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The effect of glycation on the chemical and enzymatic stability of the endogenous opioid peptide, leucine–enkephalin, and related fragments

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Abstract

Nonenzymatic glycation is a posttranslational modification of peptides and proteins by sugars, which, after a cascade of reactions, leads to the formation of a complex family of irreversibly changed adducts implicated in the pathogenesis of human diseases. The stability of the Amadori compounds, the last reversible intermediates, determines the further course of the reaction. To provide information concerning the fate of glycated opioid peptides introduced into human circulation, the enzymatic (80% human serum) and chemical (phosphate buffer) stability of three Amadori compounds related to the endogenous opioid pentapeptide, leucine–enkephalin (Tyr-Gly-Gly-Phe-Leu), and to its N-terminal fragments: *N*-(1-deoxy-D-fructos-1-yl)-L-tyrosyl-glycyl-glycyl-L-phenylalanyl-L-leucine, *N*-(1-deoxy-D-fructos-1-yl)-L-tyrosyl-glycyl-glycine, and *N*-(1-deoxy-D-fructos-1-yl)-L-tyrosine were investigated. The results obtained in human serum indicate that N-terminal glycation of leucine–enkephalin significantly enhances proteolytic stability. While leucine–enkephalin itself was rapidly degraded ($t_{1/2} = 14.8$ min), the glycated-derivative was slowly converted ($t_{1/2} = 14$ h) to the corresponding Amadori compound of Tyr-Gly-Gly and Phe-Leu. In phosphate buffer, the rate of hydrolysis of the Amadori compounds depends on the structure and length of the peptide moiety as well as on the concentration of the phosphate buffer. The hydrolysis patterns for the Amadori compounds in phosphate buffer and in human serum were not the same and appear to be specific for each substrate.

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1. Introduction

Glycation, the nonenzymatic addition of reducing sugars to the amino groups of proteins, peptides, and amino acids is the first of three stages (early, intermediate, and late) of a complex reaction known as the Maillard reaction [1,2]. Its chemistry is responsible for many aspects of the color, flavor, and texture of processed foods [3] and, more importantly, the products of nonenzymatic glycation arising in vivo have been implicated in the pathogenesis of diabetic complications and in changes associated with aging. The early stage of the reaction is initiated by nucleophilic attack of the lone pair electrons of an amino nitrogen atom at C-1 of the open-chain form of a reducing sugar such as D-glucose to yield a Schiff base, which then undergoes an Amadori rearrangement to yield a ketoamine (i.e., Amadori compound) (Fig. 1) [4]. Glycation-induced pathological conditions result from further slow reactions of oxidation, cleavage, and rearrangements of the initially formed Amadori product producing irreversibly advanced glycation end products (AGE) which change the physical, chemical, and biochemical properties of proteins, thereby affecting their

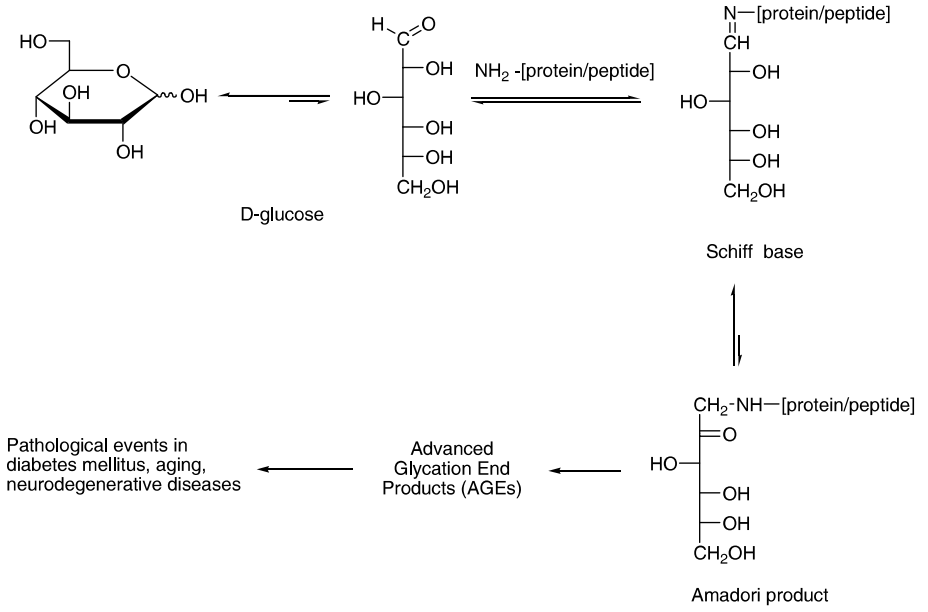

Fig. 1. Pathways for nonenzymatic glycation. The advanced glycation end products (AGEs) are generated via the Maillard reaction, which is initiated by condensation of the amino group of proteins or peptides with physiological concentrations of D-glucose, or other reducing sugars, to form Schiff bases. Subsequent rearrangement produces the so-called Amadori products. Subsequent chemical transformations lead to tissue damage.

