monitored for

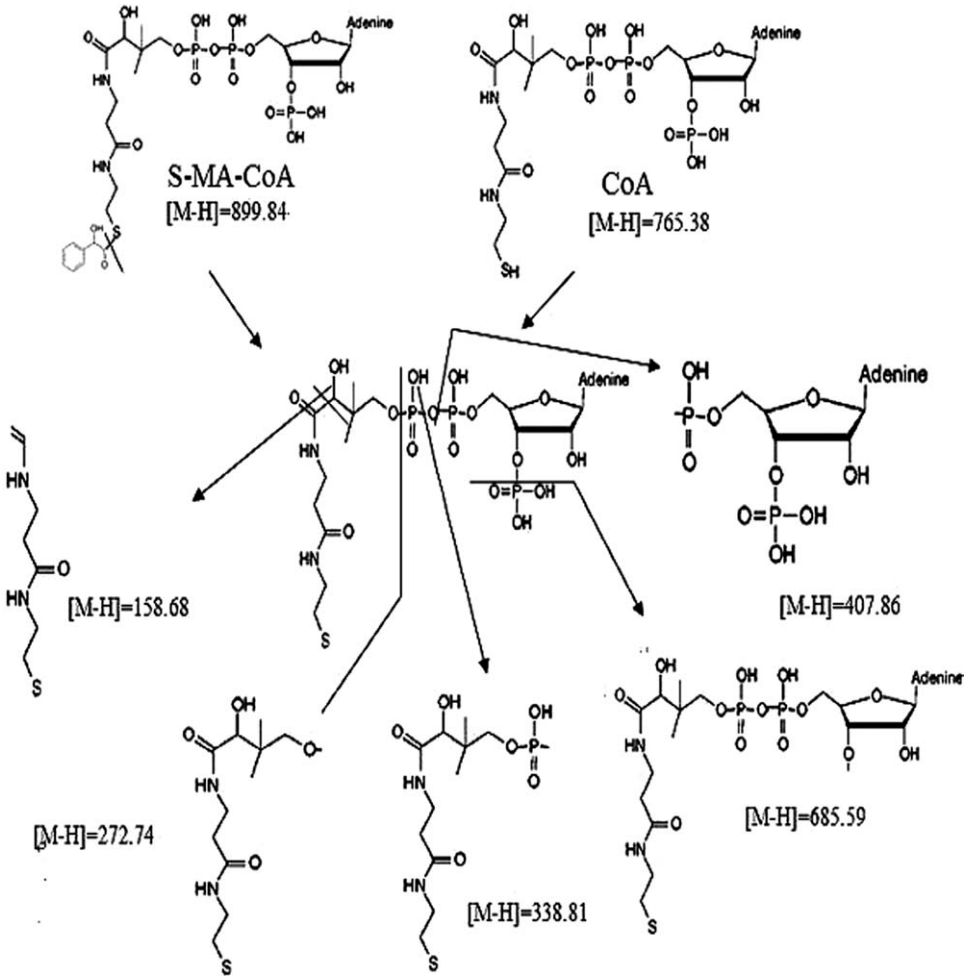

Fig. 7. Schizolysis spectrum of MA-CoA thioester and CoA.

2 h, acidified with 20 μl of 5*M* HCl, and treated as Chiral derivatization and HPLC analysis to assay for *S*- and *R*-MA.

In addition, the time-response curve and concentration-response curve of *S*-MA-CoA thioester in rat liver homogenate and subcellular fractions were also investigated. The incubation mixtures were the same with above. To perform the tests for time-response curve, the incubation mixtures were incubated for different times, 5, 15, 30, 45, and 60 min. To perform the tests for concentration-response curve, the final enzyme concentrations were set at 1, 2, 5, and 10 mg/ml (mitochondrial was 1, 2, and 4 mg/ml) and the incubation time was set for 45 min. Samples were treated as described in the previous section. Chiral derivatization and HPLC analysis to determine the *R*-MA.

RESULTS

Nonenzyme Metabolism of MA Enantiomers In Vitro

Chiral inversion did not occur between MA enantiomers after 24 h incubation in pH 7.4 and 1.5 phosphate buffers. It indicated that MA configuration is stable and *S*-MA and *R*-MA cannot be inverted to each other in gastro-intestine without enzymes.

