Comparative Analysis of Different Hemoglobins: Autoxidation, Reaction with Peroxide, and Lipid Oxidation

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Beef hemoglobin (Hb) had lower levels of deoxyHb and autoxidized much slower as compared to trout Hb at pH 6.3. Chicken Hb autoxidized at a rate intermediate between beef and trout Hb. In the presence of hydrogen peroxide, metHb formed rapidly from trout Hb whereas beef Hb was essentially nonreactive with hydrogen peroxide. The autoxidation rate of perch Hb was more rapid than trout Hb despite the low deoxyHb content of perch Hb. Perch Hb was a better catalyst of lipid oxidation than trout Hb when added to washed cod muscle based on formation of lipid hydroperoxides and thiobarbituric acid reactive substances. These studies indicate that autoxidation rate does not always increase with increasing deoxyHb content. The role of heme crevice volume in heme protein autoxidation is discussed. Among other factors, these studies suggest that rates of lipid oxidation in various muscle foods may depend on the relative ability of hemoglobins from different animal species to promote lipid oxidation.

INTRODUCTION

The heme proteins including hemoglobin (Hb) and myoglobin are potent catalysts of lipid oxidation in muscle foods. Lipid oxidation reactions need to be controlled during storage of muscle foods since various parameters of quality are negatively affected, including odor, flavor, and nutritional value (*1*). Just after slaughter, the iron atom in the heme ring of the heme proteins is primarily in the ferrous (+2) state. Conversion of ferrous heme protein to met (+3) heme protein (metHP) is a process known as autoxidation. Autoxidation appears to be a critical step in the ability of heme proteins to stimulate lipid oxidation since metHP reacts with peroxides to stimulate formation of compounds capable of initiating and propagating lipid oxidation (*2*).

A more rapid rate of autoxidation was detected when analyzing hemoglobins from cold water fish as compared to warm water fish (pH 7, 20 °C) (*3*). Hagfish had lower Hb autoxidation rates as compared to carp, tuna, and lamprey (the latter three are similar) (*4*). This suggested that monomeric forms of Hb (hagfish and lamprey) did not accelerate autoxidation rates. Gutzke and Trout (*5*) concluded that species type in mammals had little effect on myoglobin autoxidation rates among sheep, pig, deer, and cow (pH 5.5−6.5, 20−40 °C). We had found that hemoglobins from trout promoted lipid oxidation more rapidly than chicken hemoglobins in a model system consisting of washed, minced cod muscle; beef hemoglobins were poor catalysts of lipid oxidation as compared to the chicken

and trout Hb (pH 6.3, 2 °C) (*6*). More studies are needed to characterize the prooxidative nature of these different hemoglobins, for example, determination of autoxidation rates and reactions with peroxide.

There is evidence that substantial amounts of Hb from the blood are present in muscle from bled animals. In bled broilers, it was found that 100 and 86% of the heme pigment detected by aqueous extraction was Hb in breast muscle and dark muscle, respectively, on a wet weight basis (*7*). No significant difference in Hb content was found when whole muscle from bled and unbled sockeye salmon was compared (*8*). Hb made up 32% of the total heme protein on average in bled beef shank muscle (*9*). The purpose of our studies was to compare the prooxidative nature of hemoglobins from different aquatic and terrestrial animals to better understand the mechanisms by which hemoglobins contribute to quality deterioration reactions in muscle foods.

MATERIALS AND METHODS

Chemicals. Bovine Hb, tetraethoxypropane, cumene hydroperoxide, streptomycin sulfate, sodium heparin, ferrous sulfate, barium chloride, ammonium thiocyanate, and tris [hydroxymethyl] aminomethane (Tris) were obtained from Sigma Chemical A/S (St. Louis, MO). All other chemicals used were analytical grade, and distilled, deionized water was used.

Blood Collection. Blood from trout, perch, chicken, and beef cows was obtained from campus sources. Approximately 4 parts of blood was drawn via a syringe containing 1 part of 150 mM NaCl and sodium heparin (120 Units/ml). Rainbow trout (*Onchorhynchus mykiss*) (25−30 cm) and yellow perch (*Perca flavescens*) (15−25 cm) were bled from the caudal vein according to Rowley (*10*) using aminobenzoic

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Reactivity of Different Hemoglobins

J. Agric. Food Chem., Vol. 51, No. 13, 2003 **3887**

acid ethyl ester as an anesthetic. Chickens (White Rock, Leghorn, Ancona, and New Hampshire, 8−10 weeks old) were bled from the brachial vein. Beef cows (Black Angus, 3−4 years old) were bled from the jugular vein. Hemoglobins were prepared within 24 h of blood collection.