function [5]. The production of AGEs increases with aging and their accumulation was demonstrated in different tissues and organs of the human body [6], wherever reducing sugars have contact with long-lived proteins. The excessive level of glucose in diabetes accelerates the formation of AGEs in comparison to normal aging. AGEs are considered the key pathophysiological event in the onset and progression of diabetic microvascular, macrovascular, and nonvascular complications [6], producing a wide range of disorders such as diabetic nephropathy, retinopathy, neuropathy, as well as coronary, cerebrovascular, and peripheral vascular diseases. In addition, the formation of AGEs has been reported as a risk factor in the development of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis [7].

Although the complications in diabetes and other AGE-related diseases have historically been attributed to the glycation of structural proteins, recent attention has been focused on possible glycation and impaired function of relatively short-lived important peptide hormones. Substantial quantities of glycated insulin and gastric inhibitory polypeptide (GIP) were detected in plasma and biological tissues of

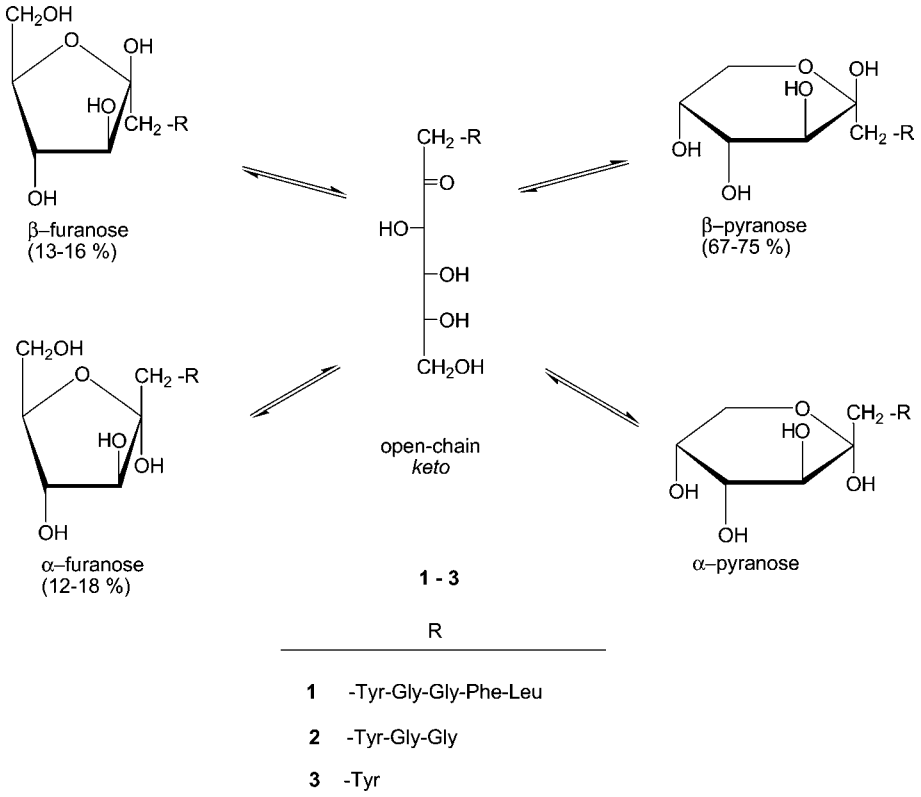

Fig. 2. Structures of Amadori compounds **1**–**3**. The equilibrium among the tautomeric forms was calculated from the intensities of the respective C-2 signals of the sugar moiety in ^{13}C NMR spectra [15].

normal and diabetic subjects [8,9]. Glycation of insulin interferes with its normal cellular functions and results in decreased biological activity [10].