Chiral Inversion of MA Enantiomers in Intestinal Bacteria

Chiral inversion of *S*-MA to *R*-MA was not observed after incubated for 24 h with SD rats intestinal bacteria *in vitro* (Fig. 3).

Chiral Inversion of MA Enantiomers in SD Rat Hepatocyte

The *in vitro* results showed that chiral inversion of *S*-MA to *R*-MA was observed in rat hepatocyte (Fig. 4). The concentrations of *R*-MA inverted from *S*-MA at different times were shown in Figure 5.

Chemical Synthesis, Racemization and Hydrolysis of S-MAndelic Acid-CoA Thioester

Synthesis and identification of S-MA-CoA thioester. The synthesized and purified *S*-MA-CoA thioester was lyophilized to a yellow powder solid. It was identified to have the parent ion fragmentation of m/z 900 ([MA-CoA-H]$^-$) with MS (Fig. 6A). Among all the individual daughter ions of parent ion fragmentation m/z 900 obtained by MS/MS, we could see that except the fragmentation of m/z 766 ([CoA-H]$^-$), the other daughter ions m/z 686, 408, 338, 159, and 134 were the same with that of CoA. In other words, the spectra obtained were in accordance with the fragmentation patterns of CoA (Fig. 6B, 6C, and 7). So it was confirmed that the synthesized and purified compound was the target molecule.

Incubation of S-MA-CoA thioester with rat liver homogenate and subcellular fractions. *S*-MA-CoA thioester was rapidly hydrolyzed by rat liver homogenate with formation of *R*-MA (Fig. 8). The same phenomenon was also observed in rat S9, cytosolic, and mitochondrial fractions (Fig. 9). It indicated the

