The Role of Histidine-Containing Compounds on the Buffering Capacity of Muscle

Eric A. Decker

Introduction

A buffer is defined as a compound that in small concentrations can impart a resistance to large pH changes. Common buffers contain two substances, a conjugate base and a conjugate acid. There are acidic buffers that contain a weak acid and the salt of a weak acid (the conjugate base) and basic buffers that contain a weak base and the salt of a weak base (the conjugate acid). The combination of a conjugate base and acid can resist large changes in pH by absorbing H^+ and OH^-. For example, the conjugated base can absorb H^+ to form the conjugate acid. Since this reaction decreases the amount of free H^+ in solution, the pH change is decreased compared to a solution with no buffer. The pHs of buffered systems do change upon the addition of an acid or a base. However, this change is much smaller than seen in unbuffered systems. The amount of pH change is dependent on the ratio of conjugate base/conjugate acid. The conjugate base/conjugate acid ratio for a buffer is 1 when the pH = pK_a. The ability of a buffer to resist pH change is greatest when the pH is at or near the buffer's pK_a. Figure 1 shows the ability of acetic acid to resist pH change when titrated with a strong base. As can be seen, the maximum resistance to pH change is near the pK_a of acetic acid (4.76).

Buffers in Skeletal Muscle

Most biological tissues are designed to operate at pH near 7.0. If a tissue has metabolic processes that can alter pH, it would be necessary for the tissue to contain buffering agents. Skeletal muscle is a good example of a tissue whose metabolic pathways change pH since prolonged exercise will cause anaerobic conditions where metabolism of stored ATP and glycolytic production of lactic acid will decrease pH. These same metabolic processes also occur during the conversion of muscle to meat resulting in pH decline. The postmortem decrease in muscle pH is dependent on numerous factors of which one is the existence of buffering agents. Table 1 lists examples of buffers found in skeletal muscle. The effectiveness of these buffers is dependent on their concentrations and pK_a's. If a buffering agent has a pK_a near seven and is in relatively high concentrations, it would be expected to provide resistance to the postmortem pH decline. The ability of skeletal muscle buffers to resist pH changes can be described as the buffering capacity. Buffering capacity is typically defined as the amount of H^+ or OH^- that causes a given change in pH. Figure 2 shows how the buffering capacity of a beef muscle homogenate is affected by pH. Near pH 7, the muscle has high buffering capacity that makes sense since the tissue would be designed to maintain physiological pH. Beef also had strong buffering capacity at pH's < 5. At these lower pH's, compounds such as lactic acid and creatine, provide buffering capacity.

The major skeletal muscle buffers that have pK_a near 7 are the phosphates and the histidine-containing compounds. Inorganic phosphates such as phosphoric acid (pK_a = 7.2) and pyrophosphate (pK_a = 6.7) will provide buffering capacity as will organic phosphates such as AMP (pK_a = 6.2) and phosphorylated monosaccharides (e.g. glucose-6-phosphate. pK_a = 6.1). Total phosphate concentrations in pork muscle are approximately 50 mmoles/g tissue (Table 1). If this phosphorous was all in inorganic forms, it would be expected to be an

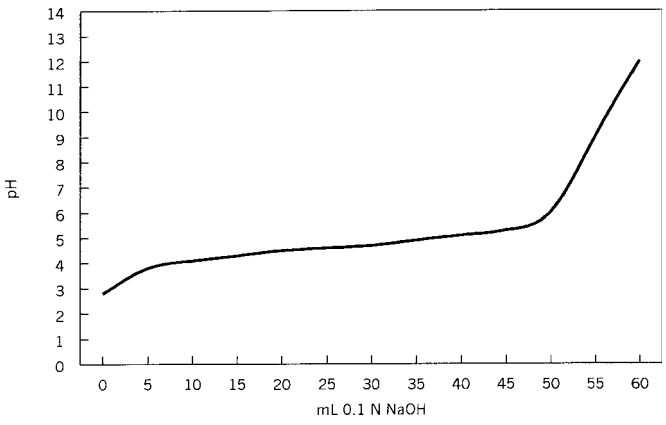


FIGURE 1. *The ability of the buffer, lactic acid, to resist pH changes upon the addition of 0.1 N NaOH.*

E. A. Decker
Department of Food Science
Chenoweth Lab
University of Massachusetts
Amherst, MA 01003
413-545-1026
FAX 413-545-1262
edecker@foodsci.umass.edu

Proceedings of the 54th Reciprocal Meat Conference (2001)

TABLE 1. Potential buffers in postmortem skeletal muscle.

