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The use of capillary electrophoresis to monitor Maillard reaction products (MRP) by glyceraldehyde and the epsilon amino group of lysine

Paula F.G. de Sa, James M. Treubig Jr., Phyllis R. Brown, Joel A. Dain *

University of Rhode Island, Kingston, RI 02881, USA

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Abstract

Capillary electrophoresis (CE) was used to monitor the formation of Maillard reaction products (MRP) from the reaction of glyceraldehyde (GA) with the epsilon amino group of lysine using *N*-α-acetyl-L-lysine (20 mM) at 37°C. Major components were separated by capillary CE using a bare capillary and detected by UV spectroscopy, at 214 nm. CE electropherograms demonstrated formation of different MRP species when conditions were varied. CE analysis was simple, rapid and needed no sample preparation.

1. Introduction

Nonenzymatic reactions involving amino acids, peptides or proteins with reducing sugars (Maillard reaction) leads to the formation of a wide variety of compounds (Yaylayan, 1997) often described as Maillard reaction products (MRP). The main components formed during the process are central to the formation of flavors, and colors as foods are cooked and processed.

The chemistry of the Maillard reaction has been investigated using a wide range of model systems involving different sugars reacting with amino acids or proteins (Al-Abed, Ulrich, Kapurniotu, Lolis & Bucala, 1995; Baxter, 1995; Bedinghaus & Ockerman, 1995). The reaction is initiated by the reversible formation of a Schiff base between a reducing sugar and the amino group of a protein. The Schiff base rearranges to a more stable compound known as an Amadori product, which undergoes through a series of complex sequence of reactions to form compounds collectively known as MRP (Fig. 1).

Some MRP selectively absorbs wavelengths of light in the region of UV/visible; thus the progressive formation of chromophores from carbohydrate/amino model systems leads to mixtures of different species that can be monitored by UV and fluorescence spectroscopy (Rizzi, 1997). However, in those mixtures there are also non-UV absorbing components with a wide range of polarities and molecular weights, making analyses of some MRP species difficult.

Separation of MRP from different model systems has been reported in such diverse fields as human pathology (Frye, Degenhard, Thorpe & Baynes, 1998), food chemistry (Meynier & Mottram, 1995), and the flavor industry (Weenem, 1998). Techniques that have been used to separate and quantify MRP in vivo and in vitro include high performance liquid chromatography (HPLC) (Bank, Verzijl, de Ross, Sakke & Tekoppele, 1997), gas chromatography (GC) (Tressl, Wondrak & Kersten, 1994), gel electrophoresis (Miksik & Deyl, 1997), radioimmunoassay (RIA) (Makita, Vlassara, Cerami & Bucala, 1992) and nuclear magnetic resonance (NMR) (Hofmann, 1998).

A technique that is currently gaining use for the separation of MRP mixtures is capillary electrophoresis (CE). The use of electrophoresis in narrow-bore capillaries provides a means to obtain rapid high efficiency separations of analytes, including biological molecules (Oda & Landers, 1997). Moreover, since these separations are often based upon electrophoretic mobility, the basis for separation selectivity is complementary to that obtained with HPLC.

The use of CE to separate MRP has been reported in the literature by several groups. Tomlinson, Mlotkiewicz and Lewis (1994) applied CE to separate components produced by the reaction of glucose and 5-hydroxymethyl

* Corresponding author. Tel.: +1-401-874-5942; fax: +1-401-874-5072.

E-mail address: jdain@chm.uri.edu (J.A. Dain).