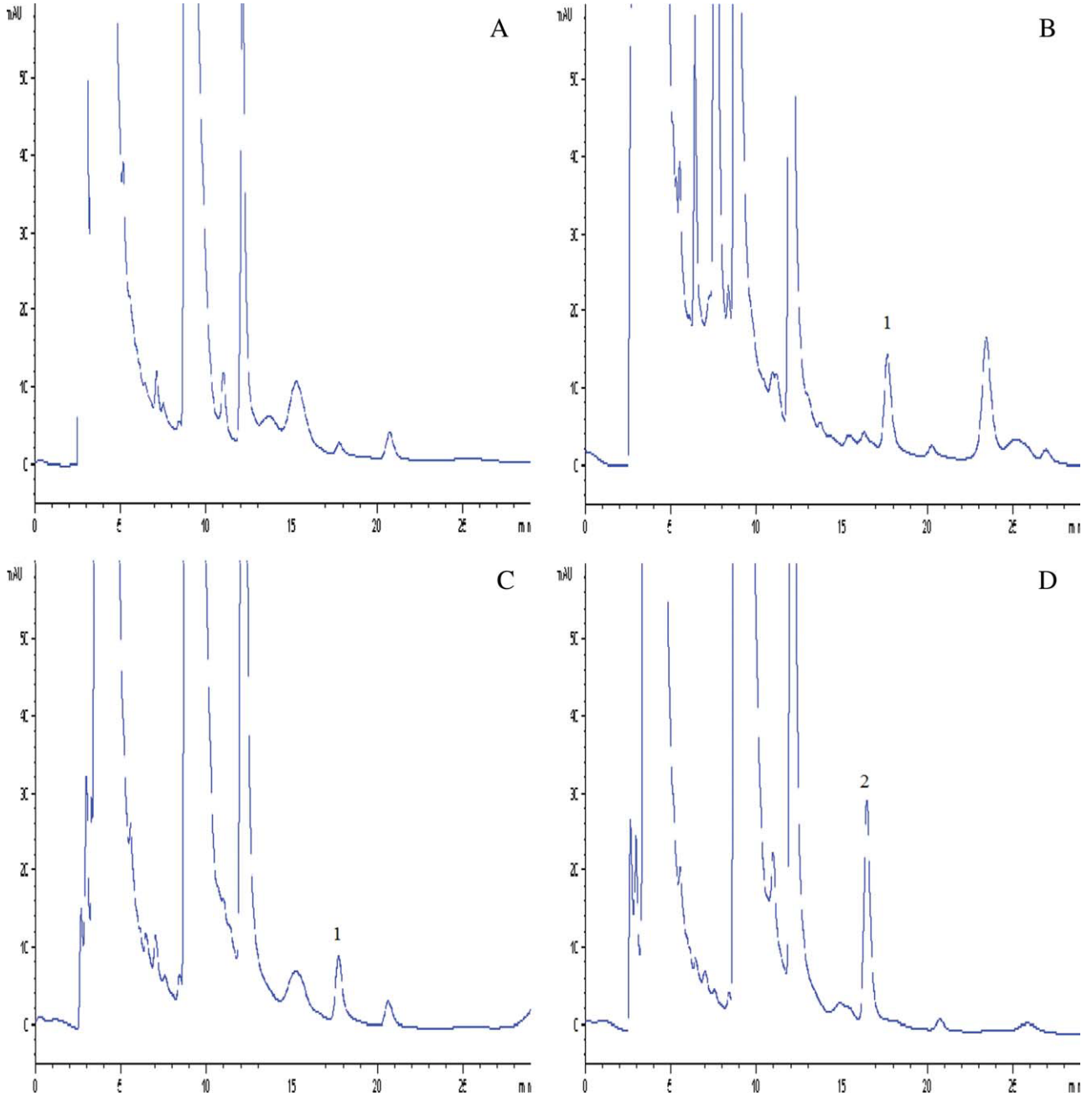

Fig. 8. Chromatograms of *S*-MA-CoA thioester incubated with SD rats liver homogenate after derivatization. (**A**) 0 h incubation; (**B**) *S*-MA-CoA incubated for 2 h; (**C**) *R*-MA standard; (**D**) *S*-MA standard. 1: *R*-MA; 2: *S*-MA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

racemization of *S*-MA-CoA thioester to *R*-MA-CoA thioester and subsequent release of *R*-MA, which suggests that one of the mechanisms of chiral inversion of MA is probably similar with that of 2-arypropionic acids.

The Time-Response Curve and Concentration-Response Curve of S-MA-CoA Thioester in Rat Liver Homogenate and Subcellular Fractions

The time-response curve and concentration-response curve for rat liver homogenate and subcellular fractions were shown in Figure 10. For the time-response curve, one could see that the reaction rate was almost linear until 45 min. For the concentration-response curve, the metabolic rate increased as a function of enzyme concentrations. From 1 to 5 mg/mL protein concentration, the curve had better linearity.

DISCUSSION

Up to now, the mechanism of one-directional chiral inversion of MA in mammal has never been able to explain, and the corresponding report is rather rare. Generally speaking, the change of chiral drug configuration may be mainly caused by three mechanisms.

One is nonenzymatic mechanism, the chiral drug is unstable and will undergo isomerization in the certain environment (acidic or basic or highlight). For instance, stiripentol in the acidic environment and thalidomide in aqueous media

