Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 336 (2005) 36–41
BBRC
www.elsevier.com/locate/ybbrc

Carnosine and anserine act as effective transglycating agents in decomposition of aldose-derived Schiff bases ☆

Benjamin S. Szwergold *

Department of Medicine, Dartmouth Medical School, Hanover, NH, USA

Received 15 July 2005
Available online 15 August 2005

Abstract

There are numerous publications describing the positive effects of carnosine (β-alanyl-histidine) and anserine (β-alanyl-1-*N*-methyl-histidine) on cell and organ function. Of special interest to us is the fact that these dipeptides act to retard and (in one instance) reverse non-enzymatic glycation. To date, the primary explanation for these anti-glycating effects has been the fact that carnosine and anserine can serve as alternative and competitive glycation targets, thereby protecting proteins from this deleterious process. In this paper, we document another mechanism by which these two peptides can retard or reverse glycation. The process involves decomposition of the very first intermediates of the non-enzymatic glycation cascade (aldosamines a.k.a. Schiff bases) by nucleophilic attack of carnosine and/or anserine on the preformed aldosamine such as glucosyl-lysine. If future research shows this reaction is to be physiologically important, this mechanism could explain some of the beneficial effects of carnosine and anserine as anti-glycating agents.

Keywords: Carnosine; Anserine; Non-enzymatic glycation; Deglycation; Transglycation; β-Alanine; Aldosamine; Ketosamine

Non-enzymatic glycation is believed to play an important role in the evolution of diabetic complications. Until fairly recently, this process was believed to be entirely non-enzymatic and irreversible. With the discovery of fructosamine-3-kinase (FN3K) [1–3] and evidence that this kinase functions as a deglycating enzyme (Fig. 1) [8–10], it is becoming clear that the unavoidable non-enzymatic glycation process is counteracted in vivo by active deglycation mechanisms. However, as indicated in Fig. 1, in addition to glucose, non-enzymatic glycation in vivo can be mediated by other carbonyl-containing compounds. These include: phosphorylated glycolytic and pentose shunt intermediates [11,12], dicarbonyls such as methylglyoxal and 3-deoxyglucosone [13,14], and oxidized lipids such as 4-hydroxynonenal and malondialdehyde [15,16]. Since FN3K-mediated deglycation can deal only with glucose-derived intermediates, this leaves open the question of how cells and organisms can deal with a wide variety of glycation products derived from other sources.

In order to address this issue, we have proposed a FN3K-independent deglycation system involving removal, by transglycation, of sugar moieties from Schiff bases by a variety of biological nucleophiles including: free amino acids, thiols, and peptides such as glutathione (Fig. 2) [17,18]. With the addition of this mechanism, the glycation/deglycation scheme of Fig. 1 becomes

☆ *Abbreviations:* 3DG, 3-deoxyglucosone; AGE, advances glycation end product; G-β-ALa, glucosyl-β-alanine; G-Ans, glucosyl-anserine; G-Car, glucosyl-carnosine; α-G-E, α anomer of glucosyl-ethylamine; β-Glc, β anomer of glucosyl-ethylamine; G-His, glycosyl-histidine; G-β-Ala, glucosyl-β-alanine; α-Glc, α anomer of glucopyranose; β-Glc, β anomer of glucopyranose; FL3P, fructoselysine-3-phosphate; FN3K, fructosamine-3-kinase; G-E, glucosyl-ethylamine; ROS, radical oxygen species.

* Fax: +1 603 650 1848.
E-mail address: Szwergold@Dartmouth.edu.

