Taylor & Francis
Taylor & Francis Group

β-Alanine suppresses heat inactivation of lactate dehydrogenase

ANKUR D. MEHTA & NORBERT W. SEIDLER

Department of Biochemistry, Kansas City University of Medicine and Biosciences, 1750 Independence Avenue, Kansas City, MO 64106-1453, USA

Abstract
β-Alanine exhibits neurotransmitter activity and is a component of the anti-glycation agent carnosine. We propose that β-alanine may have additional properties which may be of physiological significance. Interestingly, stress modulates the level of β-alanine, which regulates excitotoxicity responses and prevents neuronal cell death. We hypothesize that β-alanine's protective role may involve preservation of enzyme structure and function, suggesting that β-alanine may act as a chemical chaperone. We used light scattering, enzyme activity and intrinsic fluorescence to monitor heat-induced changes in lactate dehydrogenase (LDH) in the presence and absence of β-alanine. We observed that β-alanine suppressed heat-induced LDH inactivation, prevented LDH aggregation, ameliorated the decrease in intrinsic fluorescence and reactivated thermally denatured LDH. These observations support the hypothesis that β-alanine has chaperone-like activity and may play a cellular role in the preservation of enzyme function.

Keywords: β-*Alanine, lactate dehydrogenase, chaperone, denaturation*

Introduction

β-Alanine is a substrate of carnosine synthetase, resulting in the production of carnosine in tissue specific regions of brain [1,2]. Carnosine is an anti-glycation [3–5] and anti-lipoxidation agent [6], whose β-alanine moiety reacts directly with oxidized carbohydrates [7] and lipids [8]. Additionally, β-alanine acts as a neurotransmitter by activating glycine and GABA receptors [9]. While these are the accepted functions of β-alanine, we propose that β-alanine may have additional properties which may be of physiological significance.

Biological stress increases β-alanine concentrations in living systems. In lower organisms osmotic stress modulates β-alanine levels [10]. Tissue and plasma levels of β-alanine rise in response to stress using whole animal models [11,12]. Hippocampal tissue slices exposed to cell-damaging conditions release β-alanine, regulating excitotoxicity responses and preventing neuronal death [13]. Interestingly, stimulation of excitotoxic receptors promotes β-alanine release from oligodendrocytes [14], suggesting that β-alanine release is a compensatory mechanism for protection. Several studies provide evidence supporting β-alanine's protective role. In plants β-alanine protects against osmotic stress [15]. β-Alanine prevents hydrogen peroxide-induced [16] and exercise-induced [17] cell injury. In cortical injury astrocyte uptake of β-alanine increases [18]. Additionally, β-alanine may be a potential treatment preventing hypoxic liver injury during transplantation [19]. These studies suggest that β-alanine may work as a chemical chaperone.

The concept of chemical chaperones as distinct from molecular chaperones such as heat shock proteins has been previously described [20] although β-alanine was not mentioned. The current study

Correspondence: N.W. Seidler, Department of Biochemistry, Kansas City University of Medicine and Biosciences, 1750 Independence Avenue, Kansas City, MO 64106-1453, USA, Tel: 1 816 283 2207. Fax: 1 816 283 2357. E-mail: nseidler@kcumb.edu

RIGHTSLINK

examined the ability of β-alanine to preserve enzyme function in heat denaturing conditions using lactate dehydrogenase as our model system.

Materials and methods

Materials

β-Alanine, lactate dehydrogenase (LDH), pyruvate and NADH were purchased from Sigma Chemical Company (St. Louis, MO). LDH (rabbit muscle isoform, EC 1.1.1.27) was obtained as a lypholyzed solid and prepared in sodium phosphate buffers prior to use. All other chemicals were of reagent grade.

Thermal denaturation

The effects of prolonged exposure to heat on the conformational integrity of LDH were examined. Unless otherwise indicated the temperature was set at 55°C for 0–25 min. The following measurements were made: light scattering, intrinsic fluorescence and enzyme activity. Light scattering and intrinsic fluorescence assessed thermal unfolding, and enzyme activities measured the functional consequences of thermal unfolding. The ability of β-alanine to suppress thermal denaturation was tested using techniques described below.