Preparation of Hemoglobins. Four volumes of ice cold 1.7% NaCl in 1 mM Tris, pH 8.0, were added to heparinized blood and centrifuged (700*g* for 10 min at 4 °C) in a Beckman J-6B centrifuge (Beckman Instruments Inc., Palo Alto, CA). After the plasma was removed, the red blood cells were washed by suspending three times in 10 volumes of the above buffer (*11*). Cells were lysed in 3 volumes of 1 mM Tris, pH 8.0, for 1 h. One-tenth volume of 1 M NaCl was then added to aid in stromal removal before ultracentrifugation (28 000*g* for 15 min at 4 °C) using a Beckman L8-70M ultracentrifuge (Beckman Instruments Inc.). Hemolysates were then passed through DG-10 gel filtration columns (Bio-Rad, Hercules, CA). Hb solutions were stored at −80 °C prior to use.

Quantifying Hb Levels. The method of Brown (*12*) was adapted. Concentrated Hb solutions were diluted with 50 mM Tris, pH 8.0, buffer. Around 1 mg of sodium dithionite was added to 1.5 mL of the Hb solution and mixed in a cuvette. Carbon monoxide gas (Badger Welding, Madison, WI) was then bubbled into the samples for 30 s. The sample was then scanned from 440 to 400 nm (Soret band) against a blank that contained only buffer using a model UV-2401 double-beam spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD). The peak at 420 nm was recorded. Standard curves were constructed using twice-crystallized bovine Hb (Sigma) and a molecular mass of 68 000 Da.

Washed, Minced Cod Muscle. Cod fish (*Gadus morhua*) fillets without skins were delivered overnight via air transport from Gloucester, MA. The fillets were considered of excellent quality based on appearance and odor. All dark muscle was removed. The rest of the fillets was ground in a KS M90 mincer (Kitchen Aid Inc., St Joseph, MI) (plate diameter 5 mm). The mince was washed twice in distilled, deionized water at a 1:3 mince to water ratio (w:w) by stirring with a plastic rod for 2 min. Subsequently, the mixture was allowed to stand for 15 min before dewatering with fiberglass screen. Mince was then mixed with 50 mM sodium phosphate buffer (pH 6.3) at the same 1:3 ratio and homogenized (setting 1) using a Polytron Type PT 10/35 (Brinkmann Instruments, Westbury, NY). It was allowed to stand for 15 min and finally centrifuged (15 000*g* for 20 min at 4 °C) using a Beckman L8-70M ultracentrifuge (Beckman Instruments Inc.). The resulting pellet was then used as the washed cod muscle.

Addition of Hemoglobins to Washed Cod Muscle. An appropriate volume of the Hb stock was added to a final concentration of 12 μmol per kg washed cod and stirred with a plastic spatula for 3 min to distribute the heme protein. This level of Hb was selected since it is near the range of Hb levels found in light muscle of trout, *sartorius* muscle of bled broilers and *Longissimus dorsi* muscle in bled beef (*7, 13, 14*). Streptomycin sulfate (200 ppm) was added to inhibit microbial growth during storage. The pH of samples was checked just after addition of Hb, periodically during storage, and finally at the end of storage. To measure pH, around 0.5 g of sample was diluted in 10 volumes of distilled, deionized water and homogenized, and readings were recorded using an Accumet AR50 pH meter (Fisher Scientific, Pittsburgh, PA). The final moisture content of the samples stored at 2 °C was adjusted to 88%. pH was adjusted if necessary by addition of 1 M NaOH or 1 M HCl.

Measuring the Relative Oxygenation of Hb. Solutions containing Hb were scanned from 630 to 500 nm using a double-beam spectrophotometer model UV-2401 (PC) (Shimadzu Instruments, Inc.). The blank contained only buffer. The absorbance at the peak (575 nm) minus the absorbance at the valley (560 nm) was calculated. Larger differences indicated that the Hb was more highly oxygenated (*15*). These experiments were run at atmospheric conditions. The percentage of deoxyHb was calculated according to the equations of Benesch et al. (*16*).