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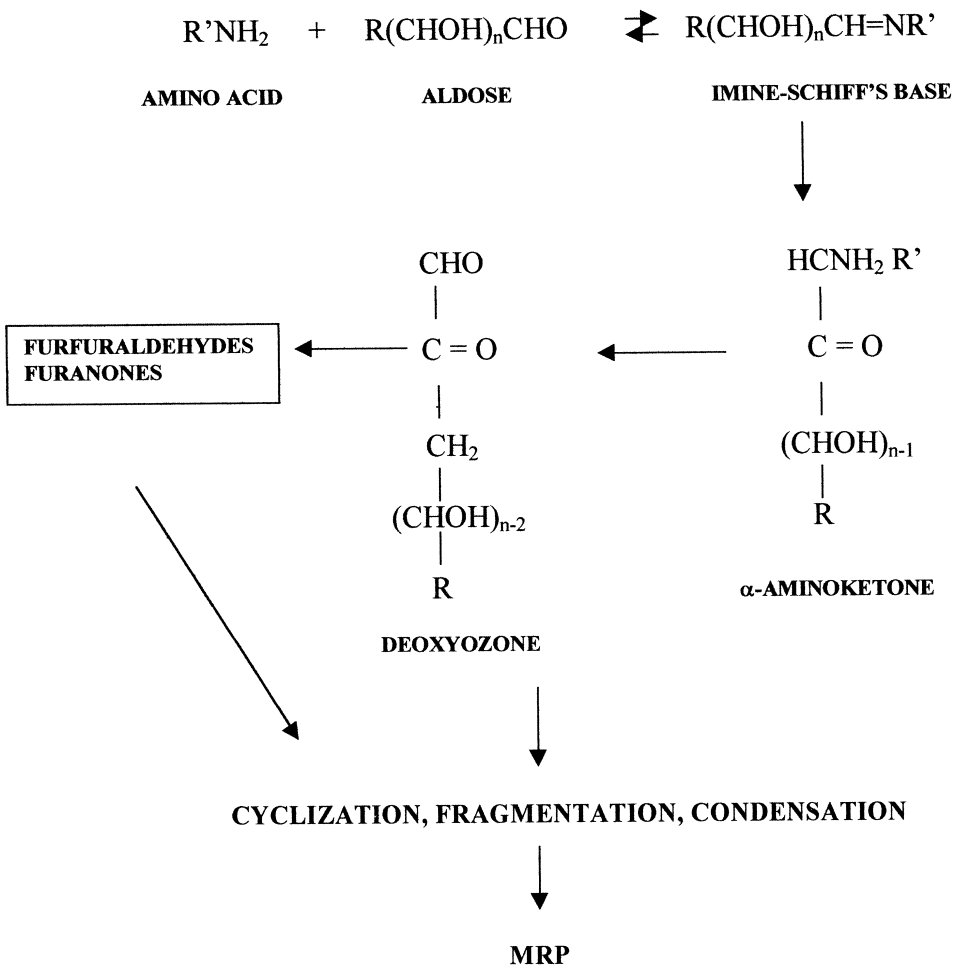

Fig. 1. Pathways of MRP formation.

furfural with glycine. Tomlinson's group also reported the effect of buffer composition, ionic strength and pH for separation of Maillard compounds (Tomlinson, Landers, Lewis & Naylor, 1993). Jones, Tier and Wilkins. (1998) applied CE to separation of MRP of β-lactoglobulin and lactose in skimmed milk powders, and Royle, Bailey and Ames (1998) compared the advantages of CE to those of reversed phase HPLC to separate reaction products for model systems of glucose or xylose and glycine.

The objective of the present study was to demonstrate the use of CE in monitoring MRP formation of a sugar with the epsilon amino group of lysine. This model system contained *N*-α-acetyl-L-lysine (NALys) and DL-Glyceraldehyde (GA). Effects of the sugar concentration on the reaction and MRP profile are also reported.

2. Materials and methods

2.1. Reagents

GA, NALys and sodium tetraborate were purchased from Sigma Chemical Company (St Louis, MO, USA). Potassium phosphate monobasic and potassium phosphate dibasic were purchased from Fisher Scientific (New Lawn, NJ, USA). All buffers and samples were prepared with a Milli-Q purified distilled water (Millipore, Bedford, MA, USA).