Light scattering

Thermal denaturation curves of LDH (200 μg/mL) were determined in a 50 mM sodium phosphate buffer (pH = 7.4) using a quartz cuvette and a programmable Peltier-heated cell holder in an Ultrospec 4000 spectrophotometer (Amersham Biosciences, Piscataway, NJ). Heat denaturation, which results in unfolding/aggregation that causes increased light scattering, was recorded at 55°C (absorbance, 450 nm; time drive, 0–22.5 min). Samples were read in the presence or absence of β-alanine (100–500 mM).

Intrinsic fluorescence

Intrinsic protein fluorescence was determined using an LS50B fluorometer (Perkin–Elmer Corporation, Shelton, CT). Real-time recordings of emission spectra (excitation, 290 nm; slit width, 5 nm) were obtained during heat exposure (0–5 min at 50°C) of LDH samples (0.1 mg/mL) in the presence or absence of β-alanine (100 mM). Fluorescence intensity, which was presented as relative fluorescence units (rfu), was measured at the wavelength of peak emission. The following settings were used: scanning speed, 600 nm/min; emission range, 300–500 nm; slit width, 2.5 nm; and stirrer rate high. Control spectra were obtained at room temperature.

Enzyme activity

LDH samples (0.2 mg/mL) in a 50 mM sodium phosphate buffer (pH = 7.4) containing β-alanine (0–500 mM) were incubated at 55°C for 0–25 min in a water bath. LDH activities were measured spectrophotometrically. Absorbance changes were monitored at 340 nm, which corresponds to enzymatic oxidation of NADH in the presence of pyruvate. Samples were assayed at 24°C in a 20 mM sodium phosphate buffer (pH = 7.4) containing 8 mM pyruvate, 0.2 mM NADH and 0.02–0.1 μg LDH using an Ultrospec 4000 spectrophotometer. Activities were calculated using the extinction coefficient for NADH (6.22 absorbance of one μmole NADH/mL at 340 nm in a 1 cm light path) and were presented as μmoles NADH oxidized/min per mg LDH.

Reactivation experiments

Unless otherwise indicated LDH (0.2 mg/mL in a 50 mM sodium phosphate buffer, pH = 7.4) was heat-denatured at 70°C for 90s using a Tecam heating block. β-Alanine (250 mM), which was preheated to 37°C, was immediately introduced to the samples that were then kept at 37°C for 5 min. Samples were incubated for an additional 30 min at 24°C under constant stirring. Matched with appropriate controls, samples were diluted and tested for enzyme activity as described above. Additionally, heat-denatured LDH (0.4 mg/mL for 10 min at 70°C) was subsequently treated with various concentrations of β-alanine (0–250 mM) and assayed for light scattering to assess protein disaggregation and refolding.

Results

Suppression of thermal denaturation

It was observed that β-alanine prevented the light scattering effects of heat-exposed LDH (Figure 1). Under these conditions (55°C) light scattering of control LDH increased over time following a sigmoidal curve. This suggests an unfolding/aggregation of the enzyme. β-Alanine (100 and 500 mM) decreased light scattering by 17.8% and 66.6%, respectively. These observations suggest that β-alanine protected against heat-induced denaturation.

Consistent with the light scattering experiments we observed that heat exposure (55°C) caused a loss of enzyme activity (Figure 2). After 15 min at 55°C only 48% of control activity remained. β-Alanine (500 mM) completely protected LDH from heat-induced inactivation. A protective trend was also evident at 100 mM β-alanine.

