JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2008; **43**: 262–264
Published online 12 October 2007 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/jms.1290

JMS

JMS Letters

Dear Sir,

Electrospray positive ionization tandem mass spectrometry of Amadori compounds

The Maillard reaction, the nonenzymatic interaction between reducing sugars and amino acids, is one of the most important reactions in food and human bodies.[1,2] Amadori compounds are *N*-substituted 1-amino-1-deoxyketoses, which are formed in the initial phase of the Maillard reaction by Amadori rearrangement, representing a key class of Maillard intermediates.[3,4] The importance of Amadori compounds results from the fact that their formation as well as decomposition can occur under physiological conditions and during food processing and storage. Amadori compounds play a central role in the production of aroma, taste, and color.[5–9] Furthermore, it has been shown that these compounds are accompanied by a reduction in the nutritive value and the formation of toxic compounds, which have a remarkable effect on the health of human, and so this type of compounds has attracted considerable attention.[10–13]

Many methods have been used to analyze Amadori compounds, such as gas chromatography with sample derivatization prior to analysis,[14] high-performance liquid chromatography,[14] high-performance anion exchange chromatography with an electrochemical detector,[15] and so on. However, the analysis of Amadori compounds was still a challenging task because they are numerous and their structures are similar. With the development of soft ionization technology applied in the field of mass spectrometry over the past decade, fast atom bombardment tandem mass spectrometry[16] and the coupling of tandem mass spectrometry to capillary electrophoresis[17] were successfully used to identify a few glucose-derived Amadori compounds. Recently, electrospray ionization tandem mass spectrometry has become an important means of analysis of organic molecules. High-performance ion exchange chromatography coupled to tandem mass spectrometry[18] has been applied to analyze several Amadori compounds. But the systemized research of mass spectrometry of Amadori compounds and the fragmentation modes of the compounds under electrospray ionization condition have not been developed yet. In this paper, the 18 Amadori compounds were prepared from the reactions between the 18 common amino acids found in food and human bodies with glucose. After purification by high-performance liquid chromatography, the mass spectrometry (ESI-MS/MS) of these compounds was carried out. Moreover, their main fragmentation pathways are summarized.

The preparation of Amadori compounds is shown in Scheme 1. Samples of individual Amadori compounds were prepared by refluxing powdered anhydrous glucose (20 mmol), malonic acid (5 mol), and the corresponding amino acid (20 mmol) in methanol (30 ml) under nitrogen for 6 h. After the solutions were cooled to room temperature, the precipitates were removed by filtration (the unreacted glucose and amino acid were filtered off). Each filtrate was concentrated to approximately 15 ml *in vacuo*. Dropwise addition of anhydrous acetone to the ice-cooled filtrate yielded the Amadori product as a hygroscopic solid. Then the melted solids were filtered out and washed with acetone a few times. The viscous solids were transferred into a flask and acetone (8 ml) was added to the flask. After the system was dried under reduced pressure, the amorphous solids were obtained. The products were stored under nitrogen. Owing to the hygroscopic nature of the Amadori products in air, all solid compounds (1 mg) were quickly weighed and then dissolved in water (100 ml), respectively. The solutions were used in the following experiment.

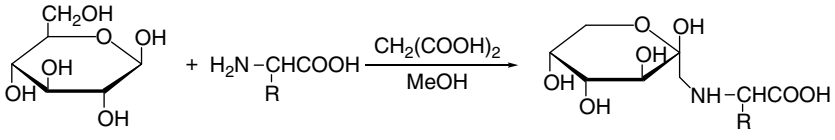
The ESI-MS/MS spectra were obtained using the 4000 Q-Trap mass spectrometer (AB/MDS Sciex, Concord, Canada). An Agilent-1200 system including a quaternary pump, autosampler, and thermostatted column compartment was integrated with the

Table 1. Optimized declustering potential and collision energy of Amadori compounds

No.	Amadori compounds	DP (V)	CE (V)
1	Fru-Gly	50	20
2	Fru-Ala	42	18
3	Fru-Val	46	20
4	Fru-Leu	48	20
5	Fru-Ile	48	20
6	Fru-Pro	42	18
7	Fru-Phe	42	23
8	Fru-Tyr	48	23
9	Fru-Met	43	20
10	Fru-Ser	47	20
11	Fru-Thr	42	20
12	Fru-Try	55	22
13	Fru-Asn	49	20
14	Fru-Gln	48	20
15	Fru-Asp	33	20
16	Fru-Glu	49	20
17	Fru-His	45	20
18	Fru-Arg	42	28

Note: *N*-(1-deoxy-D-fructos-1-yl)glycine was abbreviated as Fru-Gly. The same abbreviation method was appropriate to other Amadori compounds.