2.2. Preparation of the reaction mixtures and buffer

Phosphate buffered saline (PBS) solution was prepared by mixing equal volumes of 0.2 M of potassium phosphate monobasic and 0.2 M of potassium phosphate dibasic solution. Stock solutions of 0.1 M NALys and 0.2 M of GA were prepared in 0.2 M PBS, pH 7.2. Reaction mixtures contained NALys (20 mM) and GA (2, 10, 20 and 40 mM) were prepared in 0.2 M PBS buffer, pH = 7.2. Controls contained either the NALys or the GA. The reaction mixtures and blanks were incubated at 37°C in sealed glass vials for 10 h and then frozen.

Separating buffer for the CE consisted of 20 mM sodium tetraborate. The pH of the buffer was adjusted to 9.6 using 1 M NaOH, and then the solution was filtered with a 45 μm syringe filter from Scientific Resources Inc (North Brunswick, NJ, USA).

2.3. Fluorescence measurements

Fluorescence emission spectra of the reaction mixtures were measured using a Hitachi 880 spectrofluorometer (Hitachi Instruments, San Jose, CA). The excitation wavelength of 370 nm used for in monitor total MRP for all reaction mixtures.

2.4. Capillary electrophoresis

CE analyses were performed using a Quanta 4000 CE unit with UV detection at 214 nm from Waters Company (Milford, MA, USA). A capillary with an internal diameter of 50 μm, 600 mm total length and 500 mm effective length was cut from bulk stock purchased from Polymicro Technologies (Phoenix, AZ, USA). The capillary was conditioned by flushing for 15 min with 1 M NaOH, 10 min with Milli-Q water, followed by 5 min with the tetraborate buffer. It was rinsed between the runs with 1M NaOH for 1 min followed by the running buffer for 2 min. Buffer solution was made fresh each day, but the same NaOH solution was used during all runs.

Samples were applied to the capillary using hydrodynamic injection for 20 s at the anionic end. Electropherograms were monitored at 214 nm, and the data collected on a model SP4270 Spectra Physics Integrator (San Jose, CA, USA).

3. Results and discussion

3.1. Spectroscopy

Fluorescence spectral features typical of MRP formed after 10 h of incubation in our model system are shown in Fig. 2. Because fluorescence spectroscopy is very sensitive to background signals that may originate from endogenous sample constituents, NALys 20 mmM incubated in PBS for 10 h was used as a control. No emission peak was observed for NALys at the excitation wavelength of 370 nm. Maximum emission was observed at 445 nm for the reaction mixtures after 10 h incubation. A red shift is observed for the 40 mM reaction mixture (452 nm). This slight change can be associated either with the presence of different fluorophores and/or with the presence of different products formed from the interactions between the main fluorophore and secondary intermediate species present in the medium.

Since fluorescence intensity is quantitatively dependent on the parameters defined by the Beer–Lambert law, more products are formed for the reaction mixtures containing a higher sugar concentration.

3.2. CE of the MRP species

The intermediates and products for the reactions between NALys and various concentrations of GA were separated by CE. CE electropherograms recorded at 214 nm showed formation of different intermediates and products for each system (Fig. 3). A main product, at $t_r = 11.7$–11.8 min, was observed for all, except for the 2 mM mixture. Different secondary intermediates are formed when the concentration of sugar is twice the amino acid concentration. These results suggest that the pathway for the types MRP formed in solution is influenced by the sugar/amino acid molar ratio. When the GA was 2 mM in the reaction mixture no peaks could be detected after an incubation time of 10 h.