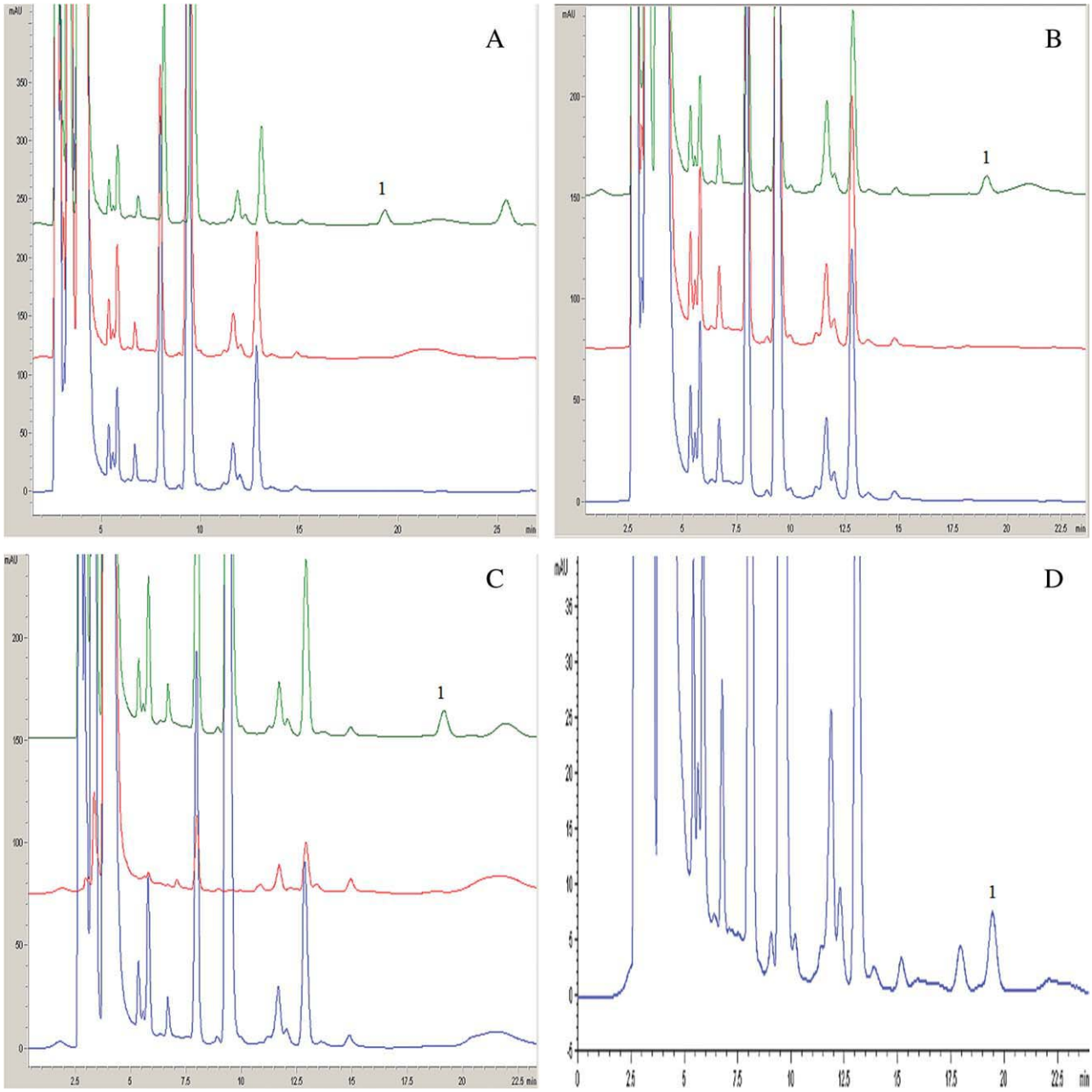

Fig. 9. Chromatograms of *S*-MA-CoA thioester incubated with SD rat liver S9, cytosol protein and mitochondria protein after derivatization. (**A**) S9; (**B**) cytosol protein; (**C**) mitochondria protein; (**D**) R-MA Standard 1: *R*-MA-naphthylethylamide derivative. The three curves from top to the bottom respectively mean chromatogram after 2 h incubation, chromatogram before incubation and chromatogram of blank system. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

can undergo chemical racemization.[15,16] In this study, we simulated the physiological environment of stomach and intestine to investigate the chiral inversion of MA. The result showed MA enantiomers were stable under the environment of pH 1.5 and 7.4, which confirmed the idea of that the chiral inversion of MA was catalyzed by the enzymes in SD rat.

The second is bacteria mechanism. Extensive metabolic inversion of MA mediated by mandelate racemase was observed in bacteria.[12,13] Intestinal bacteria are also able to contribute to inversion of one enantiomer to its antipode in the organism. The potential contribution of intestinal bacteria has been demonstrated to chiral inversion of flosequinan.[17] Chiral inversion at sulfoxide position of flosequinan enantiomers occurred in conventional rats but not in either germ-free rats or rats treated with antibiotics. Several strains of intestinal bacteria stereoselectively reduced flosequinan to give sulfoxide, followed by oxidation of the sulfoxide in the body to produce the antipode of flosequinan. Here according to our research, rat intestinal bacteria have nothing to do with the chiral inversion of MA.

The last is enzymatic mechanism, which based on two opposite metabolic processes or the reversible metabolic pathway and result in chiral inversion. According to some literatures, MA was thought to be stereoselectively metabolized by rat alcohol dehydrogenase.[18] Hence, we hypothesized that the chiral inversion of MA may mediate via oxido-reduc-