*Correspondence to: J. Wang, Technical Center, ChangYang Road 717#, Shanghai 200 082, P. R. China. E-mail: chwj75@163.com



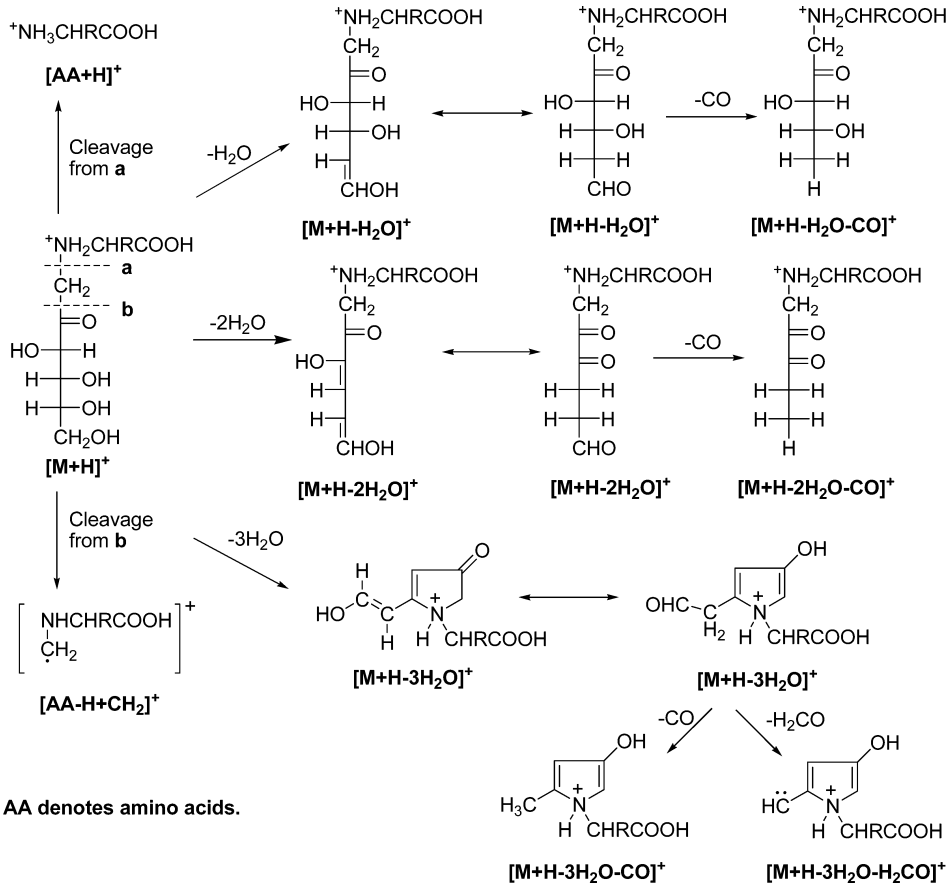
No.	AA	No.	AA	No.	AA	No.	AA
1	Gly	**6**	Pro	**11**	Thr	**16**	Glu
2	Ala	**7**	Phe	**12**	Try	**17**	His
3	Val	**8**	Tyr	**13**	Asn	**18**	Arg
4	Leu	**9**	Met	**14**	Gln		
5	Ile	**10**	Ser	**15**	Asp		

AA denotes amino acids.

Scheme 1. Preparation of the Amadori compounds.

Table 2. MS/MS spectral data of $[M + H]^+$ and the significant fragment ions of Amadori compounds