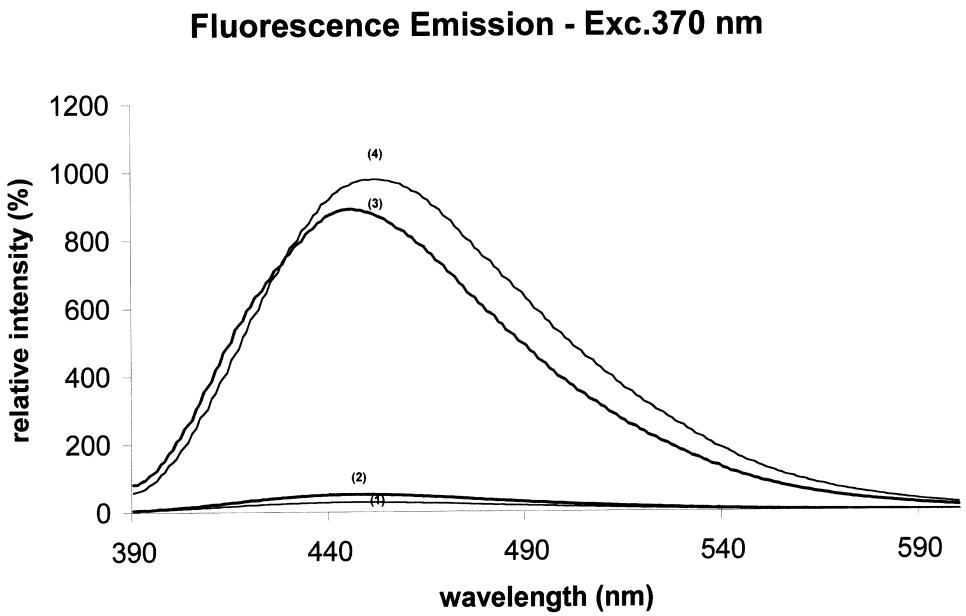

Fig. 2. Fluorescence spectra, Exc 370 nm, after 10 h of incubation for all reaction mixtures: GA 40 mM/NALys (1), GA 20 mM/NALys (2), GA 10 mM/NALys (3) and GA 2 mM/NALys (4). NALys is used as control and did not show any emission at the specific wavelength.