The intrinsic fluorescence of LDH changed upon exposure to 50°C for 5 min (Figure 3). We observed a time-dependent loss of fluorescence intensity at the wavelength of peak emission
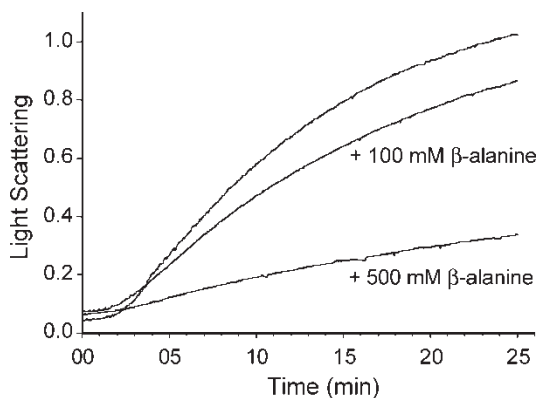
Figure 1. Suppression of heat-induced aggregation by β-alanine. LDH was exposed to heat (55°C) for 0–25 min with (lower tracings) and without β-alanine (upper tracing). Real-time absorbances (450 nm) were recorded. Data are from a representative experiment.
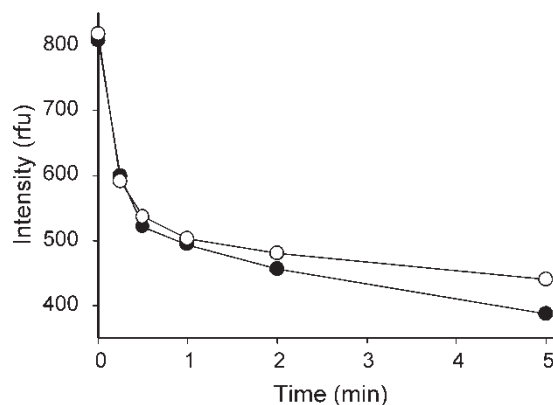

Figure 3. Effects of β-alanine on heat-induced changes in intrinsic fluorescence. Time-dependent changes in fluorescence intensity of LDH at the wavelength of peak emission (348 nm; excitation, 290 nm) were examined in the presence (open circles) and absence (closed circles) of β-alanine (100 mM).

(348 nm; excitation, 290 nm). The loss of fluorescence intensity appeared to be biphasic. These observations suggest that the initial conformational response was rapid up to 30s. The subsequent decrease in fluorescence intensity suggests a second slower conformational response to elevated temperatures. Interestingly, β-alanine (100 mM) had no effect on the early rapid change but ameliorated the subsequent loss of signal, suggesting that β-alanine showed a stabilizing effect over time.

Reactivation of heat-denatured enzyme

β-Alanine reactivated heat-denatured LDH (Figure 4). Heat-denatured LDH went directly from a saline-buffered solution at 70°C to a β-alanine-containing solution at 37°C then transferred to 24°C with continuous mixing in order to promote

a favorable refolding environment. Heat-denatured LDH that was not treated with β-alanine exhibited 53.6% of control activity. Treatment with β-alanine showed 72.4% of control activity, suggesting that β-alanine reactivated the unfolded-denatured enzyme. The activity in the presence of β-alanine ($246.3 \pm 9.19, M \pm SD$) was greater than that without β-alanine ($182.5 \pm 2.95 \, \mu mole \, min^{-1} \, mg^{-1}$) as assessed by Student's *t*-test (P < 0.0005). We observed a similar effect with heat-denatured LDH that was first allowed to cool at 24°C prior to β-alanine treatment (data not shown). β-Alanine disaggregated heat-denatured LDH as evidenced by a concentration-dependent decrease in light scattering (Figure 5). β-Alanine (250 mM) reversed protein aggregation by 43.2%. Even at β-alanine concentrations as low as 25 mM, a 27.6% reversal of protein aggregation was observed.
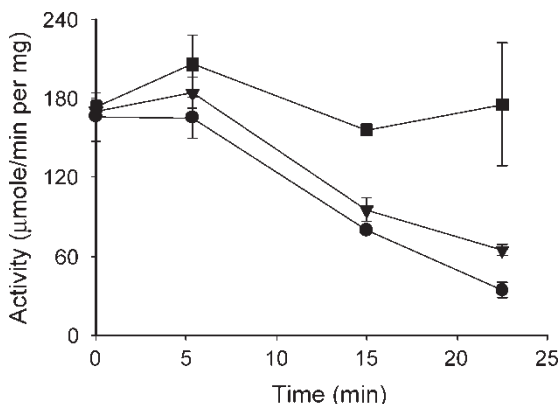