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.08.033

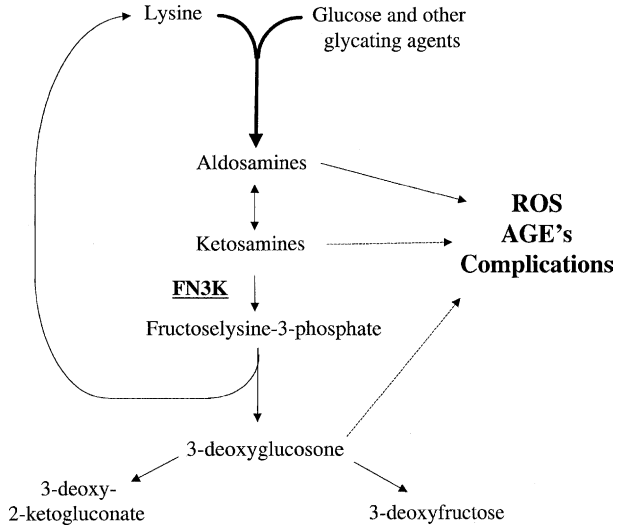

Fig. 1. Proposed role of FN3K as a catalyst in the decomposition of FL. Phosphorylation of FL to fructose-lysine-3-phosphate destabilizes the fructose-lysine linkage and leads to a spontaneous decomposition of FL to lysine, inorganic phosphate, and 3-deoxyglucosone (3DG). The potentially toxic 3DG is neutralized by reduction to 3-deoxyfructose [4,5] and oxidation to 2-keto-3-deoxygluconic acid [6,7].

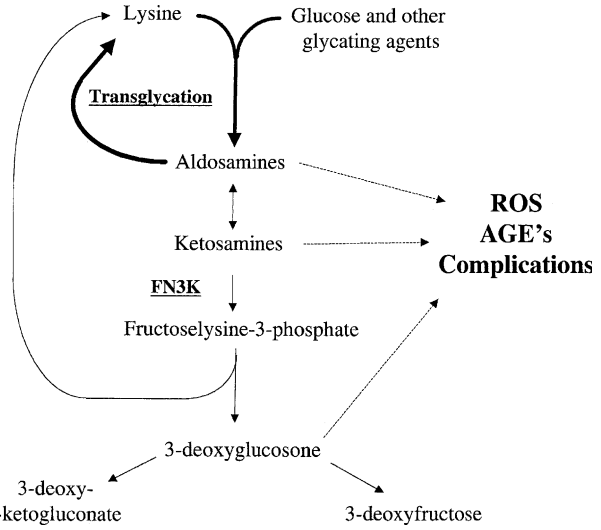

Fig. 3. Modified glycation/deglycation model of the Maillard reaction in the intracellular environment taking into account both the FN3K-mediated deglycation and a Schiff's-base deglycating mechanism. As in Fig. 1, the bidirectional arrows indicate reversible reactions and the dashed lines indicate a decreased flux of metabolites to AGE's due to the deglycation. In this scheme, with an active deglycation of aldosamines, the flux from this intermediate to AGE's is reduced significantly relative to what it would be in Fig. 1.

modified to a more comprehensive and effective deglycation system depicted in Fig. 3.

As described [17,18], we confirmed the feasibility of such a mechanism by conducting transglycation experiments in vitro with a variety of biological amines and thiols and have, by detection of glucose-cysteine in urine, obtained preliminary data suggesting that this mechanism is indeed operative in vivo.

The purpose of the current study was to examine the transglycating potential of two peptides, carnosine (β-alanyl-histidine) and anserine (β-alanyl-1-*N*-methyl-histidine), which are found in high concentrations in skeletal muscles (up to 20 mM) [19], some neural tissues (up to 2 mM) [20], and lenses (up to 0.3 mM) [21]. These compounds appear to have many extremely impressive and salutary effects on cell function including, among others: anti-oxidant activity [21,22], protection of cell membranes [23,24], delay of cell senescence [25], promotion of wound healing [26,27], and activity as a cardioprotective agent [28,29]. Especially relevant for our investigations are the numerous reports on the anti-glycating properties of these compounds [30–32], the most striking being their ability to reverse pre-existing glycation [33].

Results of our experiments indicate that both carnosine and anserine act as efficient transglycating agents. This suggests that some of the positive effects of these peptides in inhibiting non-enzymatic glycation and improving cell function may be due to their transglycating potential.