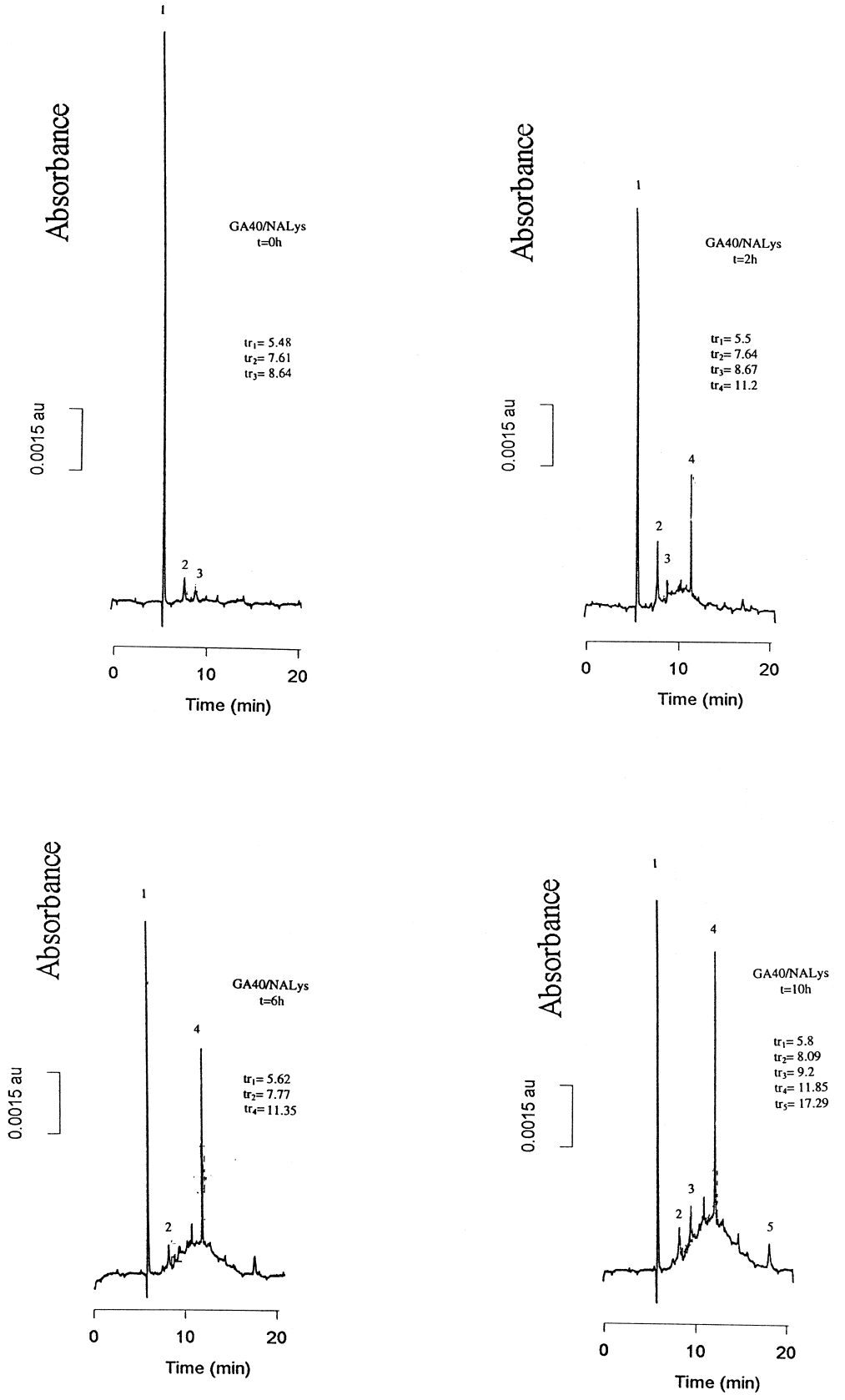

Fig. 3. Electropherograms of the reaction mixtures after incubation for 10 h at 37°C in PBS, pH 7.2. Unreacted NALys (1) and acetone (1) are used as a neutral marker. All species (2,3,4,5) are negatively charged in the following analysis conditions: silica capillary with effective length of 50 cm, hydrodynamic injection for 20 s, 20 mM borate buffer pH 9.6, 25 kV, UV detection—214 nm.