Measuring MetHb Formation. Hb solutions were diluted into 20 mM sodium phosphate buffer (pH 6.3). Spectra were obtained at regular time intervals between 700 and 500 nm using the UV-2401 spectrophotometer. The percentage of metHb was calculated according to the

Table 1. Relative Hb Autoxidation Rates from Chicken, Trout, and Beef Hb (20 μM) during 4 °C Storage[a]

Hb type	relative rate of autoxidation
trout	1.00 ± 0.08^a
chicken	0.29 ± 0.11^b
beef	0.09 ± 0.03^c

[a] Rate is derived from the slopes obtained when plotting time vs % metHb. Hb solutions were buffered with 20 mM sodium phosphate (pH 6.3). Common letters in a column indicate no significant difference. Hemoglobins from three to five different animals per group were analyzed. Samples contained 3 mmol of superoxide dismutase and catalase per mole of heme.

equations of Benesch et al. (*16*). For autoxidation studies, samples contained 3 mmol of superoxide dismutase and catalase per mole of heme to remove any superoxide and hydrogen peroxide that was produced during incubation.

Determination of Thiobarbituric Acid Reactive Substances (TBARS). TBARS were determined according to a modified procedure of Buege and Aust (*17*). Fifty percent trichloroacetic acid (TCA) containing 1.3% TBA was heated to 65 °C on the day of use to dissolve the TBA. Sample was added to the TCA−TBA mixture (1:10) (w:v) and incubated for 1 h at 65 °C. After the mixture was centrifuged (2500*g* for 10 min), the absorbance of the supernatant at 532 nm was determined. A standard curve was constructed using tetraethoxypropane.

Determination of Lipid Hydroperoxides. Between 0.4 and 0.5 g of washed cod muscle was homogenized in 5 mL of chloroform/methanol (1:1) for 30 s using a Polytron Type PT 10/35 (Brinkmann Instruments). Subsequently, the polytron was rinsed for 30 s with 5 mL of solvent. The homogenate and wash solution were then combined. Three milliliters of 0.5% NaCl was added, and the mixture was mixed for 30 s with a Vortex before centrifugation for 10 min (4 °C and 700*g*) to separate the mixture into two phases. Then, 1.33 mL of ice-cold chloroform/methanol (1:1) was added to 2 mL of the lower phase and mixed briefly. Twenty-five microliters of ammonium thiocyanate (4.38 M) and 25 μL of iron(II)chloride (18 mM) were added to the assay for lipid hydroperoxides (*18*), and samples were incubated for 20 min at room temperature before the absorbances at 500 nm were determined. A standard curve was prepared using cumene hydroperoxide. The chloroform used contained ethanol as a preservative to eliminate high blank readings (*19*).

Statistical Evaluations. All experiments were done at least in duplicate, and in each experiment, hemoglobins from at least three different animals were separately evaluated. Analysis of variance with a MIXED procedure of the SAS system was used to evaluate data from storage studies (*20*). Means were separated using differences of least squares.

RESULTS

In the first set of experiments, hemoglobins from trout, chicken, and beef animals were prepared. The concentration of each heme protein was adjusted to 20 μM. Hb autoxidation rates were then measured during storage at 4 °C (pH 6.3) (**Table 1**). Trout Hb autoxidized much more rapidly than beef or chicken Hb. Chicken Hb autoxidized more rapidly than beef Hb ($p < 0.05$). The initial absorbances at 630 nm were low but significantly higher in trout as compared to beef and chicken Hb ($p < 0.05$) (**Table 2**).

DeoxyHb in the presence of O_2 can accelerate rates of autoxidation (*21*). Thus, deoxyHb content in trout and beef Hb solutions (2 μM) was determined at pH 6.3. DeoxyHb content at a given pH can be estimated by monitoring initial absorbance spectra in the visible range. Fully deoxygenated Hb will appear as a single peak near 560 nm while fully oxygenated Hb will have sharp peaks near 576 and 540 nm forming a deep valley. Intermediately oxygenated heme proteins will have less sharp peaks at 576 and 540 nm forming a shallow valley. It can be

3888 *J. Agric. Food Chem.,* Vol. 51, No. 13, 2003

Richards and Dettmann

Table 2. Initial Absorbance Values at 630 nm from Chicken, Trout, and Beef Hemoglobins (20 μM)[a]

Hb type	absorbance
trout	0.027 ± 0.001^{a}
chicken	0.013 ± 0.002^{b}
beef	0.007 ± 0.001^{c}