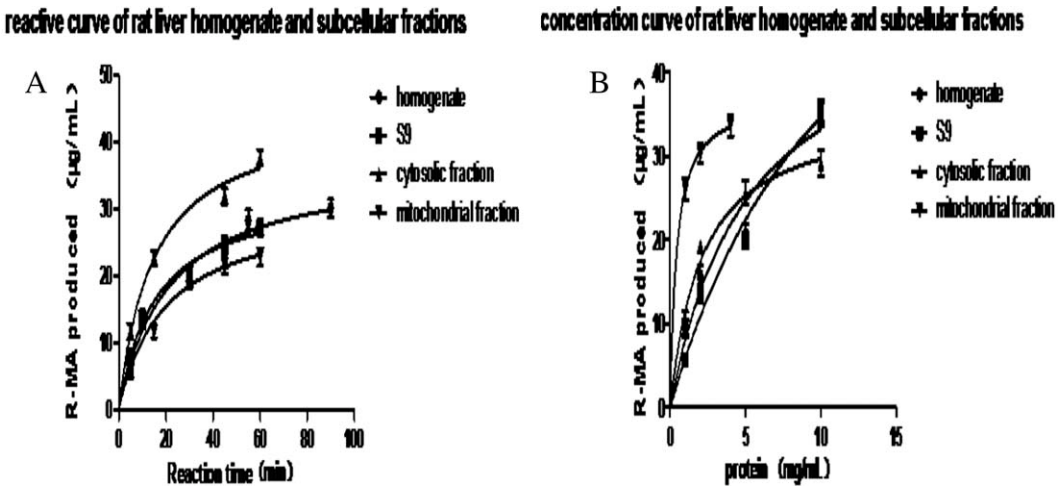

Fig. 10. The reactive curve (**A**) and concentration curve (**B**) of *S*-MA-CoA thioester metabolism by rat liver homogenate and subcellular fractions ($n = 3$).

tion of keto-alcohol. We expressed the recombinant proteins of rat alcohol dehydrogenase 1 and aldo-keto reductase 1A1 by pET *Escherichia coli* system and these recombinant proteins were incubated with MA or PGA to investigate whether the chiral inversion was mediated by alcohol dehydrogenase and aldo-keto reductase.[19] The result suggested that chiral inversion of *S*-MA in rat may not be mediated through reactions of oxidoreduction by alcohol dehydrogenase 1 and aldo-keto reductase 1A1. Whether the other isoforms of alcohol dehydrogenase and aldo-keto reductase were involved in this process needs much more in-depth investigation.

In parallel with the previous result of our laboratory,[20] we also found *S*-MA could be biotransformed to *R*-MA in SD rat hepatocytes, which showed rat liver is the right organ where the chiral inversion takes place. It has been reported that ketoprofen could undergo chiral inversion in rat hepatocytes.[21]

To date, 2-Arylpropionic acid derivatives are probably the most frequently cited drugs exhibiting the phenomenon that is best known as chiral inversion. Ibuprofen is the most extensively studied example of unidirectional chiral inversion in different animal species and man.[22,23] The molecular mechanism of chiral inversion of profens involves three steps : at first, thioesterification of *R*-enantiomer to *R*-ibuprofen CoA via an adenosine monophosphate intermediate catalyzed by the microsomal long-chain acyl-CoA synthetase; second, enzymatic epimerization of *R*-ibuprofenoyl-CoA thioester catalyzed by the cytosolic and mitochondrial 2-aryl-CoA-epimerase to *S*-ibuprofenoyl-CoA via an enolate intermediate; at last, nonstereoselective hydrolysis of the acyl-CoA thioesters by one or more hydrolases to yield free *S*-ibuprofen.[24]

The chiral structure of MA is similar with 2-Arylpropionic acid drugs. The difference is that in MA α-methyl group is changed to hydroxyl group. The polarity of hydroxyl group is much higher than that of methyl group so the chemical properties between the two drugs should have some diversity. In this article, we also investigated the chemical synthesis, racemization and hydrolysis of *S*-MA-CoA thioester to clarification whether the mechanism of the chiral inversion of MA is similar with ibuprofen.