The work reported here was prompted by the observation that hyperglycemia contributes to defective function of the endogenous opioid system [11]. So far, no attention has been given to the possibility that glycation of opioid peptide hormones may contribute to the observed dysfunctions. Here we describe the enzymatic and chemical stability of Amadori compounds **1**–**3** (Fig. 2), comprising a glycated specimen of the opioid pentapeptide leucine–enkephalin (Tyr-Gly-Gly-Phe-Leu) [12] and two structurally related smaller fragments. Previous studies of the Amadori compound **1** have shown that glycation of leucine–enkephalin at the N-terminus induces changes in the peptide conformation and results in a total loss of in vitro opioid activity [13]. In addition, AGE-associated fluorescence was detected when Amadori compounds **1**–**3** were incubated in phosphate buffer [14]. The relative concentrations of AGE fluorophores appear to be related to peptide (amino acid) structure, decreasing in the order $\mathbf{3} \gg \mathbf{2} > \mathbf{1}$. Since understanding the reactivity of the early glycation products, such as Amadori compounds, and the mechanisms of their further reactions is critical in controlling AGE product formation in physiological systems, we also report the stability and breakdown products obtained from Amadori compounds **1**–**3** in human serum, and compare them to the corresponding results in phosphate buffer.

2. Materials and methods

2.1. Materials

Human serum, leucine–enkephalin, Tyr-Gly-Gly, Phe-Leu, *p*-nitro-L-phenylalanine, captopril, human serum albumin (HSA), and $MgCl_2 \cdot 6H_2O$ were obtained from Sigma–Aldrich Chemical (St. Louis, MO, USA). *o*-Hydroxyphenylacetic acid was obtained from Fluka (Buchs, Switzerland). The syntheses of the Amadori compounds, *N*-(1-deoxy-D-fructos-1-yl)-L-tyrosyl-glycyl-glycyl-L-phenylalanyl-L-leucine (**1**), *N*-(1-deoxy-D-fructos-1-yl)-L-tyrosyl-glycyl-glycine (**2**), *N*-(1-deoxy-D-fructos-1-yl)-L-tyrosine (**3**), and their chemical characterization are described elsewhere [15]. The compounds were purified by reversed-phase high performance liquid chromatography (RP-HPLC), (Varian 9010, UV-detector 9050) using a Eurospher 100 reversed-phase C-18 semipreparative column (250×8 mm ID, 5 μm) and mobile phase A (43.5% MeOH/0.1% TFA) for Amadori compound **1** and B (15% MeOH/0.1% TFA) for Amadori compounds **2** and **3**. The flow rate was 1 ml/min. UV detection was carried out at 280 nm. HPLC effluents containing the purified product were pooled and evaporated in vacuo. Excess organic salts, present after preparative HPLC, were removed using an octadecylsilica solid-phase extraction (SPE) cartridge (500 mg, 2.8 ml). In brief, the product was dissolved in water and added to the equilibrated cartridge. The cartridge was eluted with water in order to remove the salt. The Amadori compounds were than recovered with methanol. The effluent was evaporated, dissolved in water and lyophilized.

2.2. Stability in serum and phosphate buffer

For serum stability studies, a mixture of human serum [2 ml of 80% (v/v), diluted with H_2O], the Amadori compounds (**1**–**3**) or the peptides, leucine–enkephalin, Tyr-Gly-Gly or Phe-Leu (8×10^{-4} M), and an internal standard [*o*-hydroxyphenylacetic acid (40 μg/ml) for **1**, leucine–enkephalin and Phe-Leu; or *p*-nitro-L-phenylalanine (40 μg/ml) for Amadori compounds **2**, **3**, and Tyr-Gly-Gly] was kept at 37 °C in a teflon lined screw-cap test tube. For serum stability of Amadori compound **1** in the presence of protease inhibitor, captopril at two different concentrations (1×10^{-5} and 5×10^{-5} M) was preincubated with 80% human serum for 10 min prior to addition of compound **1**. Three samples (100 μl) were removed at appropriate intervals and deproteinized by the addition of 20 μl of 48% aqueous TFA. The samples were briefly vortexed and frozen. The thawed samples were centrifuged for 10 min (15,000*g*) and the supernatants were analyzed by RP-HPLC.

The decomposition of the Amadori compounds in buffer solution was studied in triplicate, at 37 °C, under sterile conditions. Solutions of **2** or **3** (8×10^{-4} M) containing the internal standard, *p*-nitro-L-phenylalanine (40 μg/ml) and NaN_3 (0.02%) were prepared in phosphate buffers/0.1 M NaCl (pH 7.4) (PBS) of three different concentrations (0.05, 0.01, and 0.0008 M). For the determination of the stability of Amadori compounds in the presence of metal ions, to the solution of compound **3** (8×10^{-4} M) in either 0.05 M or 0.0008 M PBS was added equimolar amounts of magnesium (II) chloride at the beginning of the incubation time. The solutions were sterilized by passage through a 0.45 μm nylon filter and incubated in the dark, at 37 °C, in teflon lined screw-cap test tubes. At appropriate times, samples of the reaction mixtures were removed and chromatographed immediately.