[a] Solutions were buffered with 20 mM sodium phosphate (pH 6.3). Common letters in a column indicate no significant difference.
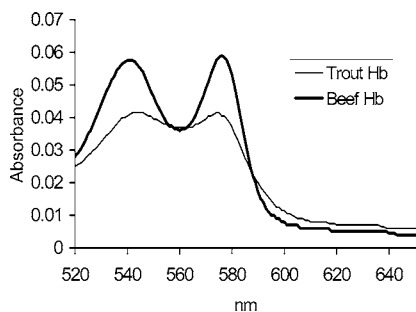
Figure 1. Spectra of trout and beef Hb at pH 6.3 in 20 mM sodium phosphate. The Hb concentration was 2 μM. A deeper valley at 560 nm is indicative of increased Hb oxygenation.
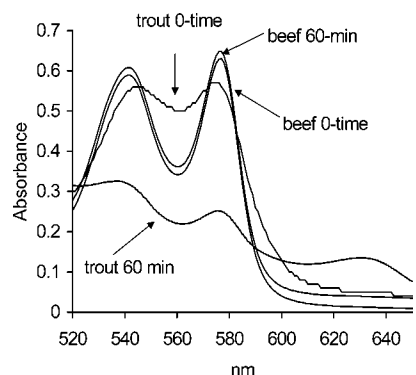
Figure 2. Spectra of beef and trout Hb (20 μM) exposed to hydrogen peroxide (60 μM) at 0 time and after 60 min of incubation at 4 °C (pH 6.3, 20 mM sodium phophate).

seen in **Figure 1** that at pH 6.3, there was a more shallow valley between the peaks at 576 and 540 nm in trout Hb as compared to beef Hb. This is indicative of a substantially higher initial content of deoxyHb in the trout Hb as compared to the beef Hb. The estimated percentages of deoxyHb in the trout and beef hemoglobins at pH 6.3 were 56 and 6%, respectively.

Beef and trout Hb in the ferrous state were added to a 3-fold excess of hydrogen peroxide at pH 6.3 and incubated at 4 °C. Reaction of ferrous heme protein with hydrogen peroxide will result in spectral changes in the visible range. Spectra were recorded immediately after addition of hydrogen peroxide and during 60 min of incubation at 4 °C (**Figure 2**). Upon incubation of trout Hb with hydrogen peroxide, the absorbance at 576 and 540 nm decreased while at 630 nm the absorbance increased, which is typical of the formation of metHb from ferrous Hb (*16*). Little change occurred in the beef Hb upon incubation with hydrogen peroxide, which was indicative of the heme protein remaining in the ferrous oxidation state and poorly reacting with hydrogen peroxide as compared to trout Hb. Sodium azide (100 μM) was added to the reaction mixtures to inhibit any catalase that may have been present. Even in the presence of azide, trout Hb was still much more reactive (data not shown). This suggested that differences in reactivity between

Table 3. Effect of Chicken Strain on Hb Oxygenation at pH 5.8 in 20 mM Sodium Phosphate or MES Buffer[a]

chicken type	activity rank	Hb oxygenation[b] (sodium phosphate)	Hb oxygenation (MES)
Ancona	4	0.218 ± 0.011	0.219 ± 0.005
New Hampshire	2	0.223 ± 0.006	0.220 ± 0.009
Leghorn	3	0.220 ± 0.004	0.217 ± 0.007
White Rock	1	0.216 ± 0.007	0.217 ± 0.008

[a] Hb concentration was 20 μM. Activity rank: four most active, one most docile. [b] Hb oxygenation is determined based on the peak at 577 nm minus the valley at 561 nm. Relative values are expressed as net absorbance units.
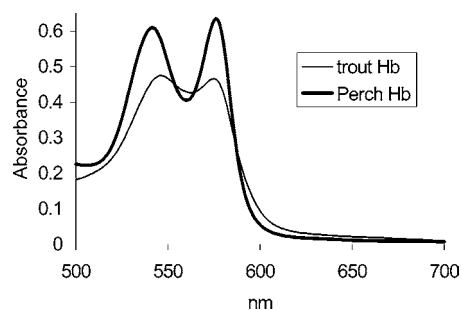
Figure 3. Spectra of trout and perch Hb at pH 6.3 in 20 mM sodium phosphate. The Hb concentration was 20 μM. A deeper valley at 560 nm is indicative of increased Hb oxygenation.

trout and beef Hb were due to differences between the hemoglobins and not variation in catalase activity.

