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Chiral separation of fluorescamine-labeled amino acids using microfabricated capillary electrophoresis devices for extraterrestrial exploration

Alison M. Skelley, Richard A. Mathies*

Department of Chemistry, University of California, 307 Lewis Hall, Berkeley, CA 94720, USA

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Abstract

Chiral separations of fluorescamine-labeled amino acids are characterized and optimized on a microfabricated capillary electrophoresis (CE) device. A standard mixture of acidic and neutral amino acids is labeled with fluorescamine in less than 5 min and the hydroxypropyl-β-cyclodextrin (HPβCD) concentration, temperature, and pH are optimized (15 mM HPβCD, 6 °C, pH < 9) to achieve high-quality and low background chiral separations in less than 200 s. All four stereoisomers formed in the labeling reaction of the chiral dye with the chiral amino acids are typically resolved. At pH > 9, isomerization of the dye chiral center is observed that occurs on the time scale of the chip separation. Typical limits of detection are ∼50 nM. These results demonstrate the feasibility of combining fluorescamine labeling of amino acids with microfabricated CE devices to develop low-volume, high-sensitivity apparatus and methods for extraterrestrial exploration.

Keywords: Enantiomer separation; Extraterrestrial life detection; Mars exploration; Chip technology; Derivatization, electrophoresis; Amino acids; Fluorescamine

1. Introduction

Recent observations suggesting the presence of water on Mars [1–3] together with previous analyses of the Mars meteorite ALH84001 have rekindled interest in questions of life on Mars [4,5]. To take the next step in the search for extraterrestrial life, a suitable molecular biomarker must be identified and direct in situ measurements performed. Initial Lunar exobiological studies targeted elements necessary for biological molecules such as carbon and nitrogen [6]. The observation of macromolecular biomolecules such as DNA and proteins is unlikely due to degradation in the harsh Martian environment. Amino acids, on the other hand, are ideal biomarkers because biomacromolecules are expected to be made up of homochiral amino acids [5], and amino acids racemize very slowly under conditions found on Mars [7]. Amino acid analysis is traditionally performed using HPLC [8], GC/GC–MS [9,10], or more recently capillary electrophoresis–electrospray mass spectrometry [11] and electrospray mass spectrometry [12], but these analytical methods do not provide the low instrument mass and analysis volume characteristics desired for current in situ space exploration.

Microfabricated lab-on-a-chip analysis systems have advanced rapidly over the past decade and would appear to be ideal for in situ exploration [13]. Microfabricated capillary electrophoresis (CE) devices were first used to perform fast and efficient amino acid composition analyses in 1993 [14]. In 1999, we extended this work to the chiral analysis of amino acids by using fluorescein isothiocyanate (FITC) as the labeling reagent and γ-cyclodextrin to provide chiral resolution [15]. Amino acid extracts from the Murchison meteorite were labeled and analyzed in a few minutes, and the enantiomeric ratios were comparable to, or better than, HPLC and GC–MS results. However, the labeling reaction with FITC was found to be slow (8–12 h), and unreacted FITC resulted in large background peaks.

The use of a fluorogenic dye with better reaction kinetics would reduce the overall amino acid analysis time and simplify the electropherogram. Possibilities for alternative fluorogenic dyes include *o*-phthaldialdehyde (OPA), naphthalene-2,3-dicarboxaldehyde

* Corresponding author. Tel.: +1-5106424192; fax: +1-5106423599.
E-mail address: rich@zinc.cchem.berkeley.edu (R.A. Mathies).

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(NDA), and fluorescamine. All three dyes react quickly with primary amines to form fluorescent products [16–19]. The OPA reaction is performed in a borate buffer with β-mercaptoethanol [17] and the product is excited maximally at 350 nm. Labeling with NDA requires KCN and gradual addition of the NDA to prevent precipitation [19]; the product is excited maximally at 445 nm. Fluorescamine reacts in seconds with amino acids in a borate buffer [20], and unreacted dye is hydrolyzed in water to yield a non-fluorescent product [21]. Fluorescamine is also advantageous because the excitation maximum of its amino acid derivatives (390 nm) overlaps well with the output of compact blue laser diodes (405 ± 10 nm). Fluorescamine has been used extensively for protein quantitation [22], for compositional analysis of amino acids [23], and for analysis of neurotransmitters [24] and a variety of other primary amine-containing molecules. The observation that sublimation of amino acids onto a cold finger coated with fluorescamine results in the rapid formation of a fluorescent amino acid adduct [25] provided further encouragement to explore this reagent. The chiral separation of fluorescamine-labeled amino acids was recently reported using HPLC [26] suggesting that it would be feasible to perform amino acid composition and chirality analyses using microchip CE.

We present here chiral separations of fluorescamine-labeled amino acids on microfabricated CE chips as well as the optimization of the device and method for in situ amino acid analysis. A novel pH-dependent isomerization of the optically active center in the dye–amino acid adduct during the separation is observed and characterized. Finally, the optimized fluorescamine separations are compared with separations using FITC demonstrating that fluorescamine is a viable labeling reagent for in situ amino acid analyses.