The effect of human serum albumin (HSA) on the stability of Amadori compounds was analyzed by incubation of compounds **2** or **3** (8×10^{-4} M) in 0.0008 M PBS containing HSA (50 mg/ml), internal standard (*p*-nitro-L-phenylalanine) (40 μg/ml), and NaN_3 (0.02%). The samples were collected and deproteinized in the same way as described for the samples in the 80% human serum.

The concentration of compounds in the incubation mixtures was monitored by RP-HPLC on a Eurospher 100 reversed-phase C-18 analytical column (250 × 4 mm ID, 5 μm), eluted at a flow rate of 0.5 ml/min with solvent A for Amadori compound **1**, leucine–enkephalin, and Phe-Leu and with solvent B for Amadori compounds **2**, **3**, and Tyr-Gly-Gly using a HP 1090 system equipped with a Diode Array Detector. UV detection was performed at 280 nm and at 215 nm. The Amadori compound or peptide concentration of samples (in triplicate) was determined by electronic integration of peak areas and calculation of analyte/internal standard peak-areas.

3. Results and discussion

The enzymatic stability of the leucine–enkephalin Amadori compound **1** and of structurally related peptides was determined in 80% human serum. The relative

amounts of intact compound **1** and of hydrolysis by-products are presented in Fig. 3. The half-lives of hydrolysis observed are listed in Table 1. As can be seen from the data presented in Fig. 3, Amadori compound **1** was slowly degraded with an estimated half-life of 14 h. The main metabolites were Tyr-Gly-Gly-related Amadori compound **2** and Phe-Leu. Only Phe-Leu was observed to be further degraded to the respective amino acids, while the other metabolite, Amadori compound **2**, showed no sign of degradation over the time span investigated (4 days). Under these conditions neither Tyr-related compound **3** nor the dipeptide Tyr-Gly-related Amadori compound [16] were identified as degradation products formed in human serum from compound **1**. The half-life determined for Amadori compound **1** in human serum is considerably shorter (by a factor of ~15) than the half-life reported earlier for **1** in 0.05 M phosphate buffer at pH 7.4 and 37 °C ($t_{1/2} = 8.7$ days) [14]. We have shown that in the aqueous model system, under physiological conditions, the main degradation pathways involve the enolization [17] of the open-chain form of the pentapeptide-related Amadori compound **1** (Fig. 2) leading to the slow formation of reactive intermediates, which is accompanied by release of the parent peptide, leucine–enkephalin (Tyr-Gly-Gly-Phe-Leu).

