SULFHYDRYL CONTENT OF TUNA MYOGLOBIN

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Crystalline tuna myoglobin preparations have been described by Huys (1954), Rossi-Fanelli et al. (1955), and Matsuura and Hashimoto (1955). Konosu et al. (1958) determined the amino acid composition of globin obtained from the myoglobin of bluefin tuna (Thunnus orientalis). Cystine and/or cysteine was estimated from the cysteic acid content of the hydrolyzate of oxidized globin. Approximately one mole of half-cystine was present per 17,000 grams, the assumed molecular weight.

We have prepared myoglobin from the red meat of yellowfin tuna (Neothunnus macropterus), and find that it contains approximately two equivalents of sulfhydryl per mole.

Preparation of Myoglobin. Red meat was obtained from frozen tuna which were being thawed in preparation for canning. The meat was ground through a meat grinder and extracted with water. Proteins were fractionated from the clarified extract with ammonium sulfate at pH 6.6 and 0-5° C. The myoglobin was soluble at 50% but insoluble at 75% saturation. The insoluble material was dissolved in water and fractionated between 70% and 75% saturated ammonium sulfate. This process was repeated until successive preparations showed a constant ratio of the absorbance at 406 mμ (the Soret peak of the metmyoglobin) to that at 275 mμ.

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Absorption Spectra. The absorption spectra of the purified yellowfin tuna myoglobin and some derivatives corresponded reasonably well with those published by Matsuura and Hashimoto (1955).

The absorption maxima and $E_{1\%}$ were as follows: metmyoglobin, 275 mμ, 13.9, 406 mμ, 80.5, 501 mμ, 4.8, 630 mμ, 2.1; carbonmonoxy-myoglobin, 420 mμ, 104.1, 538 mμ, 7.7, 568 mμ, 7.0; myoglobin, 431 mμ, 59.7, 556 mμ, 6.6. The extinction coefficients were calculated from the dried weight (vacuum oven at 70°) of thoroughly dialyzed preparations.

Sedimentation Velocity. The myoglobin preparation had a single homogeneous peak in the ultracentrifuge. The corrected s_{20} value of 1.80 S reflects the low molecular weight and is in agreement with previously published values for myoglobins from carp (Hamoir, 1953) and other animals (Svedberg and Pedersen, 1940). We are indebted to Dr. H. K. Schachman for this determination.

Iron Content. The preparation contained 0.35 ± 0.01% iron (dry weight basis) by the thiocyanate procedure (Sandell, 1944), as used on a sulfuric acid digest.

Dialysis. The yellowfin tuna myoglobin dialyzed slowly through 20/32" Visking tubing (cf. Craig et al., 1957), but more than twice as fast as did a preparation of whale myoglobin (humpback whale, Megaptera nodosa).

Amino Acids. Preliminary studies by Hirs* indicate that the amino acid composition of the yellowfin myoglobin closely resembles that of bluefin myoglobin as recorded by Konosu et al. (1958).

Sulfhydryl Content. Sulfhydryl was determined by the amperometric method essentially as described by Benesch et al. (1955). The material in tris(hydroxymethyl)aminomethane buffer, pH 7.4, was titrated with Ag^+ in the presence of 0.00025 M ethylenediaminetetraacetate(Sokol et al.,

* Hirs, C.H.W., manuscript in preparation.

1959). Oxygen-free nitrogen was bubbled through the solution during the titration. Under these conditions close to theoretical results (90-100%) were obtained with fresh solutions of glutathione and cysteine hydrochloride at room temperature. At 0° the cysteine required 130% of the expected Ag^+ titration (cf. Hoch and Vallee, 1959). Bovine serum albumin, bovine hemoglobin, and ovalbumin gave values of approximately 0.6, 2, and 4 groups per mole in accord with other published values. Whale myoglobin contained no sulfhydryl groups.

Variable sulfhydryl results were obtained with different yellowfin tuna myoglobin preparations. The electrophoretically-homogeneous one gave titrations equivalent to 1.1 to 1.3 sulfhydryl groups per 17,000 assumed molecular weight. Other preparations gave as high as 1.9 and as low as 0.2 groups. Those that were low had been subjected to more drastic conditions of time and temperature of storage. In 6 M urea the titration was slightly less than when urea was not added. The causes of these variations are being studied further.

Discussion

The yellowfin tuna myoglobin preparation may be considered to be reasonably free of non-myoglobin proteins. It resembles previously described tuna myoglobins in absorption spectra, amino acid composition, and iron content, and was homogeneous by electrophoresis. However, detailed electrophoretic and chromatographic analyses would be required to determine whether minor amounts of closely related myoglobins might be present.

The average molecular weight is 16,000 to 17,000 as indicated by the sedimentation constant, the iron content, and the rate of passage through the 20/32" Visking tubing.

All of these properties are in accord with those of previously described myoglobins. However, the presence in yellowfin tuna myoglobin of free sulfhydryl residues is unique. Presumably the

myoglobin of bluefin tuna described by Konosu et al. (1958) also contained one or more sulfhydryl groups.

Summary

A homogeneous preparation of myoglobin made from the red meat of yellowfin tuna (Neothunnus macropterus) resembled previously described myoglobins in solubility, absorption spectra, iron content, and sedimentation velocity, but, uniquely, contained one to two free sulfhydryl groups per assumed molecular weight of 16,000-17,000.

References

Benesch, R. E., Lardy, H. A., and Benesch, R., J. Biol. Chem. 216, 663 (1955).

Craig, L. C., King, T. P., and Stracher, A., J. Amer. Chem. Soc. 79, 3729 (1957).

Hamoir, G., Nature, 171, 345 (1953).

Hoch, F. L., and Vallee, B. L., in R. Benesch et al., Sulfur in Proteins, Academic Press, Inc., New York, 1959, p. 245.

Huys, J. V., Arch. Internat. Physiol., 62, 296 (1954).

Konosu, S., Hashimoto, K., and Matsuura, F., Bull. Jap. Soc. Sci. Fisheries, 24, 563 (1958).

Matsuura, F., and Hashimoto, K., Bull. Jap. Soc. Sci. Fisheries, 20, 946 (1955).

Rossi-Fanelli, A., and Antonini, E., Arch. Biochem. Biophys., 58, 498 (1955).

Sandell, E. B. Colorimetric Determination of Traces of Metals, Vol. III, Interscience Publ., New York, 1944, p. 271.

Sokol, H. A., Mecham, D. K., and Pence, J. W., Cereal Chem., 36, 127 (1959).

Svedberg, T., and Pedersen, K. O., The Ultracentrifuge, Oxford University Press, New York, 1940.