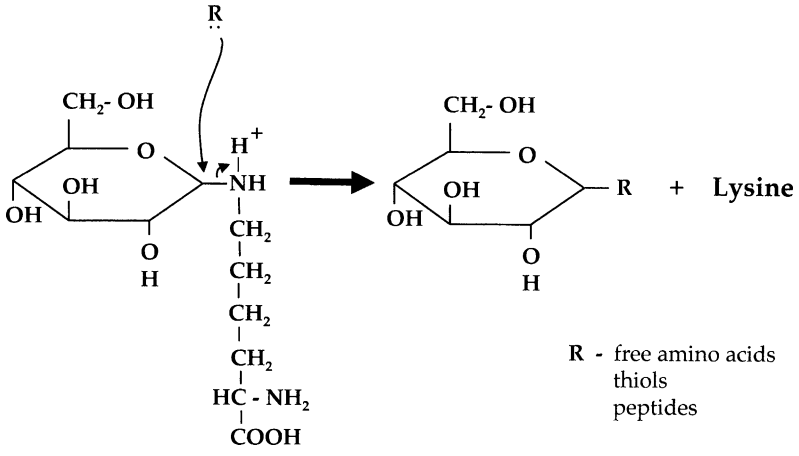

Fig. 2. Postulated mechanism of Schiff's base deglycation. The effectiveness of the scavenging compounds depends on their nucleophilicity which is, in large measure, a function of the pK_a's of their primary or secondary amines.

Methods

Glucose-ethylamine (G-E) was synthesized by incubating 500 mM glucose and ethylamine at pH 12 and 37 °C for 3 h. At the end of the incubation period, about 75% of the starting material was converted to glucose-ethylamine existing in equilibrium with the starting materials.

Transglycation reaction conditions. Since Schiff bases are unstable at physiological pH and temperature, our experiments were conducted under conditions which stabilized Schiff base enough to be able to observe them by NMR over several hours. The reaction mixture (0.5 ml in a 5 mm NMR tube) included 250 mM Hepes, pH 8.5, 10% D_2O (for the purpose of locking the NMR magnetic field) and 20 mM concentration of a compound of interest such as carnosine. The reaction was performed at room temperature and it was initiated by adding an aliquot of G-E to produce a final concentration of 20 mM at which time consecutive NMR spectra of 20 min duration were acquired using 580 scans, 60° pulses, and an interpulse delay of 2.05 s.

NMR. spectra were obtained on the Varian Unity-300 and -500 MHz machines at the Dartmouth Department of Chemistry. Spectra were analyzed using information from model compounds and chemical shift data from previously published papers [34,35].

Results

A study of the reaction involving Schiff bases is intrinsically difficult to perform due to the lability of such compounds at physiological pH's. Because of this fact, the method of choice for following Shiff base reaction unambiguously and in real-time is ^{13}C NMR spectroscopy. However, in order to utilize this technique one needs model compounds labeled with ^{13}C at an appropriate site.

Consequently, in order to test the feasibility and characteristics of the proposed transglycation reaction, we synthesized glucose-ethylamine (G-E) labeled with ^{13}C at the C-1 position of the glucose moiety and with ^{15}N at the amino group of the ethylamine (Fig. 4) as a model compound of glucosyl-lysine. In this compound, due to the spin-spin coupling between the nuclei of neighboring ^{15}N and ^{13}C atoms, the ^{13}C-labeled C-1 peak of glucose appears in the ^{13}C NMR spectrum as a doublet resonating at 90.00 ppm (Fig. 5). The unique signature doublet in the spectrum of this compound, its chemical shift as well, and the distinct ^{13}C chemical shifts of the other glucose-nucleophile adducts have allowed us to follow reactions between G-E and the various nucleophiles in real-time since cleavage of the ethylamine-glucose bond results in loss of the G-E doublet and the concomitant formation of new peaks corresponding to the new glucose-nucleophile adducts. This is illustrated in Fig. 6, which shows the end-products of transglycation of G-E by histidine, β-alanine, carnosine, and anserine after three hours of incubation. The transglycation reaction is due to the aliphatic amine in each of these compounds since incubation of G-E with imidazole does not produce any new products.

The kinetics of the transglycation reaction with carnosine, and related compounds, are illustrated in Fig. 7. It should be noted that the kinetics of decomposition of G-E in the control reaction and in the presence of imidazole are indistinguishable, confirming that the imidazole does not interact directly with G-E. However,