2. Experimental

2.1. Microfabrication

Microchip devices were prepared as previously described [15,27]. Briefly, 10 cm diameter borofloat wafers (Precision Glass and Optics, Santa Ana, CA) protected with a sacrificial layer of amorphous Si were coated with photoresist (Shipley 1818, Marlborough, MA), patterned through a chrome mask using a contact aligner (Quintel Corp., San Jose, CA), and developed (Microposit developer, Shipley). The exposed Si was removed with SF_6 plasma and the wafers were etched in 49% HF for 3 min. The mask design used [15] yields four folded separation channels 21.4 cm in length and two straight channels 7.4 cm in length. All channels were etched 21 μm deep throughout. Two of the folded channels are 152 μm wide with 47 μm wide tapered turns and two are 72 μm wide with untapered turns. All channels utilize a 0.8 cm long, 72 μm wide injection channel located 0.4 cm from the anode well. Reservoirs were drilled with 1.8 mm diameter diamond-tipped drill bits (Abrasive Technology, Lewis Center, OH), and then the etched chip was thermally bonded to a blank wafer. A 6 mm thick PDMS elastomer gasket (Corning, Sylgard 184) with 3 mm diameter holes was applied over the drilled holes to increase reservoir volume.

2.2. Sample preparation and labeling

Amino acid standards were prepared from individual 1 mM stock solutions of each amino acid enantiomer in water (Sigma-Aldrich, St. Louis, MO). Equimolar amounts of glycine, aminoisobutyric acid (AIB), and the enantiomers of valine, alanine, serine, glutamic acid, and aspartic acid were combined to make the Mars7 Standard, resulting in a total amino acid concentration of 1 mM and an individual amino acid concentration of 83 μM. The amino acids were labeled by combining 10 μl of the amino acid solution with 40 μl of a 50 mM borate buffer (pH 9.5), 10 μl of water and 50 μl of a 1.8 mM fluorescamine solution in acetone. The concentrations used are similar to those previously published for small volume derivatizations [24,28]. The solution was left to react at room temperature for approximately 5 min before the acetone was removed by reduced pressure. Amino acid samples labeled with FITC were prepared as before [15] by combining 90 μl of amino acid standard in 10 mM Na_2CO_3 (pH 10) with 10 μl of 1 mM FITC in acetone/1% pyridine. The solutions were left to react overnight. The fluorescamine-labeled solutions were diluted 1/5 (Mars7 Standard) or 1/20 (for individual amino acids) with the running buffer (consisting of 8–20 mM Na_2CO_3 pH as indicated) before running, while the FITC solutions were diluted 1/25 to 1/50 with 8 mM Na_2CO_3, pH 8.8 before running.

2.3. Separation and detection procedures

Chiral resolution of amino acids was achieved by adding a chiral cyclodextrin in the running buffer. The chiral separation of fluorescamine-labeled amino acids was first investigated by comparing the effects of α-, β-, and γ-cyclodextrin on the resolution of the amino acid enantiomers. β-Cyclodextrin was selected for further optimization because of its ability to resolve all the enantiomers present in the standard. However, β-cyclodextrin has relatively low solubility at low temperatures, so a derivatized β-cyclodextrin, HPβCD was used to maintain the resolving capabilities while improving solubility. HPβCD (Sigma-Aldrich) was dissolved in water, aliquotted into eppendorf tubes, dried down under vacuum and stored at −4 °C. The chiral separation buffer was prepared by adding the appropriate amount of running buffer to the dried HPβCD. The separation channel was filled by placing 20 μl of the buffer in the anode, sample and waste wells, applying a vacuum at the cathode to draw buffer down the channel, and then placing 20 μl of buffer in the cathode reservoir. Buffer in the

sample reservoir was then removed and replaced with sample.

Excitation and fluorescence detection was performed using a confocal fluorescence microscope [15]. The excitation source was a 404 nm blue diode laser (Power Technology, Inc., Little Rock, AR). The excitation beam was reflected off a beamsplitter (50% T at 425 nm, Chroma Technology Corp., Brattleboro, VT) and then focused on the channel using a 32× 0.4 NA objective. Fluorescence gathered by the objective was passed through a long-pass glass filter (50% T at 455 nm, Coherent, Santa Clara, CA) to the PMT (R943-02, Hamamatsu, Bridgewater, NJ) for photon counting detection. The stretched pulses were integrated and low-pass filtered using 10× gain and 10 Hz filter settings on an SRS 640 (Stanford Research Systems, Inc., Sunnyvale, CA) followed by analog-to-digital conversion. FITC excitation and fluorescence collection was performed as described previously [12].

Sample was injected using a floating injection scheme. High voltage (−2500 V) was applied at the waste reservoir, the sample and cathode reservoirs were grounded, and the anode was floated during the 10 s injection, forcing the majority of current and solution to flow from the sample well into the waste well. During the run stage, −15 000 V was applied at the cathode, resulting in a separation field of 700 V/cm, the anode was grounded and back biasing was performed by applying −2600 V at the sample and waste wells. Voltages from the in-house built power supply were computer controlled by the analog output of the DAQ card; a high-voltage solid-state relay was used to float the anode during injection. Before and between runs the channel was rinsed with 0.1 M NaOH for ∼10 min followed by water. Temperature control was achieved by using a cold plate with a circulating water/antifreeze solution [15] that cooled the chip in ∼1 min. Water condensation over the detection area was avoided by applying a thin layer of mineral oil to the glass surface.