No.	Amadori compounds	Precursor ion (m/z) $[M + H]^+$	Significant fragment ions $[MS^2]$ (m/z)
1	Fru-Gly	238	220, 202, 192, 184, 174, 156, 154, 88, 76
2	Fru-Ala	252	234, 216, 206, 198, 188, 170, 168, 102, 90
3	Fru-Val	280	262, 244, 234, 226, 216, 198, 196, 130, 118
4	Fru-Leu	294	276, 258, 248, 240, 230, 212, 210, 144, 132
5	Fru-Ile	294	276, 258, 248, 240, 230, 212, 210, 144, 132
6	Fru-Pro	278	260, 242, 232, 224, 214, 196, 194, 128, 116
7	Fru-Phe	328	310, 292, 282, 274, 264, 246, 244, 178, 166
8	Fru-Tyr	344	326, 308, 298, 290, 280, 262, 260, 194, 182
9	Fru-Met	312	294, 276, 266, 258, 248, 230, 228, 162, 150
10	Fru-Ser	268	250, 232, 222, 214, 204, 186, 184, 118, 106
11	Fru-Thr	282	264, 246, 236, 228, 218, 200, 198, 132, 120
12	Fru-Try	367	349, 331, 321, 313, 303, 283, 217, 205
13	Fru-Asn	295	277, 259, 249, 241, 231, 213, 211, 145, 133
14	Fru-Gln	309	291, 273, 263, 255, 245, 225, 159, 147
15	Fru-Asp	296	278, 260, 250, 242, 232, 214, 212, 146, 134
16	Fru-Glu	310	292, 274, 264, 256, 246, 228, 226, 160, 148
17	Fru-His	318	300, 282, 272, 264, 254, 236, 234, 168, 156
18	Fru-Arg	337	319, 301, 291, 283, 273, 187, 175



Scheme 2. Proposed fragmentation pathways of Amadori compounds.

mass spectrometer. Purification of the Amadori samples was carried out on a C-18 column (RESTEK Pinnacle-II, 5 µm, 250 × 4.6 mm). The elution condition was at a flow rate of 0.65 ml/min and the mixture of 0.1% (V/V) aqueous formic acid (40%) with methanol (60%) as eluent. The column temperature was set at 25 °C. The injection volume was 8 µl. The Amadori compounds were analyzed in positive electrospray ionization mode using product ion (MS^2) experiment. The turbo ion-spray source was operated at 550 °C with an ion-spray voltage at 5500 V, curtain gas (N_2) at 20 psi, ion source nebulizer gas at 45 psi, ion source heater gas at 55 psi, and collision

DOI: 10.1002/jms

JMS

gas at medium pressure. In the experiment, the entrance potential was set at 10 V, collision cell exit potential at 10 V, focusing lens at −11.0 V, and prefilter at −17.0 V. Declustering potential (DP) and collision energy (CE) were optimized according to Amadori compounds. The results of voltages are shown in Table 1. The Scan time was equal to 1 s. Data were treated with the manufacturer's Analyst 1.4.1 software.

The MS/MS tandem spectral data of the $[M+H]^+$ ions and the significant fragment ions of the 18 Amadori compounds are summarized in Table 2.

From the MS/MS spectral data of the Table 2, it was found that the MS^2 spectra of $[M+H]^+$ ions of the Amadori compounds showed characteristic fragment ions at m/z $[M+H-18]^+$, $[M+H-36]^+$, $[M+H-46]^+$, $[M+H-54]^+$, $[M+H-64]^+$, $[M+H-82]^+$, $[M+H-84]^+$, $[M+H-150]^+$, and $[M+H-162]^+$. According to the characteristic structure of the Amadori molecules, in which there are carbonyl and hydroxide groups, the fragmentation patterns can be deduced. The fragment ions at m/z $[M+H-18]^+$, $[M+H-46]^+$, $[M+H-36]^+$, and $[M+H-64]^+$ are $[M+H-H_2O]^+$, $[M+H-H_2O-CO]^+$, $[M+H-2H_2O]^+$, and $[M+H-2H_2O-CO]^+$ ions, respectively. The ions at m/z $[M+H-54]^+$, $[M+H-82]^+$, and $[M+H-84]^+$ belong to $[M+H-3H_2O]^+$, $[M+H-3H_2O-CO]^+$ and $[M+H-3H_2O-H_2CO]^+$ ions. The ions at m/z $[M+H-150]^+$ and $[M+H-162]^+$ are $[AA-H+CH_2]^+$ and $[AA+H]^+$ ions, which derive from the cleavage of Amadori compounds between the sugar and amino acid moieties by pathway **a** or pathway **b**. According to the observed rules of cleavage, the proposed fragmentation pathways of Amadori compounds are summarized in Scheme 2.