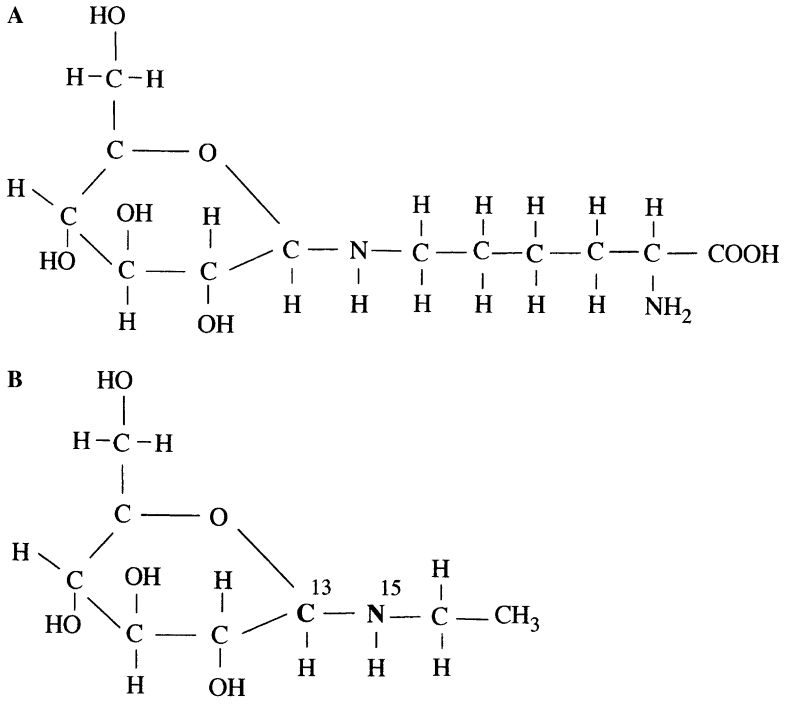

Fig. 4. Structures of (A) glucose-lysine (GL) and (B) the model compound of GL; [1-^{13}C, ^{15}N]glucosylethylamine [GE]. The ^{13}C and ^{15}N-labeled atoms are indicated as C-13 and N-15.

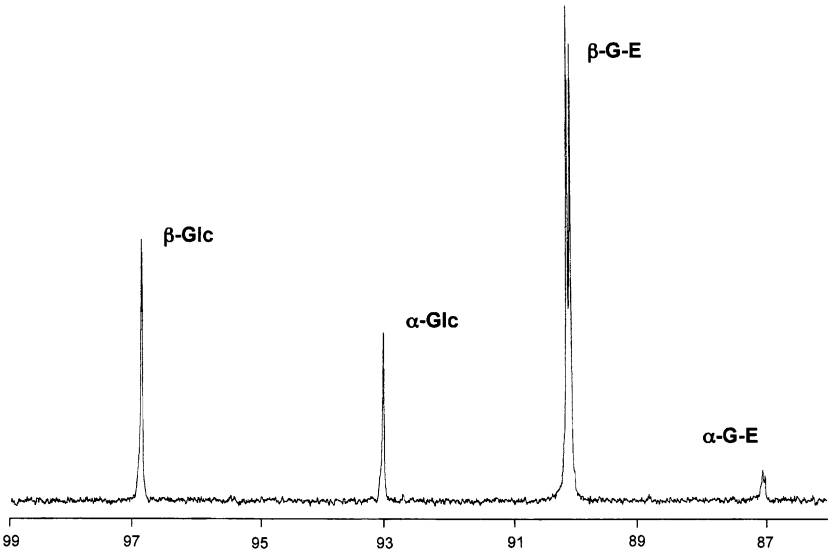

Fig. 5. ^{13}C NMR spectrum of 20 mM [1-^{13}C, ^{15}N]G-E at pH 12.0. Even at this alkaline pH, which favors the Schiff bases, G-E is found in equilibrium with glucose and ethylamine.