Because there appeared to be a relationship between deoxyHb (deoxyHb) content and both Hb autoxidation rate and reactivity with hydrogen peroxide, the deoxyHb content in different animal species was further investigated. The deoxyHb content in hemoglobins from four strains of chicken was compared. pH 5.8 was chosen since this is a typical postmortem pH in the muscle of poultry. The strains chosen ranged from highly sedentary to active, flighty birds. No significant differences in deoxyHb content were detected among the four strains of birds (**Table 3**).

The deoxyHb content in rainbow trout and yellow perch was compared at equivalent concentrations (20 μM) and pH (6.3). Perch hemoglobins were much more oxygenated than trout hemoglobins based on the absorbance spectra (**Figure 3**). The estimated percentages of deoxygenated molecules in trout and perch hemoglobins at pH 6.3 were 60 and 17%, respectively. It was expected that perch Hb would be more resistant to autoxidation at pH 6.3 than trout Hb considering the higher content of deoxyHb in the trout. However, perch Hb autoxidized more rapidly than trout Hb at pH 6.3 ($p < 0.05$) (**Figure 4**). Perch and trout Hb were then separately added to washed cod muscle to assess rates of lipid oxidation during storage at 4 °C. Lipid peroxides and TBARS were used as indicators of lipid oxidation. Perch Hb stimulated lipid oxidation more rapidly than trout hemoglobins based on both lipid peroxides (**Figure 5**) and TBARS (**Figure 6**) formation ($p < 0.05$).

DISCUSSION

Trout Hb autoxidized much more rapidly than chicken or beef Hb (**Table 1**). A key reaction in heme protein autoxidation is proton-dependent (*22*) as seen in eq 1:

$$\text{oxy}(+2)\text{Hb} + \text{H}^+ \rightarrow \text{met}(+3)\text{Hb} + \text{HOO}^\bullet \qquad (1)$$
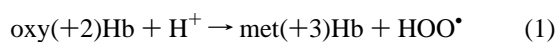
However, pH was standardized to 6.3 in our studies; hence, an alternative mechanism may be required to explain the results

Reactivity of Different Hemoglobins

J. Agric. Food Chem., Vol. 51, No. 13, 2003 **3889**
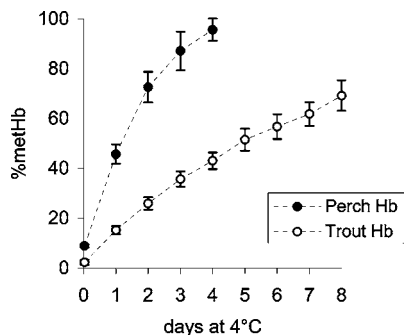
Figure 4. Formation of metHb from trout and perch Hb (pH 6.3, 20 mM sodium phosphate). Samples were stored at 4 °C. Hb concentration was 20 μM. Samples contained 3 mmol superoxide dismutase/catalase per mole of heme.
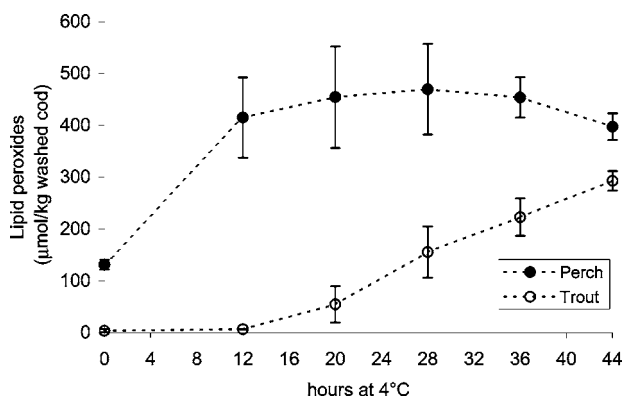
Figure 5. Lipid peroxide values of trout or perch Hb added to washed cod. The Hb level in each sample was 12 μmol/kg washed cod. Final pH was 6.3.
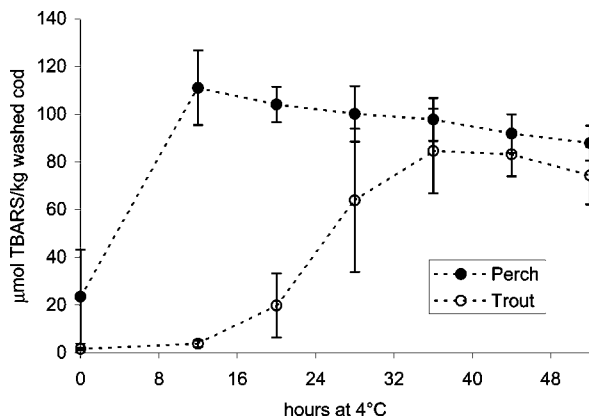
Figure 6. TBARS values of trout or perch Hb added to washed cod. The Hb level in each sample was 12 μmol/kg washed cod. The final pH was 6.3.