After being further purified by high-performance liquid chromatography, the Amadori precursor ions have been selected in terms of their mass and then every selected ion has been subjected to MS^2. The cleavage rules were summarized from the ESI-MS/MS spectra. There are remarkable characteristic rules in the main fragmentation pathways of the positive ions of the Amadori compounds. The rules are typical for the analysis and identification of Amadori compounds. The positive ion ESI-MS/MS spectrometry is an excellent method for the study of Amadori compounds.

Acknowledgements

This research project was partly supported by Shanghai Postdoctoral Scientific Program (No. 06R214213).

Yours,

JUN WANG,[1,2]* YI-MIN LU,[2] BAI-ZHAN LIU[2] and HE-YONG HE[1]

[1] Department of Chemistry, Fudan University, Shanghai 200433, P. R. China

[2] Technical Center, Shanghai Tobacco (Group) Company, Shanghai 200082, P. R. China

References

1. Hodge JE. Chemistry of browning reaction in model systems. *Journal of Agricultural and Food Chemistry* 1953; **1**: 928.
2. Ledl F, Schleicher E. New aspects of the Maillard reaction in foods and in the human body. *Angewandte Chemie International Edition sin English* 1990; **29**: 565.
3. Hodge JE. The Amadori rearrangement. *Advances in Carbohydrate Chemistry and Biochemistry* 1955; **10**: 169.
4. Wrodnigg TM, Eder B. The Amadori and Heyns rearrangement: landmarks in the history of carbohydrate chemistry or unrecognized synthetic opportunities. *Topics in Current Chemistry* 2001; **215**: 115.
5. Martins S, Jongen W, Boekel M. A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science and Technology* 2001; **11**: 364.
6. Ames JM. Applications of the Maillard reaction in the food chemistry. *Food Chemistry* 1998; **62**: 431.
7. Blank I, Devaud S, Matthey-Doret W, Robert F. Formation of odorants in Maillard systems based on L-Proline as affected by PH. *Journal of Agricultural and Food Chemistry* 2003; **51**: 3643.
8. Fogliano V, Monti SM, Musella T, Randazzo G, Ritieni A. Formation of coloured Maillard reaction products in a gluten-glucose model system. *Food Chemistry* 1999; **66**: 293.
9. Homoki-Farkas P, Örsi F, Kroh LW. Methylglyoxal determination from different carbohydrates during heat processing. *Food Chemistry* 1996; **59**: 157.
10. Huyghues-Despointes A, Yaylayan VA, Keyhani A. Pyrolysis/GC/MS analysis of 1-[(2′-carboxy)pyrrolidinyl]-l-deoxy-D-fructose (Proline Amadori compound). *Journal of Agricultural and Food Chemistry* 1994; **42**: 2519.
11. Hofmann T, Bors W, Stettmaier K. Studies on radical intermediates in the early stage of the nonenzymatic browning reaction of carbohydrates and amino acids. *Journal of Agricultural and Food Chemistry* 1999; **47**: 379.
12. Yaylayan VA, Huyghues-Despointes A. Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions and spectroscopic properties. *Critical Reviews in Food Science and Nutrition* 1994; **34**: 321.
13. Davidek T, Clety N, Aubin S, Blank I. Degradation of the Amadori compound N-(1-Deoxy-D-fructos -1-yl)glycine in aqueous model systems. *Journal of Agricultural and Food Chemistry* 2002; **50**: 5472.
14. Eichner K, Reutter M, Wittmann R. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*, Finot PA, Aeschbacher HU, Hurrel RF, Liardon R (eds). Birkhäuser Verlag: Basel, 1990; 63.
15. Davidek T, Clety N, Devaud S, Robert F, Blank I. Simultaneous quantitative analysis of Maillard reaction precursors and products by high-performance anion exchange chromatography. *Journal of Agricultural and Food Chemistry* 2003; **51**: 7259.
16. Staempfli AA, Blank I, Fumeaux R, Fay LB. Study on the decomposition of the Amadori compound N-(1-Deoxy-D-fructos -1-yl)-glycine in model systems: quantification by fast atom bombardment tandem mass spectrometry. *Biological Mass Spectrometry* 1994; **23**: 642.
17. Hau J, Devaud S, Blank I. Detection of Amadori compounds by capillary eletrophoresis coupled to tandem mass spectrometry. *Electrophoresis* 2004; **25**: 2077.
18. Davidek T, Kraehenbuehl K, Devaud S, Robert F, Blank I. Analysis of Amadori compounds by high-performance cation exchange chromatography coupled to tandem mass spectrometry. *Analytical Chemistry* 2005; **77**: 170.