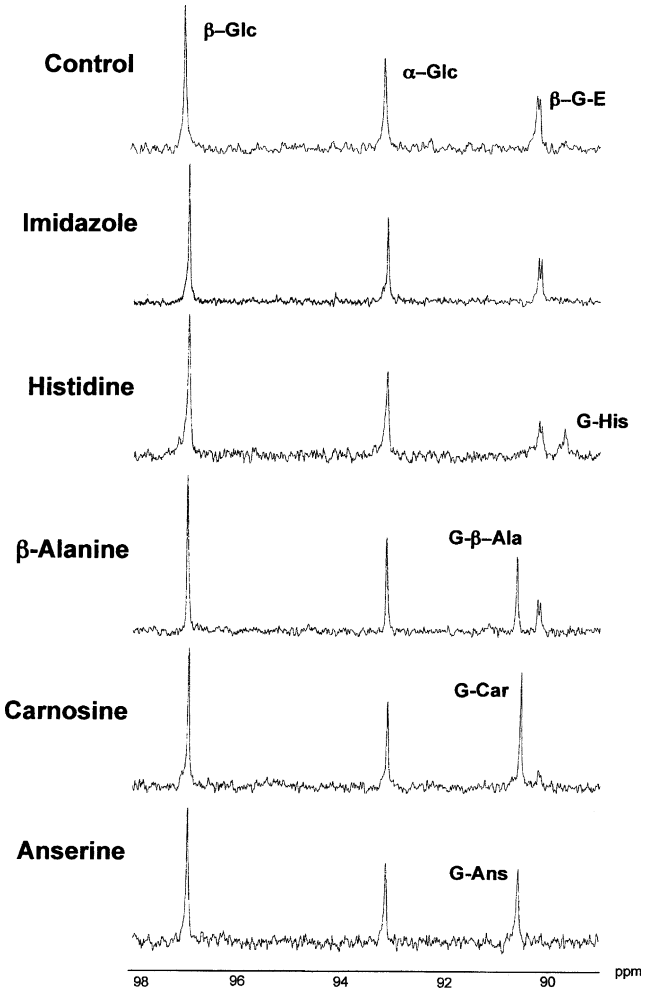

Fig. 6. ^{13}C NMR spectra of 20 mM G-E and equal concentrations of carnosine and related compounds after 180 min of incubation at pH 8.5 at RT.

histidine in the dipeptides does appear to play a role by enhancing the kinetics of transglycation in comparison with β-alanine and histidine, and by stabilizing both glucosyl-carnosine and glucosyl-anserine relative to glucosyl-histidine.

Discussion

Both carnosine and anserine have many beneficial effects on cell and tissue function [21–33]. Previously published data suggest that some of these protective properties may be due to the fact that these peptides inhibit glycation and react with carbonyl groups on proteins [36–38] thereby preventing crosslinking and possibly acting as signals for degradation of aged proteins [39].

The results of our study suggest, that in addition to their many documented and postulated properties as pH buffers, anti-oxidants, and chelators of metal ions, carnosine and anserine are potent transglycating agents accounting for their ability to reverse pre-existing, glycation-induced, crosslinking [33] and probably checking the non-enzymatic glycation cascade at the earliest point (Fig. 3).

We believe that our findings are of potential value in understanding the functions of carnosine and anserine, and may provide another rationale for the high levels of these dipeptides in some tissues. However, as is very often the case in science, these results raise new questions. Two such obvious questions are the role of histidine in transglycation by carnosine and anserine, and the fate of glucosyl-carnosine and glucosyl-anserine after their formation.

As suggested by Hobart et al. [40] the conjugates of carnosine and anserine glucose are probably stabilized