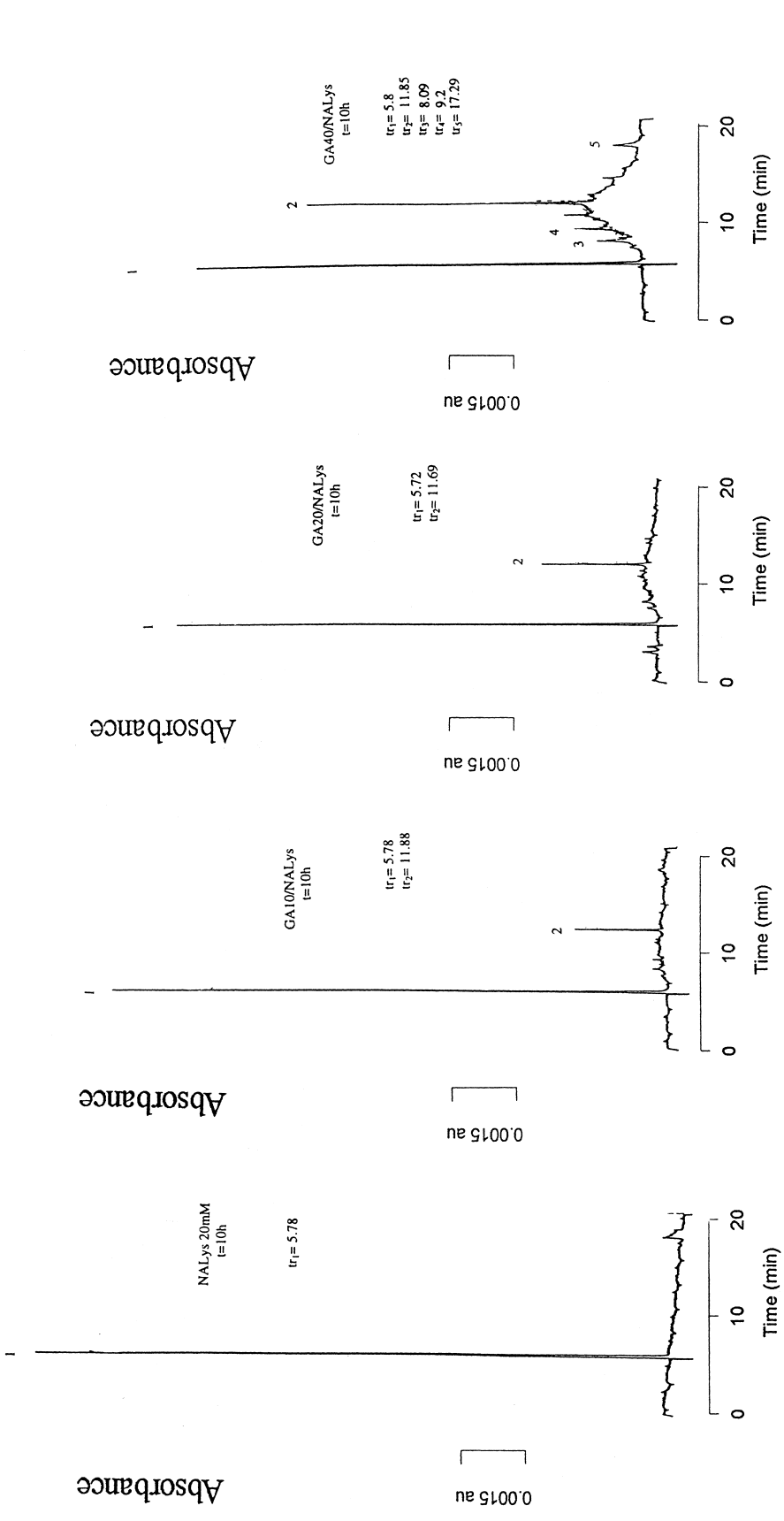

Fig. 4. Monitoring of MRP formation pathway for GA40 mM/NALys using CE. The peak intensity of the main product (t_r = 11.2–11.8) increased with the incubation time. Silica capillary with effective length of 50 cm, hydrodynamic injection for 20s, 20 mM borate buffer pH 9.6, 25 kV, UV detection—214 nm.

Acetone was used as a neutral marker for the CE analysis of the mixtures. It had a migration time of 5.78 min. At pH 9.6, NALys is neutral, and had the same migration time as acetone. The majority of reaction products had longer migration times than NALys, suggesting the presence of negatively charged species at that specific pH.

The high reactivity of GA is clearly demonstrated by the formation of some products after only 2 h of incubation for the 40 mM reaction mixture. GA does not form a stable hemiacetal in solution so that its carbonyl group is readily susceptible for chemical modifications. Although GA is one of the simplest carbohydrates the complexity of the Maillard reaction yields many different compounds in solution. Some of these compounds continue to react to form new compounds and a new cascade is initiated. The electropherograms in Fig. 4 show this random change in intensity of some components during the Maillard reaction between GA and NALys mixtures.

4. Conclusions

A large number of MRP from reaction mixtures of GA and NALys can be separated by CE. Under our analysis conditions we demonstrated the anionic character of intermediates and products at pH 9.6. Since tetraborate ion rapidly forms complexes with vicinal hydroxyl groups of sugars, GA can not be detected at 214 nm, suggesting the potential application of this methodology for kinetic studies by monitoring NALys consumption during MRP formation.

The fluorescence spectra recorded for reaction mixtures containing different sugar/amino acid ratio, indicated that the GA concentration affects the Maillard reaction rate. In addition, the electropherograms demonstrated that the GA concentration affects the MRP profile due the formation of different intermediates in the reaction medium.