Grams 32 (Thermo Galactic, Salem, NH) was used to calculate retention times, peak widths, peak areas, efficiencies and resolutions. The pH-dependent diastereomerization process [29,30] was analyzed using the approximation functions developed by Trapp and Schurig to calculate interconversion rates from the retention time (t_R), peak and plateau heights ($h_{A,B,plateau}$), and peak width at half-height ($w_{A,B}$) parameters [31,32]. These formulas, developed for enantiomerizations, are relevant for this diastereomerization as only two species are interconverting in a 1:1 ratio.

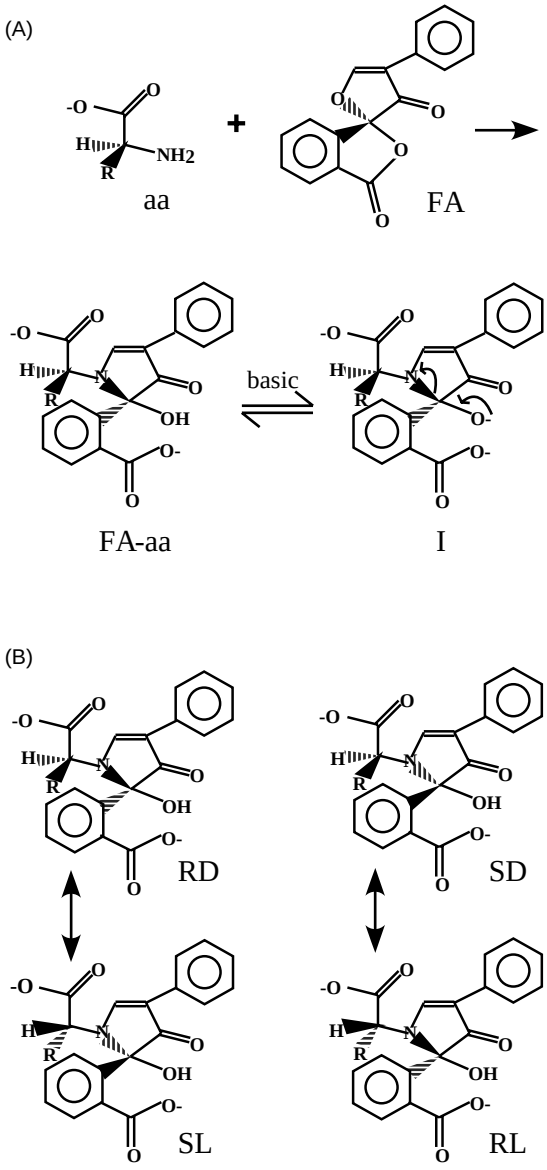

Fig. 1. Labeling of amino acids (aa) with fluorescamine (FA). (A) Fluorescamine reacts with the amino acids by addition of the amine nitrogen to the double bond [21]. Rearrangement yields the final product (FA-aa). At high pH, racemization of the dye chiral center occurs presumably via deprotonation of the dye alcohol to form the intermediate (I) followed by opening of the dye chiral center. (B) Structures of the four fluorescent stereoisomers formed by the fluorescamine reaction. Within the four stereoisomers there are two pairs of enantiomers indicated by the arrows.

3. Results

Reaction of the chiral non-fluorescent fluorescamine with a chiral amino acid (Fig. 1A) begins by nucleophilic addition of the amine nitrogen to the dye double bond [21] resulting in a breakage of the C–O bond and loss of the dye chiral center. Closing of the 5-membered ring by a second nucleophilic attack of the amino acid nitrogen on the (former) chiral carbon forms a fluorescent product with two chiral centers (FA-aa) and hence four stereoisomers as shown in Fig. 1B. To optimize the separation of a mixture of fluorescamine-labeled amino acids, the Mars7 Standard was developed consisting of equimolar amounts of the achiral amino acids glycine and AIB, as well as the enantiomers of valine, alanine, serine, aspartic acid, and glutamic acid. The composition of this standard is representative of that expected in Martian soil samples [15]. Fig. 2A presents the separation of this mix-