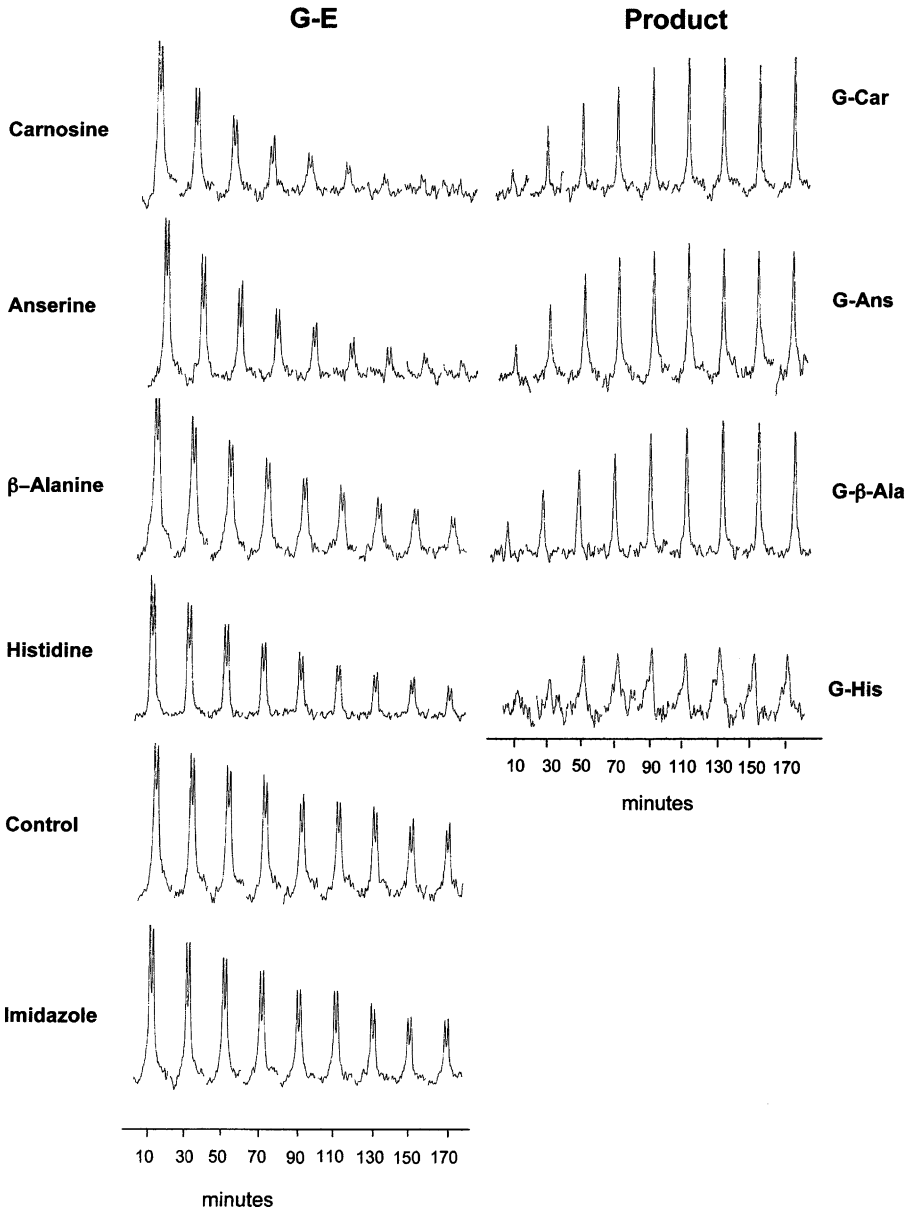

Fig. 7. Kinetics of transglycation of G-E by carnosine and related compounds. In the left column are shown serial spectra of G-E as a function of time whereas in the right column are serial spectra of the respective transglycation products. Note that with imidazole no transglycation products are formed (Fig. 6) and the kinetics of decomposition of G-E are indistinguishable from control.

by histidine and, as evident in Fig. 7, the presence of histidine in these dipeptides also enhances the rate of transglycation.

Regarding the fate of glucosyl-carnosine and glucosyl-anserine, there are at present no data but it is possible that such adducts could either be transported out of cells or metabolized further, providing thereby an irreversible set of reactions for removal of aldosamines from proteins and phospholipids such as phosphatidylethanolamine and phosphatidylserine.

In summary, our observation that carnosine and anserine can act as efficient transglycating agents provides another possible mechanism by which these peptides exert their anti-glycating effect.

Acknowledgments

This work was supported by grants from NIH (NIDDK-R21DK062875), the Juvenile Diabetes Research Foundation and the American Diabetes Association.

References

[1] B.S. Szwergold et al., Identification of a novel protein kinase activity specific for Amadori adducts on glycated proteins, Diabetes 46 (1997) 108A.
[2] G. Delpierre et al., Identification, cloning and heterologous expression of a mammalian fructosamine-3-kinase, Diabetes 49 (2000) 1627–1634.