In this report we demonstrated the use of CE as a powerful tool for separating main components produced by the Maillard reaction of GA with the ε-amino group of lysine. The technique is simple, separation is rapid, and no sample preparation is necessary.

References

Al-Abed, Y., Ulrich, P., Kapurniotu, A., Lolis, E., & Bucala, R. (1995). Model studies of Maillard reaction of Arg-Lys with D-Ribose. *Bioorganic and Medical Chemistry Letters, 5*(23), 2929–2930.

Bank, R. A., Verzijl, N., de Roos, J. A. D. M., Sakke, A. N., & TeKoppele, J. M. (1997). Fluorimetric quantitation of pyrralyne and pentosidine in lenses by HPLC. *Journal of Chromatography B, 703*, 37–44.

Baxter, J. H. (1995). Free amino acid stability in reducing sugar system. *Journal of Food Science, 60*(2), 405–408.

Bedinghaus, A. J., & Ockerman, H. W. (1995). Antioxidative Maillard reaction products from reducing sugars and free amino acids in cooked ground pork patties. *Journal of Food Science, 60*(5), 992–995.

Frye, E. B., Degenhard, T. P., Thorpe, S. R., & Baynes, J.W. (1998). Role of the Maillard reaction in aging tissue proteins. *Journal of Biological Chemistry, 273*(30), 18714–18719.

Hofmann, T. (1998). Characterization of the chemical structure of novel colored Maillard reaction products from furan-2-carboxyaldehyde and amino acids. *Journal of Agricultural and Food Chemistry, 46*, 932–940.

Jones, A. D., Tier, C. M., & Wilkins, J. P. G. (1998). Analysis of Maillard reaction products of β-lactoglobulin and lactose skimmed milk powder by CE and electrospray mass spectroscopy. *Journal of Chromatography. A, 822*, 147–154.

Makita, Z., Vlassara, H., Cerami, A., & Bucala, R. (1992). Immunochemical detection of advanced glycosylation endproducts in vivo. *Journal of Biological Chemistry, 267*(8), 5133–5138.

Meynier, A., & Mottram, D. S. (1995). The effect of pH on the formation of volatile compounds in meat-related model systems. *Food Chemistry, 52*, 361–366.

Miksik, I., & Deyl, Z. (1997). Post-translational non-enzymatic modification of proteins II. Separation of selected protein species after glycation and other carbonyl-mediated modifications. *Journal of Chromatography. B, 699*, 311–345.

Oda, R. P. & Landers, J. P. (1997). Handbook of capillary electrophoresis, ed. J. P. Landers. CRC Press, New York, pp 4–19.

Rizzi, G. P. (1997). Chemical structures of colored Maillard reaction products. *Food Review International, 13*(1), 1–28.

Royle, L., Bailey, R. G., & Ames, J. M. (1998). Separation of Maillard reaction products from xylose-glycine and glucose-glycine model by capillary electrophoresis and comparison to reverse phase HPLC. *Food Chemistry, 62*(4), 425–430.

Tomlinson, A. J., Landers, J. P., Lewis, I. A. S., & Naylor, S. (1993). Buffer condition affecting the separation of Maillard reaction products by capillary electrophoresis. *Journal of Chromatography. A, 652*, 171–177.

Tomlinson, A. J., Mlotkiewicz, J. A., & Lewis, I. A. S. (1994). Application of capillary electrophoresis to the separation of coloured products of Maillard reactions. *Food Chemistry, 49*, 219–223.

Tressl, R., Wondrak, G., & Kersten, E. (1994). Structure and potential cross linking reactivity of a new pentose specific Maillard product. *Journal of Agricultural Food Chemistry, 42*, 2692–2697.

Weenem, H. (1998). Reactive intermediates and carbohydrate fragmentation in Maillard Chemistry. *Food Chemistry, 62*(4), 393–401.

Yayalayan, V. A. (1997). Classification of the Maillard reaction: A conceptual approach. *Trends in Food Science & Technology, 8*, 13–18.