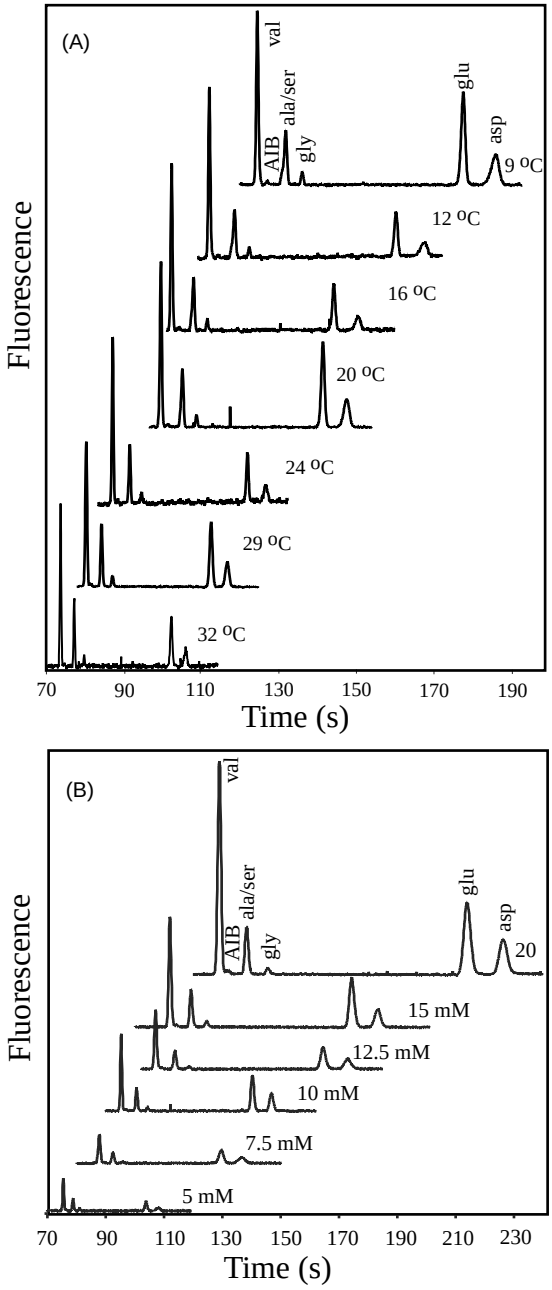

Fig. 2. (A) Temperature dependence of the capillary zone electrophoresis separation of the Mars7 Standard. Run conditions: buffer 10 mM CO_3^{2-}, pH 9.8, 700 V/cm, effective separation length 19.25 cm. (B) Ionic strength dependence of the separation of the Mars7 Standard. Run conditions: buffer pH 9.6, 700 V/cm, $T = 8\,^{\circ}C$, effective separation length 19.25 cm.

ture of amino acids at various temperatures. The separation occurs quickly (<110 s) at 32 °C but as the temperature is lowered to 9 °C, the separation slows down to 190 s. There is a linear increase in elution time (102–178 s for glutamic acid) and a slight increase in resolution (from 4.2 to 5.3 for glutamic and aspartic acid) with decreasing temperature.

Fig. 2B presents the separation of the Mars7 Standard as a function of buffer concentration. In a 5 mM CO_3^{2-} buffer, the standard elutes in only 120 s due to the high electroosmotic flow (EOF). Increasing the buffer concentration to 20 mM CO_3^{2-} causes the separation time to slow to 240 s due to decreased EOF. The increased buffer concentration increases the amino acid resolution slightly (from 3.5 to 4.5 for aspartic and glutamic acid).

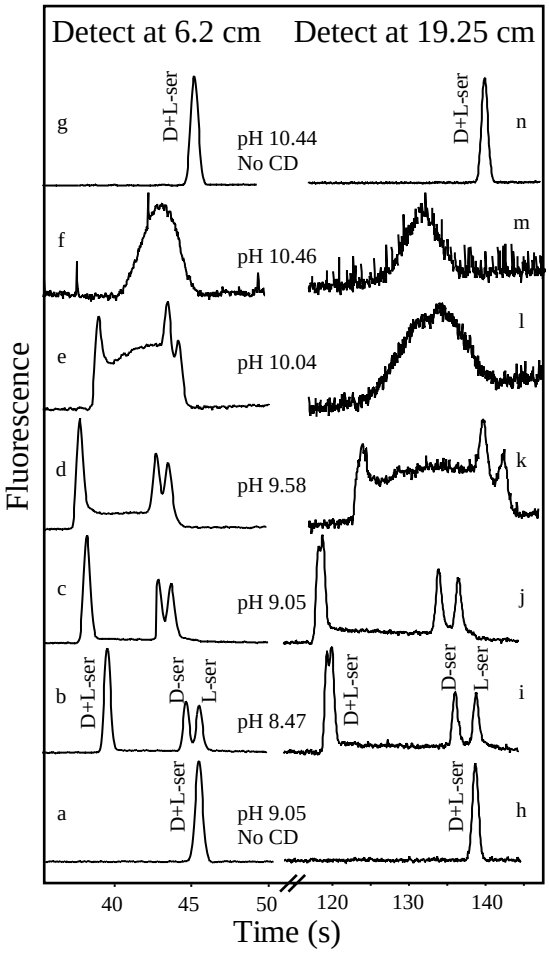

Fig. 3. Effect of pH on the chiral separation of D- and L-serine detected at 6.2 and 19.25 cm, respectively. The stage was manually shifted to record electropherograms at different separation distances. At high (g, n) and low (a, h) pH and with no cyclodextrin present, only one peak is observed at the two detection distances. With cyclodextrin included in the separation buffer, the four different stereoisomers are observed at pH 8.47 (b, i). At higher pHs, the resolution between the four stereoisomers decreases (b → f) and this effect is more evident at the longer separation distance (i → m). Run conditions: buffer 10 mM CO_3^{2-}, 20 mM HPβCD, 700 V/cm, $T = 13\,^{\circ}C$.