The direct synthesis of *S*-MA-CoA thioester was tried using several procedures according to literatures about 2-APA-CoA thioester synthesis. At first, we tried Ulrik Sidenius method,[25] 1,1′-Carbonyldiimidazole was used to react with the carboxylic acid at room temperature in a nitrogen atmosphere, then to the reaction mixture was added CoA to

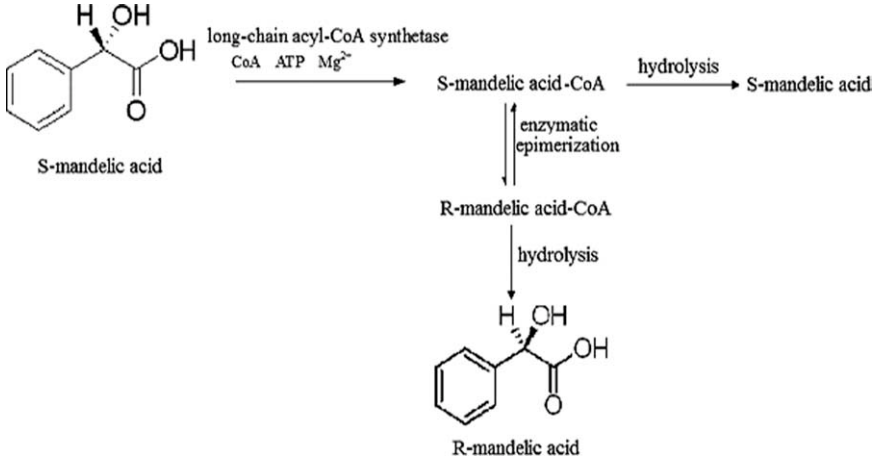

Fig. 11. The probable mechanism of chiral inversion of *S*-MA according to our investigation.

form the acyl-CoA. The acyl-CoA thioesters of eight carboxylic acids [ibuprofen, clofibric acid, indomethacin, fenbufen, tolmetin, salicylic acid, 2-phenoxypropionic acid and (4-chloro-2-methyl-phenoxy) acetic acid (MCPA)] were successfully synthesized by this method, but it did not work for *S*-MA-CoA thioester synthesis.

Then we tried Carabaza et al.'s method[26]: oxalyl chloride was added to a solution of MA in methylene chloride, the solution was stirred for 1 h under an argon atmosphere and then evaporated to an oily residue with a stream of argon. The residue was added dropwise into a stirred solution of sodium Coenzyme A in H_2O adjusted to pH 8.9 with 0.5*M* NaOH. Martín et al.'s method[27] was performed also: trifluoroacetic acid, anhydride, and thioglycollic acid were used to react with ibuprofen formed ibuprofen-thioglycollate and finally react with CoA to form ibuprofen-CoA. Unfortunately, none of them was successful for MA-CoA thioester synthesis.

In the end, we decided to try the Hall's method[14] to synthesis *S*-MA-CoA thioester indirectly. A series of α-hydroxythiol esters were successfully prepared by this method. First, equilibration of the glyoxal with the thiol was carried out in the presence of Mg^{2+} (0.5 equiv) in DMF solvent. The resulting α-ketohemithiol acetal, which is presumably chelated to the Mg^{2+}, is subsequently converted to the corresponding α-hydroxythiol ester by the addition of 2,6-dimethylpyridine. Increased isolated yields were realized by the presence of trace amounts of hydroquinone that retarded oxidation of the thiols to dimmers and by the use of the sterically hindered base 2,6-dimethylpyridine, rather than pyridine or triethylamine, which catalyzes the hydrolysis of the α-hydroxythiol esters during work-up.

S-MA-CoA thioester was racemized and hydrolyzed to *R*-MA in rat liver homogenate. Thus it gives us a hint that one of the mechanisms of MA chiral inversion might be similar with that of 2-Arylpropionic acid drugs. Thioesterification of S-MA to *S*-MA-CoA thioester was taken place in priority, and then isomerized to *R*-MA-CoA thioester via an enolate intermediatetic, at last nonstereoselective hydrolyzed of the *R*-MA-CoA thioesters to yield free *R*-MA (Fig. 11).[22,23,27,28]

S-MA-CoA thioester was also racemized and hydrolyzed to *R*-MA in rat S9, cytosolic and mitochondrial fractions. It is paralleled with the report of that *R*-ibuprofen-CoA thioester hydrolyzed to *S*-ibuprofen in rat microsome and mitochondrial fractions as same as in liver homogenate.[29] Either of the experimental result demonstrates the chiral inversion of MA could happen in rat liver homogenate, mitochondrial fractions and so on. There is no location restriction for the chiral inversion. According to literature, there are a number of hydrolases which could possibly catalyze the hydrolysis reaction of thioesters. Therefore, whether the hydrolases catalyze the hydrolysis reaction of *S*-MA-CoA thioester and *R*-ibuprofen-CoA thioester are similar still remains unknown and needs more investigation.

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