Buffer	pK_a	Concentration (mmole/g tissue)
Phosphate-containing buffers		
Adenosine 5'-phosphate (AMP)	6.1	<1
Adenosine 5'-diphosphate (ADP)	6.3	0
Adenosine 5'-triphosphate (ATP)	6.5	0
Glucose 6-phophate	6.1	6.5
Fructose 6-phosphate	6.1	1.8
Orthophosphate	7.2	30–60
Amine-containing buffers		
Carnosine	6.8	0-31
Anserine	7.0	0-55
Creatine	2.3	30-50
Hypoxanthine	8.9	5
Organic acids		
Lactic acid	3.7	70–90

excellent buffer source since the pK_a's of inorganic phosphates are near 7. The organic phosphates would be less effective buffers since their pK_a values are closer to 6.0 and their concentrations are depleted by postmortem metabolic pathways. Inorganic phosphates have been estimated to provide 22-32% of the buffering capacity of pork muscle (Abe, 2000).

Histidine has a pK_a of 6.2. However, this pK_a changes when the histidine is bound to other amino acids resulting in a pK_a range of 5-8 (Abe, 2000). At physiological pH's, histidine is the major buffering component of proteins. Proteins have been estimated to provide 25, 27, and 24% of the buffering capacity of beef, pork and chicken breast muscle. Sarcoplasmic proteins contribute to 75-80% of the total protein buffering capacity (Abe, 2000).

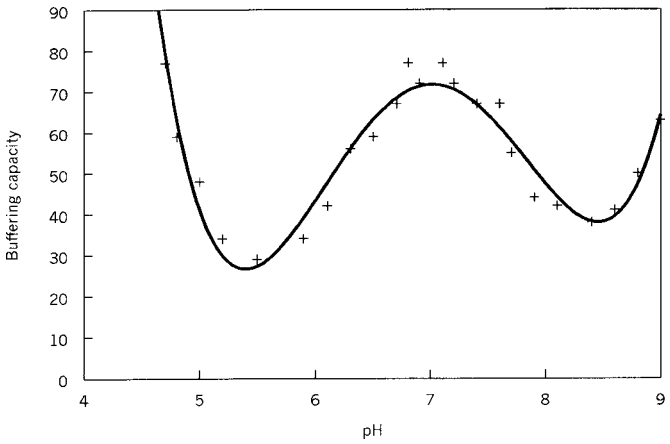


FIGURE 2. *An example of a typical profile of the buffering capacity of beef muscle homogenate at different pHs. Adapted from Puolanne and Kivikari (2000).*

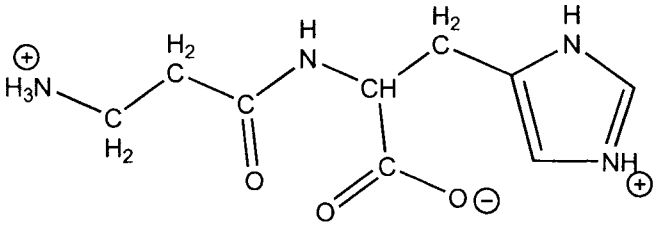
Histidine-Containing Dipeptides in Skeletal Muscle