The effect of pH on the fluorescamine–amino acid separations is presented in Fig. 3. Increasing the pH has no effect on migration times or relative mobilities of the amino acids in the standard when the separation is performed without the chiral resolving agent in the running buffer. This result is evident by comparing traces a and h at pH 9.05 with traces g and n at pH 10.44. The pH has a more profound effect on the chiral fluorescamine–amino acid separations. At low pH, and with HPβCD included in the buffer, three stereoisomers are resolved at 6.2 cm (trace b) and all four are visible at 19.25 cm (trace i). The identities of the

peaks (D or L) were assigned by spiking. These traces reveal that we are resolving both amino acid enantiomers and the dye isomers although we do not know which dye isomer is responsible for which set of D/L peaks. As the pH of the running buffer is increased (trace b → f), the region between the two sets of peaks fills in and chiral resolution is lost. The peak profile is not consistent with poor plug definition, which would result in tailing, but is instead consistent with an interconversion or exchange between the peaks as first observed by Keller and Giddings [29]. This explanation is further supported by the rapid return to baseline after the L-ser peak. The interconversion between peaks is more apparent at the longer detection distance (i → m), indicating that the interconversion is taking place on the time scale of the separation. This interconversion is attributed to the racemization of the dye chiral center. As illustrated in Fig. 1A, the racemization is most likely achieved by the deprotonation of the alcohol on the dye chiral center, resulting in a rearrangement and loss of the chiral center through intermediate I.

To test this racemization hypothesis, Fig. 4 presents the chiral separation of glycine as a function of pH. Since glycine is achiral, the reaction with fluorescamine produces a product with only two stereoisomers (enantiomers) due to the chiral center of the dye. At high pH and with no cyclodextrin, only one peak is observed at short (a) and long (g) separation distances. With HPβCD included in the buffer (pH 8.47) the two enantiomers are well resolved (b, h). As the pH increases, the resolution degrades (b → f), and the interconversion between the peaks is more apparent and complete at longer separation times (h → l). This experiment clearly shows that the rapid interconversion is due to dye center diastereomerization rather than amino acid racemization. Using the chromatographic parameters for L-ser at pH 9.05, 9.58, and 10.04 and the formulas developed by Trapp and Schurig [31,32], the forward rate constants for peak interconversion are calculated to be $0.006 \pm 0.001\,s^{-1}$, $0.012 \pm 0.001\,s^{-1}$, and $0.041 \pm 0.008\,s^{-1}$, respectively. These data follow the trend observed in Figs. 3 and 4 of increasing rate constants for interconversion with increasing pH, and also quantitate that the racemization can be effectively avoided by running at pH < 9.

The chiral separation of the Mars7 Standard was further optimized by exploring the effect of HPβCD concentration (Fig. 5). At an HPβCD concentration of 10 mM, the valine and alanine/serine peaks split, while the acidic peaks shift to shorter retention times and are poorly resolved. At 15 mM, the enantiomers of valine, alanine/serine, aspartic acid and glutamic acid are all visible. In addition, AIB is clearly resolved from the L-ala/ser peak and L-asp. The resolution of AIB from the L-ala/ser peak is lost as the HPβCD concentration increases to 20 mM and higher, and the resolution of the valine enantiomers decreases. At all concentrations of HPβCD, glycine coelutes with the second set of alanine/serine peaks. The optimal concentration for chiral resolution of the fluorescamine-labeled Mars7 Standard is thus ∼15 mM HPβCD.

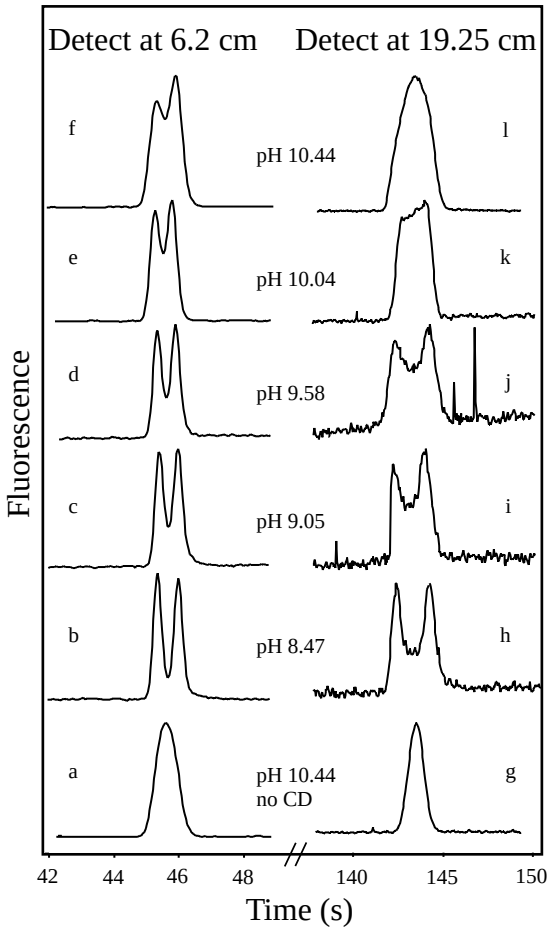

Fig. 4. Effect of pH on the chiral separation of glycine detected at 6.2 and 19.25 cm. At high pH and with no cyclodextrin present, only one peak is observed (a, g). With cyclodextrin included in the pH 8.47 running buffer, a clear splitting is observed due to the two enantiomers of the dye (b, h). As the pH increases, the resolution of the two enantiomers decreases (b → f) and this effect is more pronounced at the longer separation distance (h → l). Run conditions as in Fig. 3.