above. Heme protein autoxidation is also dependent on deoxyHb content (*21*). In the absence of oxygen, metHP will form slowly, but deoxyHb in the presence of O$_2$ is susceptible to rapid autoxidation (*22*) according to eq 2:

$$\text{deoxy}(+2)\text{Hb} + \text{O}_2 \rightarrow \text{met}(+3)\text{Hb} + \text{O}_2^{-\bullet} \qquad (2)$$

Thus, fully oxygenated heme proteins should be more resistant to autoxidation than heme proteins containing substantial amounts of deoxyHb. Trout hemoglobins are known to have exaggerated Bohr effects, which is an increase in deoxyHb content with decreasing pH (*23*). Relatively minimal Bohr effects occur in mammalian hemoglobins (*24*). The substantial
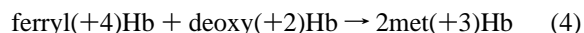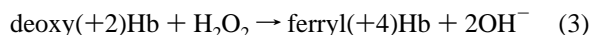
amounts of deoxyHb in trout as compared to beef Hb at pH 6.3 can be observed from the spectra in **Figure 1**, which at least partially explains the rapid autoxidation rates in trout as compared to beef Hb.

Chicken Hb autoxidation was intermediate between trout and beef Hb (**Table 1**). In certain birds, "supercooperativity" of oxygen binding has been described. This is a tetramer−tetramer association that results in decreased oxygen affinity (*25*). We had previously examined deoxyHb content of chicken between pH 5.5 and pH 7.5 and found that deoxyHb content was intermediate between beef and trout Hb at pH 6.3 (*6*). This further substantiates the ability of deoxyHb to accelerate rates of autoxidation.

Fish from sluggish waters have non-Bohr effect hemoglobins while fish in active streams possess Bohr effect hemoglobins (*26*). This suggests that active animals contain larger amounts of deoxyHb at reduced pH due to Bohr effects than sedentary animals. Therefore, it was tested if active or flighty birds might have hemoglobins with higher deoxyHb content at postmortem pH values as compared to less active birds. Apparently, the range of activity in the chicken species evaluated was not enough to induce differences in deoxyHb content at pH 5.8 as seen in **Table 3**. Examining hemoglobins from a migratory bird may produce different results.
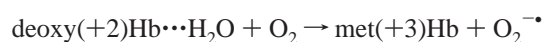
There was a slight but significantly higher initial absorbance at 630 nm in trout Hb as compared to beef Hb, which suggests that the trout hemoglobins started to autoxidize almost immediately at pH 6.3 (**Table 2**). It has been documented that hemoglobins with low oxygen affinity are easily oxidized (*27*). These researchers found that in carp Hb (pH 6, 25 °C) one-quarter of the hemes was oxidized within 3 min. Hemoglobins are prepared from blood at pH 8.0 since the heme proteins are resistant to autoxidation at elevated pH. This is likely due to the lack of both H$^+$ (eq 1) and deoxyHb (eq 2) at elevated pH regardless of species or strain. Deoxygenated heme proteins have a slightly higher extinction coefficient at 630 nm than their oxygenated counterparts (*28*). This can also explain the slightly higher initial absorbance values at 630 nm when examining the trout hemoglobins that possess high deoxyHb content at pH 6.3.