Figure 2. Prevention of heat inactivation by β-alanine. LDH was exposed to heat (55°C) for 0–22.5 min with (100 mM, triangles; 500 mM, squares) and without β-alanine (circles). At defined time points multiple enzyme activity measurements were recorded. Data points represent means and standard error from 1-3 readings.
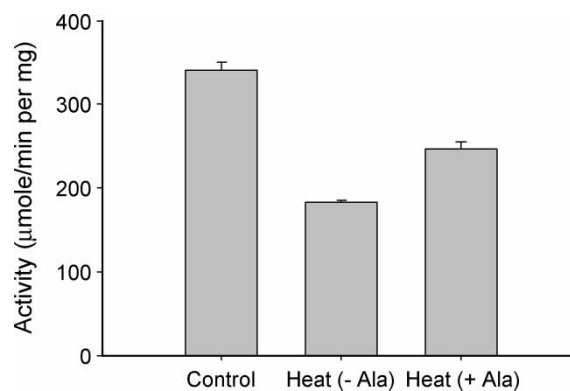

Figure 4. Reactivation of LDH following heat denaturation. Activity measurements of LDH samples were made prior to (control) and following heat exposure (90s at 70°C) in the presence (+ Ala) and absence (−Ala) of β-alanine. Preheated β-alanine (250 mM) was immediately introduced to the samples, kept for 5 min at 37°C and incubated for 30 min at 24°C prior to assaying for enzyme activity.
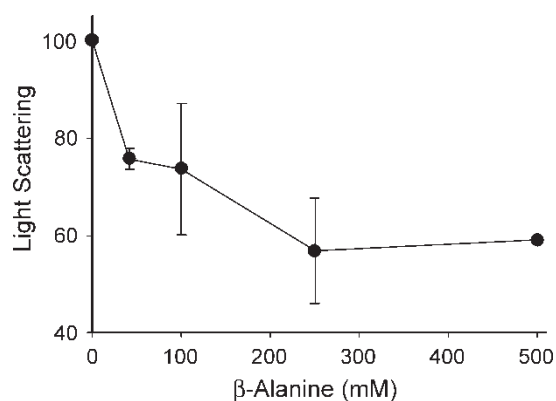
Figure 5. Disaggregation of heat denatured LDH by β-alanine. LDH was denatured (10 min at 70°C) and allowed to cool to room temperature prior to treatment with β-alanine and measurement of light scattering (absorbance at 450 nm).

Discussion

We observed that β-alanine suppressed the loss of structural integrity associated with heat exposure to LDH. β-Alanine also prevented heat-induced loss of enzyme activity. Additionally, β-alanine reactivated heat denatured LDH.

In our system, β-alanine (500 mM) protected LDH denaturation (55°C) by approximately 70% (Figure 1). Glycerol and trehalose (each at 0.5 M) provides about 50% and 80% protection, respectively, using a model of thermal denaturation (44°C) with malate dehydrogenase (MDH) [20]. The denaturation conditions using MDH are 11°C less than that used in the current study, suggesting that β-alanine's suppression of protein denaturation was greater than that of glycerol or trehalose. The difference in protective effects of β-alanine and the polyols (glycerol and trehalose) may be attributed to β-alanine's functional groups.

β-Alanine (500 mM) provided 100% protection against heat-induced enzyme inactivation (25 min at 55°C; Figure 2). Contrary to this observation, proline concentrations greater than 2 M are required to completely protect against heat inactivation of MDH [20]. The difference between β-alanine and proline may be due to the relative molecular orientation of the functional groups. Both contain charged amino and carboxyl groups, which presumably provide thermal stability by forming electrostatic interaction with various amino acid side chains on the enzyme. Proline's zwitterionic groups are in rather close proximity as compared with those of β-alanine, suggesting that the functional groups on proline may be limited in their ability to interact with the enzyme.

The molecular chaperone α-crystallin completely suppresses heat denaturation (48°C) of alcohol dehydrogenase at a molar ratio 1:1 [21]. In the current study β-alanine suppressed heat denaturation of LDH by 70% at a molar ratio of approximately 300:1 (β-alanine:LDH). These observations suggest that while β-alanine does not exhibit the same effectiveness as a heat shock protein β-alanine may nevertheless have a physiological role as a chemical chaperone.