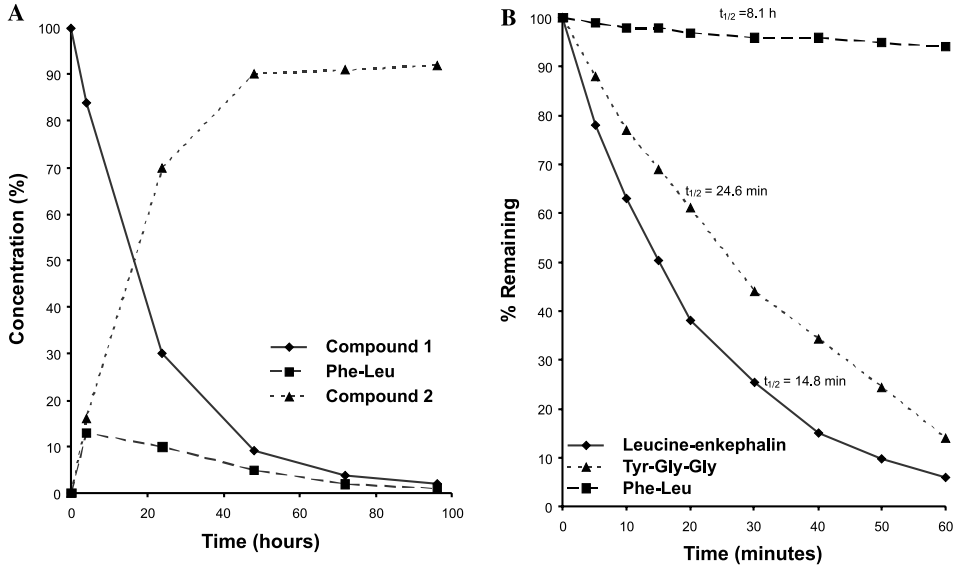

Fig. 3. Degradation of Amadori compound **1** and of structurally related peptides in 80% human serum. (A) Degradation of leucine–enkephalin-related Amadori compound **1** (◆) and formation of metabolites Phe-Leu (■) and compound **2** (▲). (B) Rate of degradation of leucine–enkephalin (◆), Tyr-Gly-Gly (▲), and Phe-Leu (■). The incubation temperature was 37 °C. The initial concentration of the Amadori compound or of the peptides was 8×10^{-4} M. Samples were collected in triplicate, deproteinized with trifluoroacetic acid, and centrifuged. The supernatant was then analyzed by RP-HPLC measuring the ratio between the area of the peak corresponding to the analyte and the peak area of *o*-hydroxyphenylacetic acid or *p*-nitro-L-phenylalanine, previously added to serum as internal standards.

Table 1
Half-lives of hydrolysis of Amadori compounds **1**–**3** in human serum and in phosphate (0.05–0.0008 M, pH 7.4) buffered 0.1 M saline (PBS) at 37 °C

Media	Compound ($t_{1/2}$)		
	1	**2**	**3**
80% human serum	14.0 h	Stable[a]	Stable[a]
80% human serum (10 μM captopril)	1.8 days		
80% human serum (50 μM captopril)	4.8 days		
PBS 0.05 M	8.7 days[b]	2.2 days	18.8 h
PBS 0.05 M (800 μM $MgCl_2$)	ND	ND	6.5 h
PBS 0.01 M	ND	5.7 days	30.1 h
PBS 0.0008 M	ND	9.5 days	40.3 h
PBS 0.0008 M (800 μM $MgCl_2$)	ND	ND	16.3 h
PBS 0.0008 M (HSA 50 mg/ml)	ND	Stable[c]	Stable[d]

ND, not determined.
[a] Stable within a period of 4 days.
[b] Data taken from [14].
[c] Eight percentage of the compound **2** hydrolyzed within a period of 4 days.
[d] Five percentage of the compound **3** hydrolyzed within a period of 4 days.

Enkephalins are rapidly degraded in organisms by various enzymes as illustrated in Fig. 4. Metabolism is dependent on both species and tissue type [18]. At least seven enzymes hydrolyzing different peptide bonds in the enkephalin molecule have been found in human blood plasma [19]. These include aminopeptidases, dipeptidyl aminopeptidases, and dipeptidyl carboxypeptidases. The predominant (~80%) route for the degradation of leucine–enkephalin is reportedly the hydrolysis of the N-terminal Tyr^1-Gly^2 bond by aminopeptidases [18]. In agreement with these results, we found

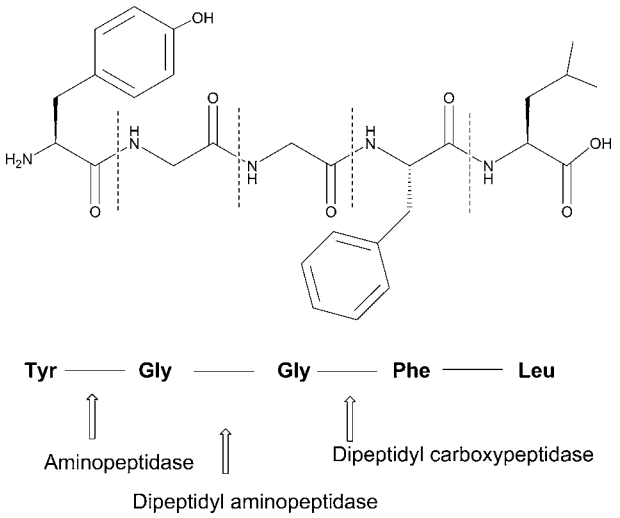

Fig. 4. Enzymatic degradation pathways for leucine–enkephalin in human serum. The arrows indicate the cleavage positions.

rapid leucine–enkephalin disappearance ($t_{1/2} = 14.8$ min) in 80% human serum (Fig. 3B), parallel by the appearance of Tyr and peptides whose amino acid composition is consistent with the N-terminal leucine–enkephalin hydrolysis by-products. The serum half-life of leucine–enkephalin determined here is higher than published earlier for human plasma. However, this difference can be related to the origin of the serum, since large variations in the plasma half-life of leucine–enkephalin, dependent on the batch and treatment of the plasma used, have been reported [20]. Also, as the degradation of leucine–enkephalin proceeded, the amount of Tyr-Gly-Gly formed decreased much faster than that of Phe-Leu which reached a maximum after 4 h, and decreased there after. In fact, a comparison of the enzymatic stability of those peptides in 80% human serum (Fig. 3B) showed large differences in the rate of hydrolysis. Specifically, the half-lives found for Tyr-Gly-Gly and Phe-Leu were 24.6 min and 8.1 h, respectively. Since Phe-Leu is not a substrate for carboxypeptidases [21], it is assumed that the observed difference in the reaction rates is attributable to the structure of the peptide, and to the type and concentration of the individual aminopeptidase involved in the degradation reaction.