Trout Hb was more reactive with hydrogen peroxide than beef Hb at pH 6.3 (**Figure 2**). Spectra resembling metHb were obtained from the trout hemoglobins after 60 min of incubation with hydrogen peroxide while beef Hb remained as oxyHb during the incubation period. Formation of metHb in the presence of hydrogen peroxide was previously measured at various oxygen partial pressures, and it was found that with increasing oxygen partial pressure the rate of metHb formation decreased (*29*). This indicated that deoxyHb reacted more readily with hydrogen peroxide than oxyHb. Hydrogen peroxide can react more rapidly with deoxyHb as compared to oxyHb since accessibility of the heme iron is inhibited by the O$_2$ ligand with oxygenated molecules (*30*). Furthermore, oxyHb is more compact than deoxyHb and greater heme pocket flexibility exists in the deoxygenated form (*31, 32*). Because trout hemoglobins contained high levels of deoxyHb at pH 6.3 as compared to beef Hb, the formation of metHb could occur by the following mechanism as proposed by Yusa and Shikama (*33*):

$$\text{deoxy}(+2)\text{Hb} + \text{H}_2\text{O}_2 \rightarrow \text{ferryl}(+4)\text{Hb} + 2\text{OH}^- \qquad (3)$$

$$\text{ferryl}(+4)\text{Hb} + \text{deoxy}(+2)\text{Hb} \rightarrow 2\text{met}(+3)\text{Hb} \qquad (4)$$

Unique amino acid sequences near the heme crevice can explain the fact that perch Hb had a low deoxyHb content and a high autoxidation rate (**Figure 4**). Typically, low deoxyHb

content is associated with low autoxidation rate by limiting reaction 2. Site-directed mutagenesis techniques have been used to examine the effect of amino acid substitutions in heme proteins on prooxidative parameters. For example, histidine[97] is located at the exterior of the proximal heme pocket in myoglobin. Disruption of the hydrogen-bonding network of His[97] by mutation to a smaller side chain had little effect on ligand binding properties (e.g., oxygen affinity) but created an opening of a channel to the heme crevice that better facilitated entry of H_2O into the heme crevice (*34*). Even though a given heme protein has high oxygen affinity, there still are substantial amounts of O_2 dissociations per second. The deoxyHb that forms upon dissociation will bind an O_2 molecule (association) more readily as residence of H_2O molecules in the heme pocket decreases. A larger heme crevice volume however should increase access of H_2O to the heme pocket, accelerating metHb formation according to eq 5 as described previously (*22*):

$$\text{deoxy}(+2)\text{Hb} + H_2O \rightleftharpoons \text{deoxy}(+2)\text{Hb}\cdots H_2O \quad (5)$$

$$\text{deoxy}(+2)\text{Hb}\cdots H_2O + O_2 \rightarrow \text{met}(+3)\text{Hb} + O_2^{-\bullet}$$

Thus, a relatively small amino acid in Hb at the site analogous to His[97] in myoglobin might explain why perch Hb rapidly autoxidized despite high oxygen affinity. Studies are needed to characterize the amino acid sequence and crystal structure of perch Hb.

The ability of deoxyHb to accelerate autoxidation (metHb formation) leads to a number of events that can accelerate lipid oxidation. Resistance to heme loss results from reduction of the heme iron from its met to ferrous form (*35*). Using a linoleic acid−myoglobin model system, denaturation of myoglobin was believed to expose heme to the environment or release heme and accelerate lipid oxidation (*36*). Myoglobin unfolding is around 60 times more likely when examining met forms as compared to ferrous forms (*37*). Furthermore, metHb reacts with peroxides to form ferryl forms of Hb that can initiate lipid oxidation (*38*). These findings may explain the rapid rate of lipid oxidation induced by the perch hemoglobins (**Figures 5** and **6**) that were prone to rapid autoxidation (**Figure 4**). The possibility of more efficient decomposition of lipid hydroperoxides by deoxyHb as compared to oxyHb should also be considered as a viable mechanism of deoxyHb-mediated lipid oxidation that is independent of metHP formation (*39*).

It is generally believed that the major forces controlling rates of lipid oxidation in muscle foods are endogenous antioxidant capacity and the amount of polyunsaturated fatty acids that are present. In water-washed fibers from fish, chicken, and beef, the rate of Fe^{2+} or horse metmyoglobin-mediated lipid oxidation increased with increasing fatty acid unsaturation in the fibers (fish > chicken > beef) (*40*). Our current and previous studies indicate a wide variation in the ability of different hemoglobins to promote lipid oxidation in washed cod muscle (perch > trout > poultry > beef) (*6*). It is suggested that this variation among different hemoglobins could contribute to the variation in rates of lipid oxidation that are observed when comparing different muscle foods. Future work should further characterize the role of deoxyHb in lipid oxidation processes that occur in muscle food systems.

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Reactivity of Different Hemoglobins

J. Agric. Food Chem., Vol. 51, No. 13, 2003   **3891**

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