The chiral separation of the Mars7 Standard as a function of temperature is presented in Fig. 6. Chiral resolution of the enantiomers of ala/ser at 42 s and asp at 46 s is observed at 13 °C; however, the D-asp and D + L-glu peaks are not well resolved. The AIB peak was too weak to be observed. The resolution improves at lower temperatures. At 5 °C the valine peak begins to split, the resolution of the enantiomers of ala/ser is maintained, and L- and D-asp at 56 s are baseline resolved from each other and from the glutamic acid peak. These trends are also evident at 19.25 cm (not shown).

Figs. 3–6 also illustrate that the amino acid side chain determines the amount of the *R* and *S* products formed during the labeling reaction. Glycine, alanine and serine all exhibit equal *R*/*S* peak areas, indicating no steric preference during the dye–amino acid adduct formation (Figs. 3 and 4). AIB, an achiral amino acid, only exhibits one broad

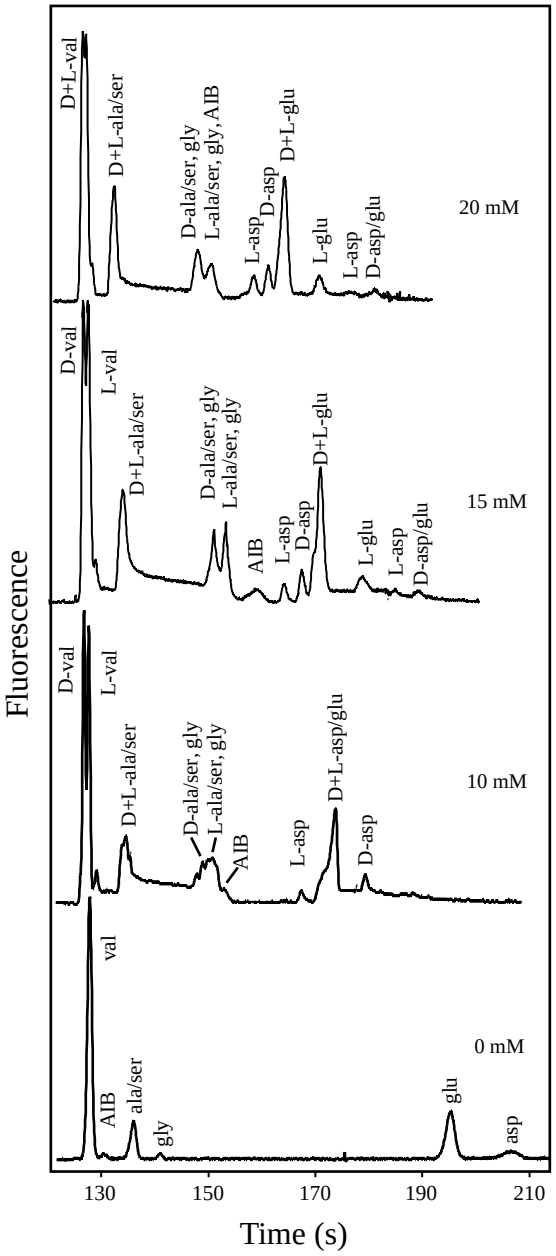

Fig. 5. Effect of HPβCD concentration on the separation of the Mars7 Standard. Traces have been shifted slightly in time (<10 s) to align the valine peaks for ease of comparison. Run conditions: buffer 10 mM CO_3^{2-}, pH 8.9, 700 V/cm, $T = 14\,^{\circ}C$, effective separation length 19.25 cm.

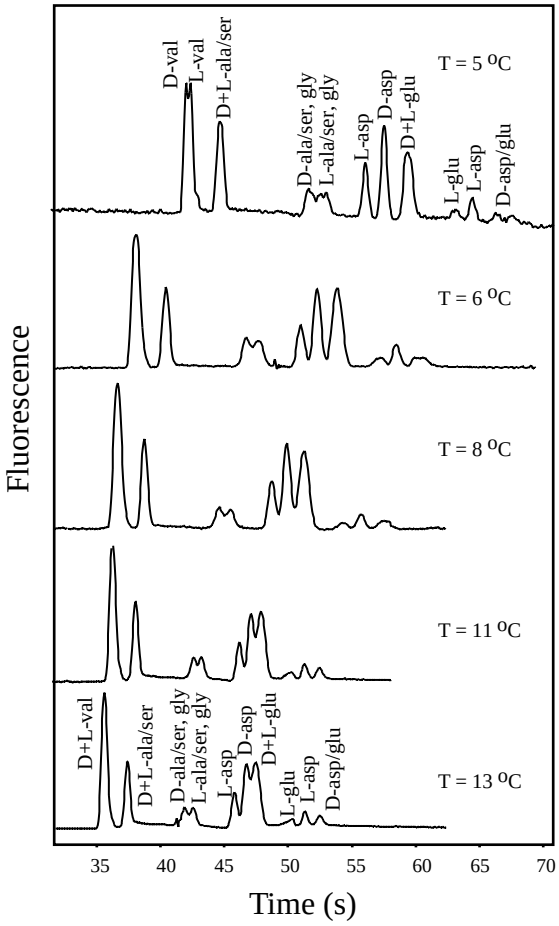

Fig. 6. Chiral separation of Mars7 Standard as a function of temperature. Run conditions: buffer 10 mM CO_3^{2-}, 15 mM HPβCD, 700 V/cm. Effective separation length 6.2 cm. At 19.25 cm effective separation length all enantiomers are resolved at all temperatures (not shown).