β-Alanine suppressed a heat-induced change in protein conformation (Figure 3). After 5 min of heat denaturation (50°C) LDH retained greater emission intensity in the presence of β-alanine. We observed a biphasic change in intrinsic fluorescence upon heat exposure (50°C) of LDH. There was an initial rapid drop in fluorescence intensity that occurred within 30s followed by a more gradual decrease over time. This observation suggests that there were two distinct changes in LDH conformation, and it may be that the functional loss was associated with the second slower change in conformation. β-Alanine, which prevented heat-induced loss of enzyme activity, suppressed the decrease in emission intensity in the latter phase. We previously demonstrated that methylglyoxal-induced decrease in intrinsic fluorescence correlates with the loss of activity using aspartate aminotransferase as the model enzyme [22].

β-Alanine reactivated LDH following heat-induced loss of enzyme activity (Figure 4) suggesting that β-alanine promoted functional refolding of the enzyme. Proline reactivates urea-unfolded MDH [20]. These observations suggest that amino acids may possess chaperone-like activity; however, the amino group at the β-position may enhance the protective properties of these putative chemical chaperones. We consider this area warrants further research particularly as it applies to preserving enzyme activity.

Another promising area of investigation involves the search for effective disaggregating agents, which may by useful therapies in misfolding diseases. We previously demonstrated that carnosine disaggregates glycated α-crystallin [23]. In the current study β-alanine, a component of carnosine, disaggregated heat denatured LDH (Figure 5), suggesting that the β-alanine moiety may contain the chemical properties necessary for protein disaggregation.

In summary, we demonstrated that β-alanine suppressed heat-induced inactivation and protein aggregation of LDH. β-Alanine prevented the decrease in intrinsic fluorescence of heat-exposed LDH. Additionally, β-alanine reactivated thermally denatured LDH. These observations support the hypothesis that β-alanine may play a cellular role in preserving enzyme activity.

Acknowledgements

References

[1] Hoffmann AM, Bakardjiev A, Bauer K. Neurosci Lett 1996;215:29–32.

[2] Bonfanti L, Peretto P, De Marchis S, Fasolo A. Prog Neurobiol 1999;59:333–353.

[3] Seidler NW. J. Biochem. Mol Toxicol 2000;14:215–220.

[4] Seidler NW, Yeargans GS. Life Sci 2002;70:1789–1799.

[5] Swearengin TA, Fitzgerald C, Seidler NW. Arch Toxicol 1999;73:307–309.

[6] Aldini G, Granata P, Carini M. J Mass Spectrom 2002;37:1219–1228.

[7] Hobart LJ, Seibel I, Yeargans GS, Seidler NW. Life Sci 2004;75:1379–1389.

[8] Carini M, Aldini G, Beretta G, Arlandini E, Facino RM. J Mass Spectrom 2003;38:996–1006.

[9] Wu FS, Gibbs TT, Farb DH. Eur J Pharmacol 1993;246:239–246.

[10] Walther M. Comp Biochem Physiol A Mol Integr Physiol 2002;133:179–190.

[11] Decker R, von Stuckrad-Barre S, Milakofsky L, Hofford JM, Harris N, Vogel WH. Life Sci 1995;57:1781–1790.

[12] Milakofsky L, Hare TA, Miller JM, Vogel WH. Life Sci 1985;36:753–761.

[13] Saransaari P, Oja SS. Neurochem Res 1999;24:407–414.

[14] Bakardjiev A. Glia 1998;24:346–351.

[15] Rathinasabapathi B, Fouad WM, Sigua CA. Plant Physiol 2001;126:1241–1249.

[16] Weinberg JM, Varani J, Johnson KJ, Roeser NF, Dame MK, Davis JA, Vankatachalam MA. Am J Pathol 1992;140:457–471.

[17] Dawson Jr., R, Biasetti M, Messina S, Dominy J. Amino Acids 2002;22:309–324.

[18] Robitaille Y, Kemball K, Sherwin AL. J Neurol Sci 1995;134:95–101.

[19] Vairetti M, Carini R, De Cesaris MG, Splendore R, Richelmi P, Berte F, Albano E. Biochim Biophys Acta 2002;1587:83–91.

[20] Diamant S, Eliahu N, Rosenthal D, Goloubinoff P. J Biol Chem 2001;276:39586–39591.

[21] Horwitz J. Proc Natl Acad Sci USA 1992;89:10449–10453.

[22] Seidler NW, Kowalewski C. Arch Biochem Biophys 2003;410:149–154.

[23] Seidler NW, Yeargans GS, Morgan TG. Arch Biochem Biophys 2004;427:110–115.