[3] B.S. Szwergold, S.K. Howell, P.J. Beisswenger, Human fructosamine-3-kinase [FN3K]: purification, sequencing, substrate specificity and evidence of activity in vivo, Diabetes 50 (2001) 2139–2147.
[4] H. Kato, N. van Chuyen, T. Shinoda, F. Sekiya, F. Hayase, Metabolism of 3-deoxyglucosone, an intermediate compound in the Maillard reaction, administered orally or intravenously to rats, Biochem. Biophys. Acta 1035 (1990) 71–76.
[5] M. Takahashi, J. Fujii, T. Teshima, K. Suzuki, T. Shiba, N. Taniguchi, Identity of a major 3-deoxyglucosone-reducing enzyme with aldehyde reductase in rat liver established by amino acid sequencing and cDNA expression, Gene 127 (1993) 247–253.
[6] K. Sato, A. Inazu, S. Yamaguchi, T. Nakayama, Y. Deyashiki, H. Sawada, A. Hara, Monkey 3-deoxyglucosone reductase: tissue distribution and purification of three multiple forms of the kidney enzyme that are identical with dihydrodiol dehydrogenase, aldehyde reductase, and aldose reductase, Arch. Biochem. Biophys. 307 (1993) 286–294.
[7] M. Oimoni, F. Hata, N. Igaki, T. Nakamichi, S. Baba, S. Kato, Purification of a-ketoaldehyde dehydrogenase from the human liver and its possible significance in the control of glycation, Experientia 45 (1989) 463–466.
[8] G. Delpierre, F. Collard, J. Fortpied, E. Van Schaftingen, Fructosamine-3-kinase is involved in an intracellular deglycation pathway in human erythrocytes, Biochem. J. 365 (2002) 801–808.
[9] G. Delpierrre, Identification of fructosamine residues deglycated by fructosamine-3-kinase in human hemoglobin, J. Biol. Chem. 279 (2004) 27613–27620.
[10] B.S. Szwergold, S.K. Howell, P.J. Beisswenger, Nonenzymatic Glycation/enzymatic deglycation. A novel hypothesis on the etiology of diabetic complications, in Proceeding of the 7th International Symposium on Maillard Reaction, Elsevier, Amsterdam, New York, vol. 1245C (2002) pp. 143–152.
[11] M.S. Swamy, C. Tsai, A. Abraham, E.C. Abraham, Glycation mediated lens crystallin aggregation and cross-linking by various sugars and sugar phosphates in vitro, Exp. Eye Res. 56 (1993) 177–185.
[12] B.S. Szwergold, S. Lal, A.H. Taylor, F. Kappler, B.Y. Su, T.R. Brown, ^{31}P NMR evidence of an activated hexose-monophosphate shunt in hyperglycemic rat lenses in vivo, Diabetes 44 (1995) 810–815.
[13] P.J. Beisswenger, S.K. Howell, R.G. Nelson, M. Mauer, B.S. Szwergold, Alpha-oxoaldehyde metabolism and diabetic complications, Biochem. Soc. Trans. 31 (2003) 1358–1363.
[14] P.J. Thornalley, Glycation in diabetic neuropathy: characteristics, consequences, causes, and therapeutic options, Int. Rev. Neurobiol. 50 (2002) 37–57.
[15] D.A. Slatter, N.C. Avery, A.J. Bailey, Identification of a new cross-link and unique histidine adduct from bovine serum albumin incubated with malondialdehyde, J. Biol. Chem. 279 (2004) 61–69.
[16] A.S. Januszewski, N.L. Alderson, A.J. Jenkins, S.R. Thorpe, J.W. Baynes, Chemical modification of proteins during peroxidation of phospholipids, J. Lipid Res. 46 (2005) 1440–1449.
[17] B.S. Szwergold, Intrinsic toxicity of glucose due to non-enzymatic glycation is controlled in-vivo by deglycation systems including FN3K-mediated deglycation of fructosamines and transglycation of aldosamines, Med. Hypotheses 65 (2005) 337–348.
[18] B.S. Szwergold, S.K. Howell, P.J. Beisswenger, Transglycation—a potential new mechanism for deglycation of Schiff's bases, in: J. Baynes, V.M. Monnier, J.M. Ames (Eds.), The Maillard Reaction: Eighth International Symposium Ann. NY. Acad. Sci. Vol. 1043 (2005) 845–864.
[19] A.R. Hipkiss et al., Pluripotent protective effects of carnosine, a naturally occurring dipeptide, Ann. NY Acad. Sci. 854 (1998) 37–53.
[20] L. Bonfanti, P. Peretto, S. De Marchis, A. Fasolo, Carnosine-related dipeptides in the mammalian brain, Prog. Neurobiol. 59 (1999) 333–353.