The results of the degradation of leucine–enkephalin-derived Amadori compound **1** in human serum revealed that glycation of leucine–enkephalin at the N-terminal position with a ketose moiety fully stabilizes the parent opioid peptide against cleavage by aminopeptidase(s). The consequences of this derivatization are that the N-terminal amino group is transformed into a secondary amino group and the Tyr^1-Gly^2 bond becomes *N*-alkylated, making the glycated pentapeptide a poor substrate for aminopeptidases. However, compound **1** turned out to be degraded by dipeptidyl carboxypeptidase(s), which cleaves the Gly^3-Phe^4 bond in leucine–enkephalin, playing a minor although significant role in degrading enkephalins in human plasma [18]. The involvement of dipeptidyl carboxypeptidase(s) in degradation of leucine–enkephalin-related Amadori compound **1** was demonstrated by using the specific inhibitor captopril (Table 1). Addition of captopril to human serum at two different concentrations significantly reduced the formation of Tyr-Gly-Gly-related Amadori compound **2**. In the presence of 10 and 50 μM captopril, 83 and 90%, respectively, of compound **1** remained intact after 14 h incubation in human serum. Identification of the main metabolites in the degradation of **1** also suggest no involvement of the recently discovered FN3K (fructosamine 3-kinase) and FN3KRP (FN3K-related protein) in enzymatic deglycation. These enzymes catalyze the breakdown of lysine-related Amadori compounds on proteins by the formation of fructosamine 3-phosphates, which subsequently decompose and regenerate unmodified lysine residues [22,23].

The observation that the main metabolite of **1** in serum, identified as the Tyr-Gly-Gly-related Amadori compound **2**, undergoes no further degradation was confirmed by the stability in 80% human serum over a period of 4 days (Table 1). From a chemical point of view this result was unexpected. This observation raises the question of why the chemical hydrolysis of compound **2** in serum to the parent tripeptide Tyr-Gly-Gly is not subject to enzymatic degradation by the aminopeptidase to Tyr and Gly-Gly. To address this question, a series of reaction mixtures was prepared containing identical amounts of Amadori compounds but various concentrations of

phosphate ions in 0.1 M saline (PBS) in order to determine the effect of buffer concentration on the chemical stability of tripeptide-derived Amadori compound **2** as well as of tyrosine-analog **3**, at pH 7.4 and 37 °C. Experiments were conducted in 0.05, 0.01, as well as in 0.0008 M PBS, to mimic the physiological phosphate concentration in 80% human serum. Table 1 summarizes the half-lives of Amadori compounds **2** and **3** determined at various phosphate concentrations. In agreement with previous results [24], we found that phosphate buffer catalyzes the degradation of Amadori compounds **2** and **3**. This catalytic effect was concentration dependent, as evidenced by the increased half-lives of Amadori compounds in 0.0008 M as compared to 0.05 M phosphate. These observations are consistent with the evidence that phosphate buffer increases the proportion of the open-chain, reactive, form of reducing sugars [25], and promotes the enolization of sugars in solution [26]. Phosphate buffer also catalyzes reactions during later steps of the Maillard reactions, as measured by the kinetics of development of brown color and fluorescence [24]. Although the initial structure of the reducing sugar has an important bearing on the chemical stability of the initially formed Amadori products, the data in Table 1 also indicate that the rates of decomposition of Amadori compounds **1**–**3** depend on the structure and the length of the peptide moiety. Amadori compound **1** derived from a pentapeptide is much more stable than the analogues derived from smaller peptides or a single amino acid. The chemical stability decreases in the order $\mathbf{1} > \mathbf{2} > \mathbf{3}$.