Carnosine and anserine are N-ß-alanyl-L-histidine and N-ß-alanyl-3-methyl-L-histidine dipeptides (Figure 2), respectively. Carnosine and anserine are found exclusively in the muscle and nervous tissues of animals. The concentrations of carnosine and anserine vary greatly with tissue type and species (Table 2). Some species only contain one type of dipeptide such as human skeletal muscle that only contains carnosine and salmon muscle that only contains anserine. Other species have combinations of anserine and carnosine but the proportions of the dipeptides are not consistent. For instance, beef, pork, and turkey contain more carnosine than anserine while chicken and lamb have more anserine than carnosine (Chan and Decker, 1994). Muscle fiber type also influences anserine and carnosine concentrations with fast twitch muscle fibers generally having higher dipeptide concentrations than slow twitch muscle fibers. For example, chicken breast and leg muscle have combined dipeptide concentrations of 1.2% (71 mmole/g tissue) and 0.2% (12.2 mmole/g tissue), respectively, of the wet weight of skeletal muscle (Crush, 1970). Differences in anserine and carnosine concentrations in pork also occur as a function of muscle fiber type. Carnosine and anserine concentration are 33 and 21 mmoles/g tissue in porcine *longissimus dorsi* (LD; high in fast twitch muscle fibers) and *vastus intermedius* (high in slow twitch muscle fibers) muscle, respectively. The role of carnosine and anserine as buffers could explain why higher concentrations are found in fast twitch muscle fibers where anaerobic metabolism is common.

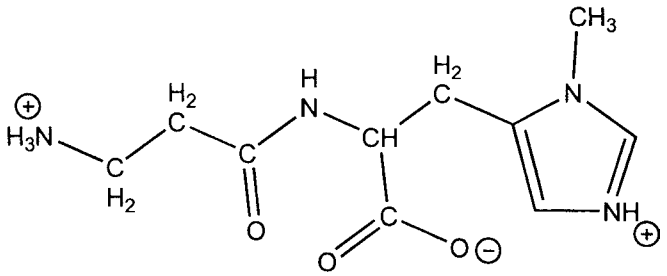
Since the pK_a's of the imidazole ring of carnosine and anserine are 6.83 and 7.04, respectively, these histidine-containing dipeptides exhibit excellent buffering capacity at physiological pH values. The buffering capacity of muscle has been correlated with carnosine concentrations in horses, dogs, fish, cows, pigs, and poultry (Harris et al., 1990; Abe, 2000). Anserine and carnosine have been estimated to provide 12-23% of pH-buffering capacity of bovine, porcine, and chicken skeletal muscle (Abe, 2001). Anserine and carnosine have been considered to be the main compounds responsible for differences in buffering capacity both within and between species. Puolanne and Kivikari (2000) found that porcine LD muscle (high in fast twitch muscle fibers) had a buffering capacity of

TABLE 2. Carnosine and anserine concentrations (mmole/g muscle) in various skeletal muscle sources (for review see Chan and Decker, 1994).

Species	Muscle	Carnosine	Anserine
Atlantic Salmon		0	22.2
Chicken	Leg	2.9	9.3
	Pectoral	16.4	54.6
Beef	Leg	8.8	2.8
Swine	Shoulder	9.2	1.1
Human	Quadriceps	21.3	0



CARNOSINE



ANSERINE

FIGURE 3. *The structures of the histidine-containing dipeptides carnosine and anserine.*

65.4 mmol H^+/(pH*kg) compared to 57.4 mmol H^+/(pH*kg) for *triceps brachii* (TB; higher in slow twitch fibers). These muscles also differ in carnosine concentrations with LD carnosine concentrations being approximately 11 mmoles/g muscle higher than TB. This difference in carnosine concentrations gives a theoretical buffering capacity of 6.5 mmol H^+/(pH*kg) that is 81% of the observed 8 mmol H^+/(pH*kg) difference in buffering capacity between LD and TB. Differences in carnosine concentrations have also been predicted to be responsible for 80% of the buffering capacity differences between chicken light and dark muscle (Puolanne and Kivikari, 2000). Differences in buffering capacity between species is also related to anserine and carnosine concentrations. The buffering capacity and histidine dipeptide concentration of chicken breast muscle are both 1.4 fold greater than porcine *biceps femoris* muscle suggesting that the major difference in the buffering capacity between these two species is their differences in carnosine and anserine concentrations (Abe, 2000).