[21] M.A. Babizhayev, Antioxidant activity of L-carnosine, a natural histidine-containing dipeptide in crystalline lens, Biochim. Biophys. Acta 1004 (1989) 363–371.
[22] H.J. Stuerenburg, K. Kunze, Concentrations of free carnosine (a putative membrane-protective antioxidant) in human muscle biopsies and rat muscles, Arch. Gerontol. Geriatri. 29 (1999) 107–113.
[23] V.N. Korobov, R.B. Maurisio, I.O. Mukalov, S.L. Stvolinskii, Carnosine stabilization of the normal erythrocyte membranes and in experimental diabetes, Patol. Fiziol. Eksp. Ter. 2 (2000) 13–15.
[24] V.V. Rusakov, V.T. Dolgikh, O.V. Korpacheva, The membrane-protective effect of carnosine in the post-resuscitation period after acute lethal blood loss, Vopr. Med. Khim. 39 (1993) 26–28.
[25] G.A. McFarland, R. Holliday, Retardation of the senescence of cultured human diploid fibroblasts by carnosine, Exp. Cell Res. 212 (1994) 167–175.
[26] P.R. Roberts, K.W. Black, J.T. Santamauro, G.P. Zaloga, Dietary peptides improve wound healing following surgery, Nutrition 14 (1998) 266–269.
[27] K. Nagai, T. Suda, Realization of spontaneous healing function by carnosine, Methods Findings Exp. Clin. Pharm. 10 (1988) 497–507.
[28] J.W. Lee, H. Miyawaki, E.V. Bobst, J.D. Hester, M. Ashraf, A.M. Bobst, Improved functional recovery of ischemic rat hearts due to singlet oxygen scavengers histidine and carnosine, J. Mol. Cell. Cardiol. 31 (1999) 113–121.
[29] V.D. Prokop'eva, B.I. Laptev, S.A. Afanas'ev, The protective effect of carnosine in hypoxia and reoxygenation of the isolated rat heart, Biokhimiia 57 (1992) 1389–1392.
[30] H. Ukeda, Y. Hasegawa, Y. Harada, M. Sawamura, Effect of carnosine and related compounds on the inactivation of human Cu,Zn-superoxide dismutase by modification of fructose and glycolaldehyde, Biosci. Biotech. Biochem. 66 (2002) 36–43.
[31] N.W. Seidler, Carnosine prevents the glycation-induced changes in electrophoretic mobility of aspartate aminotransferase, J. Biochem. Mol. Toxicol. 14 (2000) 215–220.
[32] T.A. Swearengin, C. Fitzgerald, N.W. Seidler, Carnosine prevents glyceraldehyde3-phosphate-mediated inhibition of aspartate aminotransferase, Arch. Toxicol. 73 (1999) 307–309.
[33] N.W. Seidler, G.S. Yeargans, T.G. Morgan, Carnosine disaggregates glycated alpha-crystallin: an in vitro study, Arch. Biochem. Biophys. 427 (2004) 110–115.
[34] C.I. Neglia, H.J. Cohen, A.R. Garber, S.R. Thorpe, J.W. Baynes, Characterization of glycated proteins by ^{13}C NMR spectroscopy. Identification of specific sites of protein modification by glucose, J. Biol. Chem. 260 (1985) 5406–5410.
[35] V.V. Mossine, G.V. Glinsky, M.S. Feather, The preparation and characterization of some Amadori compounds [1-amino-1-deoxy-D-fructose derivatives] derived from a series of aliphatic α-amino acids, Carbohydr. Res. 262 (1994) 257–270.
[36] J.H. Kang, K.S. Kim, S.Y. Choi, H.Y. Kwon, M.H. Won, T.C. Kang, Protective effects of carnosine, homocarnosine and anserine against peroxyl radical-mediated Cu,Zn-superoxide dismutase modification, Biochim. Biophys. Acta 1570 (2002) 89–96.
[37] A.R. Hipkiss, C. Brownson, M.J. Carrier, Carnosine, the anti-ageing, anti-oxidant dipeptide, may react with protein carbonyl groups, Mech. Ageing Dev. 122 (2001) 1431–1445.
[38] A.R. Hipkiss, C. Brownson, M.F. Bertani, E. Ruiz, A. Ferro, Reaction of carnosine with aged proteins: another protective process? Ann. NY Acad. Sci. 959 (2002) 285–294.
[39] A.R. Hipkiss, C. Brownson, Carnosine reacts with protein carbonyl groups: another possible role for the anti-ageing peptide? Biogerontology 1 (2000) 217–223.
[40] L.J. Hobart, I. Seibel, G.S. Yeargans, N.W. Seidler, Anti-crosslinking properties of carnosine: significance of histidine, Life Sci. 75 (2004) 1379–1389.