As presented in Fig. 5A, no degradation of Amadori compound **2** was observed in 80% human serum after 4 days, at 37 °C. In contrast, ~30% of **2** was hydrolyzed over the same period in 0.0008 M PBS, indicating higher chemical stability of the examined compound in human serum. An even faster degradation in PBS is seen for tyrosine-derived compound **3**. Almost 75% of **3** was hydrolyzed in 0.0008 M PBS at the end of the 4-day incubation period (Fig. 5B), whereas no hydrolysis occurred in 80% human serum under identical experimental conditions. One possible explanation for the absence of chemical hydrolysis of compounds **2** and **3** in human serum is suggested by the findings that Amadori compounds have the ability to complex metal ions [27,28]. If the complexation between Amadori compounds and metal ions involved the cyclic pyranose form of the sugar moiety [28], it could be argued that the open-chain form, which is required for hydrolysis, is less likely to be available for enolization and subsequent amino acid/peptide release. Another explanation may be that the studied Amadori compounds bind to serum proteins, such as serum albumin, as previously postulated for leucine–enkephalin [29] and its imidazolidinone prodrugs [30]. To shed some light on these possibilities, the degradation of Amadori compounds **2** and **3** was investigated in the presence of either magnesium ions, or in the presence of human serum albumin (HSA). Surprisingly, the addition of equimolar amounts of Mg^{2+} considerably accelerated the degradation rate of compound **3** in phosphate buffer (Table 1). While 40 h was required to degrade 50% of **3** in 0.0008 M PBS at 37 °C, only 16 h were needed to reach the same degree of degradation in the presence of Mg^{2+} ions. A similar effect of Mg^{2+} on the degradation of **3** was observed at increased phosphate concentration. Interestingly, addition of HSA at the concentration similar to that found in human serum, almost completely protected Amadori

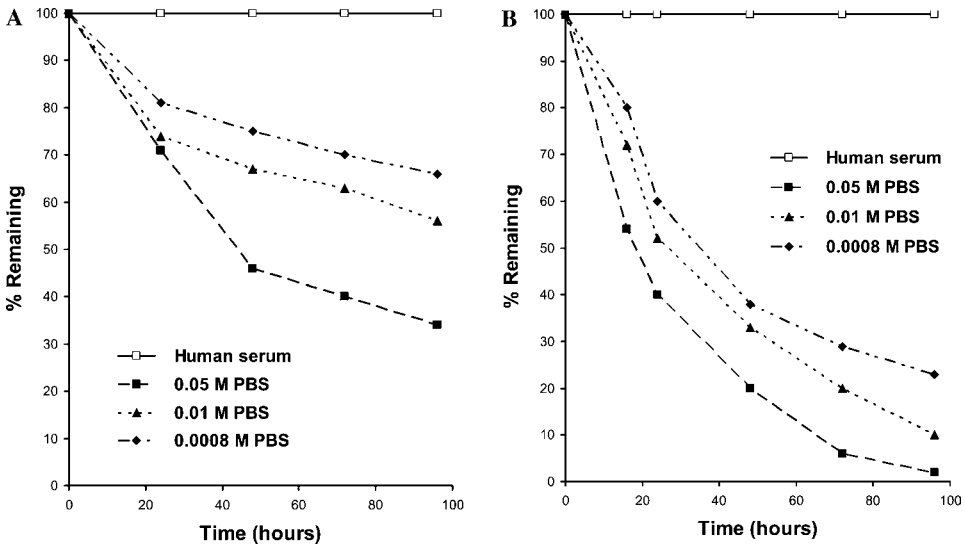

Fig. 5. Kinetics of degradation of Amadori compounds **2** (A) and **3** (B) in 0.05 M (■), 0.01 M (▲), and 0.0008 M (◆) phosphate buffer/0.1 M NaCl (PBS) (pH 7.4), and in 80% human serum (□) at 37 °C. The initial concentration of the Amadori compounds was 8×10^{-4} M. Samples (in triplicate) incubated in phosphate buffer were taken at appropriate time intervals and analyzed by RP-HPLC. Samples from human serum were analyzed as described in Fig. 3. The concentration of the Amadori compounds was determined by integration of peak areas and calculation of analyte/internal standard (*p*-nitro-L-phenylalanine) peak area ratios.

compounds **2** and **3** from the hydrolysis in phosphate buffer. Only 8 and 5% of compounds **2** and **3** were hydrolyzed after 4 days incubation at 37 °C in 0.0008 M PBS containing HSA (Table 1). The fact that compounds **2** and **3** were recovered unchanged under our experimental conditions suggests that interactions between HSA and Amadori compounds are mostly ionic in kind.

In conclusion, the stability of well-defined Amadori compounds has been investigated in serum. The data obtained indicate that glycation of the endogenous opioid pentapeptide leucine–enkephalin protects the N-terminal amino acid residue of enkephalin from enzymatic cleavage by aminopeptidases, such as those present in human serum. The fact that degradation of compound **1** was significantly reduced in the presence of captopril indicates that the dipeptidyl carboxypeptidases are mainly involved in the hydrolysis of the pentapeptide-derived Amadori compound. Additionally, evidence is obtained indicating human serum albumin-bound Amadori compounds derived from small peptide fragments or amino acids are almost completely protected from hydrolysis. Taken together, our results suggest that the accumulation of the unchanged low molecular weight Amadori compounds under physiological conditions due to binding to serum proteins might have important effects on protein backbone conformation, rates of metabolism, interaction with target molecules, and excretion.