peak in the electropherogram. However, for the acidic amino acids there is a steric preference towards one form of the dye, as seen by the unequal peak areas (Fig. 6, top trace, L-asp peaks at 55 and 63 s). Finally, for valine, only one set of D/L isomers is observed. In all cases, the separation of the D/L peaks is significantly smaller than that of the *R*/*S* peaks so the presence of the two sets of enantiomeric peaks does not interfere with our ability to determine chirality ratios.

4. Discussion

Probing for chemical signs of life on the surface of Mars through in situ analysis requires definition of the target molecule(s) and the development of instrumentation capable of dealing with potentially low analyte concentrations as well as the harsh Martian conditions. Viking, which landed on Mars in 1976, carried a GC–MS instrument for the detection of organics [33] as well as biology experiments for detection of microbial life [34]. The GC–MS experiment failed to detect any organic molecules after heating a soil sample to 500 °C, even though the detection limits for hydrocarbons and other organics were nominally in the low parts-per-billion range [33]. These negative results may be explained by insufficient practical sensitivity [35], UV destruction or oxidation of organics [33], and/or the formation of non-volatile organic salts [36]. More recent in situ experiments, such as the alpha proton X-ray spectrometer on the Mars Pathfinder in 1997, analyzed inorganic chemical composition of the Martian soil [37], while recent orbiters, such as the Mars Odyssey, have successfully targeted the

detection of water [38]. It is evident that a more complete analysis of the surface and subsurface organic molecules on Mars is needed.

One of the proposed in situ instruments for analyzing surface organic molecules is the Mars Organic Detector (MOD) [25]. This instrument heats a soil sample in a chamber causing polyaromatic hydrocarbons, amines and amino acids to sublime onto a cold finger where they are detected by fluorescence [39]. Sublimation can also be performed in the presence of acid vapor in order to enhance isolation of amino acids from the sample matrix [39]. This isolation approach is advantageous because (i) the yield can be as high as 80–100%, (ii) no amino acid racemization occurs, and (iii) the organic molecules are isolated from the bulk matrix and concentrated onto the cold finger. The presence of amines and amino acids is signaled by their reaction with the fluorogenic dye, fluorescamine, which is coated on the surface of the cold finger.

While the MOD I approach is very valuable for detecting the presence of amino acids it does not provide information about amino acid composition or chirality. We have, therefore, proposed the use of a microfabricated capillary electrophoretic separation device to analyze the composition and chirality of the captured products [15] and this combined analyzer is called the Mars Organic Analyzer (MOA). While our earlier studies demonstrated successful amino acid composition and chirality analysis [15], this work was performed using FITC labeling which involves somewhat complex and slow reaction conditions and undesirable backgrounds. We were thus interested in determining whether it would be possible to perform amino acid composition and chirality measurements directly on fluorescamine-labeled amino acids derived from MOD I.

The work presented here shows that fluorescamine-labeled amino acids can be effectively analyzed for composition and chirality using microchip CE. The achiral separations of fluorescamine-labeled amino acids under optimum pH, temperature and buffer concentration conditions produce electropherograms that exhibit similar migration orders, times, and resolution to those observed earlier for FITC [15]. The chiral separations using fluorescamine are more complex because the fluorescamine reaction produces an amino acid adduct having four stereoisomers. Furthermore, the pH-dependent intensities of the peaks and plateaus in the diastereomeric separation are reminiscent of the classic pattern expected for enantiomerization or interconversion of two species [29–31,40], suggesting that there is an interconversion that could involve either the dye or the amino acid chiral center. Because this interconversion was also observed in the chiral separation of glycine, we conclude that the dye chiral center is the one racemizing at high pH. By examining the pH dependence of this process, we were able to identify conditions (pH < 8.9) where a well-defined and stable electrophoretic pattern is observed allowing reliable composition and chirality determination. Quantitative analysis of the interconversion rates at the different pHs using model functions [32] confirms that at pH < 8.9 the interconversion time becomes longer than the separation time ($\sim$200 s). Because the microfabricated devices used here allow us to perform separations that are faster or on the same time scale as the dye interconversion, we were able to characterize these diastereomerization processes that were undetected in previous HPLC studies [26]. This time scale advantage has also recently been exploited to observe sub-second antigen–antibody dissociation [41].