While carnosine and anserine are known to be major contributors to the buffering capacity of skeletal muscle, information about their concentrations in commercial livestock is just beginning to become available. The major porcine skeletal muscle types for which carnosine concentrations have been reported are *longissimus dorsi* and *trapezius.* The carnosine concentrations of *L. dorsi* and *trapezius* range from 11.8 to 30.9 and 6.5 to 14.2 respectively (Table 3). Only one study has been published on carnosine concentrations and pork meat quality, and no differences in carnosine concentrations were observed in normal, dark, firm and dry, pale, soft and exudative, red, soft and exudative meat (Moya et al., 2001). While animal breeds are listed for some of the carnosine concentration data, a systematic approach has not be completed to evaluate genetic variations in muscle carnosine concentration. The influence of animal age has also not been investigated.

Impact of Nutrition on Skeletal Muscle Histidine-Dipeptide Concentrations

If muscle carnosine concentrations could be increased, it may be possible to improve meat quality. Histidine deficiency in rats reduces skeletal muscle carnosine concentration (Fuller et al., 1947; Tamaki et al., 1984). Skeletal muscle carnosine concentrations in pigs are unaffected by low levels of histidine supplementation (<1%) (Easter and Baker, 1977), but can be increased 2.8-fold in rats supplemented with 5% histidine (Tamaki et al., 1984). Dietary supplementation of equal molar concentrations of histidine (0.4%) and b-alanine (0.23%) to pigs had little to no effect on carnosine and anserine concentrations in the LD and *vastus intermedius* muscle (Mei et al., 1998). These data suggest that it may be difficult to increase muscle carnosine concentrations with dietary histidine in an economically practical manner.

Carnosine, but not anserine, is absorbed intact by a specific active transport system in brush border membranes of the small intestine (Ferraris et al., 1988). Absorbed carnosine is transported through the blood where it is either utilized by peripheral tissue or is hydrolyzed into ß-alanine and histidine by carnosinase, which is present in the blood, kidney, and liver (Jackson et al., 1991; Wolos et al., 1982), although the kidney seems to be the main organ responsible for the catabolism and excretion of the dipeptide (Abe, 1991). Low

TABLE 3. Carnosine concentrations (mmole/g muscle) in different pork muscle groups

L. dorsi	Trapezius	Breed	Reference
30.9	NR	Hampshire x Yorkshire	Mei et al., 1998
13.7	8.0	Pietrain x Landrace	Aristoy and Toldra, 1998
22.1	NR	Large White Face x Landrace	Moya et al., 2001
25.4	14.2	??????	Carnegie et al., 1982
11.8	6.5	??????	Cornet and Bousset, 1999

concentrations of dietary carnosine (0.9%) did not increase skeletal or heart muscle carnosine concentrations but did increase carnosine in the liver (Chan et al., 1994). Dietary carnosine (1.8%) resulted in fivefold increases in carnosine and twofold increases in histidine in the soleus muscle of rats (Maynard et al., 2001). A higher dietary carnosine concentration (5%) was capable of doubling rat skeletal muscle carnosine concentrations (Tamaki et al., 1984). While high concentrations of dietary carnosine may be able to increase skeletal muscle carnosine concentrations, this approach may not be economically feasible due to the current high cost of carnosine (>$100/kg).

Conclusions

The major buffering agents in skeletal muscle are phosphates, proteins, and histidine-containing dipeptides. Even though histidine-containing dipeptide concentrations have been strongly correlated with the buffering capacity of skeletal muscle, very little is known about their concentrations in the skeletal muscle of livestock. Since pH is an important factor in the quality of muscle foods such as pork, variations in muscle dipeptide concentrations could be an important factor in the acceptability and functional properties of pork products. More research is needed to determine if muscle histidine-containing dipeptide concentrations in skeletal muscle vary as a function of animal breed, age, and sex. Also, the impact of dietary factors such as dietary histidine concentrations should be investigated to determine if the levels of dietary histidine in commercial livestock diets can impact muscle carnosine concentrations. By finding situations where histidine-containing dipeptide concentrations in animals vary, it may be possible to determine if these buffers have an impact on meat quality.