Acknowledgment

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References

[1] F. Ledl, E. Schleicher, Angew. Chem. Int. Ed. Engl. 29 (1990) 565–594.
[2] J.A. Gerrard, Aust. J. Chem. 55 (2002) 299–310.
[3] V.A. Yaylayan, Food Sci. Technol. Res. 9 (2003) 1–6.
[4] Š. Horvat, A. Jakas, J. Peptide Sci. 10 (2004) 119–137.
[5] P.A.C. Cloos, S. Christgau, Matrix Biol. 21 (2002) 39–52.
[6] A.W. Stitt, A.J. Jenkins, M.E. Cooper, Expert Opin. Investig. Drugs 11 (2002) 1–19.
[7] S. Kikuchi, K. Shinpo, M. Takeuchi, S. Yamagishi, Z. Makita, N. Sasaki, K. Tashiro, Brain Res. Rev. 41 (2003) 306–323.
[8] J.R. Lindsay, A.M. McKillop, M.H. Mooney, F.P.M. O'Harte, P.M. Bell, P.R. Flatt, Diabetologia 46 (2003) 475–478.
[9] M.H. Mooney, Y.H.A. Abdel-Wahab, L.M. Morgan, F.P.M. O'Harte, P.R. Flatt, Biochim. Biophys. Acta Gen. Subj. 1569 (2002) 75–80.
[10] A.C. Boyd, Y.H.A. Abdel-Wahab, A.M. McKillop, H. McNulty, C.R. Barnett, F.P.M. O'Harte, P.R. Flatt, Biochim. Biophys. Acta Gen. Subj. 1523 (2000) 128–134.
[11] F. Fallucca, G. Tonnarini, N. Di Biase, M. D'Alessandro, M. Negri, Metab. Clin. Exp. 45 (1996) 1065–1068.
[12] R.J. Bodnar, M.M. Hadjimarkou, Peptides 23 (2002) 2307–2365.
[13] Š. Horvat, Curr. Med. Chem. CNS Agents 1 (2001) 133–154.
[14] A. Jakas, Š. Horvat, Biopolymers 69 (2003) 421–431.
[15] A. Jakas, Š. Horvat, J. Chem. Soc. Perkin Trans. 2 (1996) 789–794.
[16] L. Varga-Defterdarović, G. Hrlec, Carbohydr. Res. 339 (2004) 67–75.
[17] S.I.F.S. Martins, A.T.M. Marcelis, M.A.J.S. van Boekel, Carbohydr. Res. 338 (2003) 1651–1663.
[18] F. Bolacchi, M. Marini, A. Urbani, L.G. Roda, Neurochem. Res. 20 (1995) 991–999.
[19] M. Marini, G. Roscetti, L. Bongiorno, A. Urbani, L.G. Roda, Neurochem. Res. 15 (1990) 61–67.
[20] M. Marini, A. Urbani, E. Trani, L. Bongiorno, L.G. Roda, Peptides 18 (1997) 741–748.
[21] H. Ostrowska, Thromb. Res. 86 (1997) 393–404.
[22] G. Delpierre, E. Van Schaftingen, Biochem. Soc. Trans. 31 (2003) 1354–1357.
[23] B.S. Szwergold, P.J. Beisswenger, Biochem. Soc. Trans. 31 (2003) 1428–1432.
[24] D.V. Zyzak, J.M. Richardson, S.R. Thorpe, J.W. Baynes, Arch. Biochem. Biophys. 316 (1995) 547–554.
[25] H.S. Burton, D.J. McWeeny, Nature 197 (1963) 266–268.
[26] P. Thornalley, S. Wolff, J. Crabbe, A. Stern, Biochim. Biophys. Acta 797 (1984) 276–287.
[27] M. Tonković, A. Jakas, Š. Horvat, Biometals 9 (1996) 55–59.
[28] J. O'Brien, P.A. Morrissey, Food Chem. 58 (1997) 17–27.
[29] V. De Marco, R. Possenti, F. Vita, B. Rapposelli, M. D'Alagni, L.G. Roda, Neurochem. Res. 10 (1985) 1355–1369.
[30] A. Bak, M. Fich, B.D. Larsen, S. Frokjaer, G.J. Friis, Eur. J. Pharm. Sci. 7 (1999) 317–323.