The pH-dependent interconversion of the chiral center on the dye and the relative intensities of the various isomeric species can be understood. The fluorescamine-labeling mechanism begins by addition of the amine nitrogen to the dye double bond [21]. In order to reach the final structure with the amine nitrogen included in a 5-membered ring, the reaction must proceed via a ring opening where the chiral center on the dye is lost. At high pH, the deprotonation of alcohol on the dye chiral center might lead to an open-ring structure through intermediate I (Fig. 1A) which would allow interconversion of the dye center without loss or isomer-

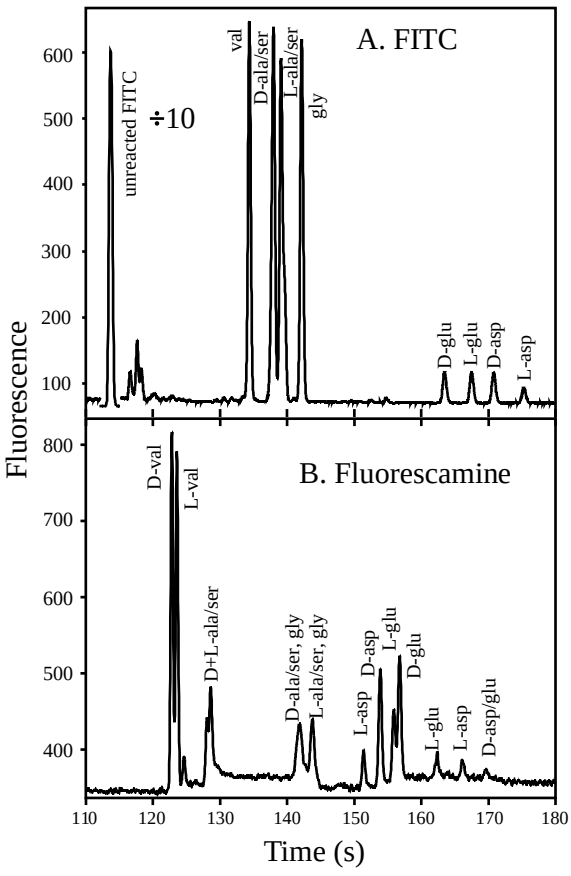

Fig. 7. Comparison of separations of the Mars7 Standard labeled with FITC in 5 mM γ-CD (A) and labeled with fluorescamine in 15 mM HPβCD (B). Run conditions: buffer 10 mM CO_3^{2-}, pH 8.9, 700 V/cm, $T = 6\,^{\circ}C$, effective separation length 19.25 cm. Injected concentrations of amino acids (valine, alanine, serine, glutamic acid and aspartic acid are 6 μM; glycine and AIB are 3 μM) are identical for the FITC and fluorescamine separations. Fluorescence scales for the FITC and fluorescamine separations are directly comparable.

ization of the amino acid. The smaller amino acids, such as glycine, alanine, and serine, produce all stereoisomeric combinations with no preference as evidenced by the equivalent peak areas of the *R* and *S* dye structures. Furthermore, these smaller side chains demonstrate the pH-dependent racemization, supporting the idea that there is no steric preference. For larger amino acid side chains such as glutamic acid and aspartic acid (and also valine), one dye chirality is preferred. In these cases, racemization is not observed, suggesting that there is a steric preference.

The results presented here demonstrate that fluorescamine is a very useful and advantageous dye label for performing amino acid composition and chirality analyses. The dye has excellent labeling properties because it reacts rapidly in both the aqueous and solid phases to form a fluorescent compound. This was a key property dictating its selection in MOD I. Furthermore, fluorescamine labeling produces low backgrounds because it yields a fluorescent adduct only when coupled to primary amines. Comparison of the FITC-labeled Mars7 Standard separation with the fluorescamine-labeled separation in Fig. 7 illustrates this point. The fluorescamine separations do not exhibit any contaminating peaks from unreacted reagent or hydrolysis products that plague the FITC separations although there is an increased background due to water Raman scattering. While the chiral separation of fluorescamine-labeled amines is somewhat more complex than the FITC separations for small amines, high-quality chiral resolution of valine, alanine, serine, glutamic and aspartic acid was obtained at pH < 9.0. FITC still exhibits 2- to 10-fold superior sensitivity for the amino acids in the Mars7 Standard. For example, the injected concentration of valine and aspartic acid after dilution was 6 μM in both traces; however, the *S*/*N* of the FITC-valine peak is 700 while that of the combined fluorescamine-valine peaks is 270, and the *S*/*N* of FITC-D-aspartic acid peak is 55 while that of fluorescamine-D-aspartic acid peak is 46. In addition, under identical ideal achiral separation and detection conditions (8 mM CO_3^{2-}, pH 8.8, $T = 6\,^{\circ}C$, effective separation length 19.25 cm), the limit of detection ($S/N = 3$) for valine determined by serial dilution is 10 nM for FITC and 83 nM for fluorescamine (an eightfold difference), and for glutamic acid the limit of detection is 50 nM for FITC and 83 nM for fluorescamine (less than a twofold difference). Nevertheless, preliminary results on soil sample extracts from the Atacama Desert indicate that fluorescamine labeling can be used to detect amino acids at 100 ppb concentrations in soil. In combination with a sublimation apparatus for isolation and concentration, this detection limit should improve significantly. In summary, our demonstration that fluorescamine labeling can be used to perform high-sensitivity composition and chirality analysis of amino acid samples establishes the feasibility of the direct interfacing between the MOD I sublimation apparatus and the microchip CE device and points the way to the development of more universal bioorganic analyzers [42].

Acknowledgements

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