References

Abe, H. 1991. Interorgan transport and catabolism of carnosine and anserine in rainbow trout. Comp. Biochem. Physiol., 100B(4):717-20.

Abe, H. 2000. Role of Histidine-Related Compounds as Intracellular Proton Buffering Constituents in Vertebrate Muscle. Biochem. (Moscow), 65(7):757-765.

Aristoy, M-C; Toldrá, F. 1998. Concentration of Free Amino Acids and Dipeptides in Porcine Skeletal Muscles with Different Oxidative Patterns. Meat Science, 50(3):327-332.

Carnegie, P. R.; Hee, K. P.; Bell, A. W. 1982. Ophidine (b-Alanyl-L-3-methylhistidine, 'Balenine') and Other Histidine Dipeptides in Pig Muscles and Tinned Hams. J. Sci. Food Agric., 33:795-801.

Chan, K.M.; Decker, E.A. 1994. Endogenous Skeletal Muscle Antioxidants. Crit. Rev. Food Sci. and Nutr., 34:403-426.

Chan, W.K.M.; Decker, E.A.; Chow, C.K.; Biossonneault, G.A. 1994. Effect of Dietary Carnosine on Endogenous Antioxidant Concentrations and Oxidative Stability of Rat Skeletal Muscle. Lipids, 29:461-466.

Cornet, M.; Bousset, J. 1999. Free amino acids and dipeptides in porcine muscles: differences between 'red' and 'white' muscle. Meat Science, 51(3):215-219.

Crush, K.G. 1970. Carnosine and related substances in animal tissues. Comp. Biochem. Physiol., 34:3-30.

Easter, R.A.; Baker, D.H. 1977. Nitrogen metabolism, tissue carnosine concentration and blood chemistry of gravid swine fed graded levels of histidine. J. Nutr., 107(1):120-5.

Ferraris, R.P.; Diamond, J.; Kwan, W.W. 1988. Dietary regulation of intestinal transport of the dipeptide carnosine. Am. J. Physiol., 225(2):G143-50.

Fuller, A.T.; Neuberger, A.; Webster, T.A. 1947. Histidine deficiency in the rat and its effect on the carnosine and anserine content of muscle. Biochem. J., 41:11-9.

Harris, R.C.; Marlin, D.J.; Dunnett, M.; Snow, D.H.; Hultman, E. 1990. Muscle buffering capacity and dipeptide content in the thoroughbred horse, greyhound dog and man. Comp. Biochem. Physiol. A., 97(2):249-51.

Jackson, M.C.; Kucera, C.M.; Lenney, J.F. 1991. Publications and properties of human serum carnosinase. Clinica Chimica Acta, 196:193-206.

Maynard, L.M.; Boissonneault, G.A.; Chow, C.K.; Bruckner, G.G. 2001. High levels of dietary carnosine are associated with increased concentrations of carnosine and histidine in rat soleus muscle. J. of Nutr., 131(2):287-290.

Mei, L.; Cromwell, G.L.; Crum, A.D.; Decker, E.A. 1998. Influence of Dietary -Alanine and Histidine on the Oxidative Stability of Pork. Meat Sci., 49:55-64.

Moya, V.J.; Flores, M.; Aristoy, M.C.; Toldra, F. 2001. Pork meat quality affects peptide and amino acid profiles during the ageing process. Meat Science, 58(2):197-206.

Puolanne, E.; Kivikari, R. 2000. Determination of the buffering capacity of postrigor meat. Meat Science, 56(1):7-13.

Tamaki, N.; Funatsuka, A.; Fujimoto, S.; Hama, T. 1984. The utilization of carnosine in rats fed on a histidine-free diet and its effect on the levels of tissue histidine and carnosine. J. Nutr. Sci. Vitaminol., 30:541-51.

Wolos, A.; Jablonowska, C.; Faruga, A.; Jankowski, J. 1982. Postnatal ontogenetic studies on kidney and liver carnosinase activity and carnosine content in muscles of turkey. Comp. Biochem. Physiol., 71A:145-8.