

REVIEW OF BASIC RESEARCH ON OXIDATIVE ENZYMES IN FISH TISSUE^{1/}

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ABSTRACT

Basic research on oxidative enzymes in fish tissue now has been undertaken to lay a sound foundation for effective applied research.

INTRODUCTION

Some aspects of the collaborative research program between the Department of Food Technology of the University of California and the Seattle Fishery Technological Laboratory of the U. S. Bureau of Commercial Fisheries have been described in the previous paper in this series (Stansby and Brown 1958). The present report deals specifically with one of the newer phases of investigation; namely, a study of the oxidative enzymes of fish. Knowledge of such enzymes has most direct application to two closely related areas of interest to fishery technology; (1) the nature and properties of the enzymes that survive the death of the fish and (2) the enzymes and pathways of intermediary metabolism in fish.



Fig. 1 - Measuring activity of a glycolytic enzyme by spectrophotometric assay.

A knowledge of the nature and properties of surviving enzymes is needed because of the possibility that these catalysts may bring about certain biochemical transformations, such as the oxidation of carbohydrate intermediates in fishery products that are held under refrigeration. Such transformations could be either detrimental or beneficial to the final product. In either case, an understanding of them is a prerequisite to the control of them.

A knowledge of the intermediary metabolism of fish would provide an understanding of the details of enzymic reactions involved in the synthesis and breakdown of proteins, fats, and carbohydrates. A fundamental investigation of these pathways is essential. Because of the many enzymes involved and the detail required in such a study, it is neither brief nor simple. A thorough understanding of the chemistry of fish metabolism, however, would be of great value because it would afford a basis of understanding and application to problems found in fields of investigation such as fish nutrition and commercial handling, processing, and preservation. An example pertinent to this discussion is that an understanding of fish metabolism is required in the study of surviving enzymes.

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Our initial research was directed specifically at a study of the surviving enzymes of fish. Preliminary studies were aimed at identifying such enzymes in several species of frozen fish, including yellowfin, albacore, and skipjack tuna, king salmon, ocean perch, and black rockfish. It was apparent in the early stages of this study, however, that knowledge of the fundamental pathways of metabolism in live fish would contribute greatly to a study of surviving enzymes. Consequently, this portion of the research has been suspended, pending completion of certain basic studies on intermediary metabolism. The remainder of this report will deal more specifically with the investigations of the enzymes of intermediary metabolism of fish, with emphasis on oxidative enzymes.

Suitable test fish for use in the fundamental enzymic studies have to fulfill certain requirements, including continuous availability and ease of maintenance in aquaria in a size suitable for laboratory manipulations. Members of the minnow family (*Cyprinidae*), particularly the common carp, fulfill these requirements and have been used in these studies. In addition to the routine use of the test fish, extensions have been and will continue to be made to various commercial food fishes by using various species of them as test animals for the study of any enzyme or reaction judged to be of particular importance, or simply by comparing various enzymic activities in commercial fish with those of our test carp.

PRINCIPAL FINDINGS

An examination of the literature revealed little in the way of information about oxidative enzymes in fish. Reports that had appeared were widely scattered, and no compilation of the existing material had been made. An extensive review of the literature therefore was completed and now is being written. This material will be published shortly.

Although little information is available about intermediary metabolism in fish, the literature contains an extensive biochemical background dealing with metabolic pathways in other animals as well as in bacteria and in plants. It appeared likely that the pathways occurring in fish would most closely resemble those found in other animals. Our initial approach, therefore, was to study certain selected areas known to be of great importance in animal metabolism, using the techniques and enzymic assays developed by other investigators in this general area. Fish are directly compared to mammals; any differences revealed by the study can be explored more intensively to gain a thorough knowledge of pathways peculiar to aquatic animals. The research to date on this problem has been concentrated on the two general areas of carbohydrate metabolism and fatty acid metabolism. The general plan of research in these studies has been to assay for activities of specific enzymes known to be catalysts in the metabolic pathways that were of interest. In general, the assay methods used for all the various enzymes follow the same type of procedure and involve some measure of the rate of the enzyme-catalyzed reaction. Since the majority of enzymes of interest are oxidative, for example, oxygen uptake could be measured in a system that contained substrate, enzyme preparation, and any necessary cofactors, all maintained in a suitably-controlled environment.

CARBOHYDRATE METABOLISM: Three main pathways in carbohydrate metabolism are of particular importance: (1) glycolysis, (2) the hexose monophosphate shunt, and (3) the tricarboxylic acid cycle. In each pathway, multienzyme systems catalyze a sequence of reactions resulting in the transformation of a given metabolite to chemical energy and products. Activities of many of the enzymes of these pathways have been measured in our preparations, which usually were homogenates of fish tissue.

Glycolysis refers to several reactions in an organism that are catalyzed by a multienzyme system and that result in the breakdown of starch or glycogen to pyruvic acid, with the accompanying production of compounds rich in energy. The

pyruvate formed may be transformed into lactic acid as is done in animal muscle. Additionally, it may enter into the tricarboxylic acid cycle (see below) after having been changed to an activated two-carbon form. In the over-all process of glycolysis, a large number of individual enzymic reactions are involved. In our fish preparations, assays have been made and activity found for the following glycolytic enzymes: lactic acid dehydrogenase, alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, aldolase, and two phosphohexoisomerases; namely phosphomannose isomerase and phosphoglucose isomerase. The presence of these representative enzymes indicates that glycolysis is functioning in fish.

Glycolysis is generally considered to be the main pathway for the breakdown of monosaccharides to pyruvic acid. It is now well recognized, however, that an alternative pathway called the hexose monophosphate shunt exists in some organisms. It was of interest to know if this pathway functioned in fish tissue; assays for two of its enzymes therefore were made: glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase. Activities of these enzymes were found, indicating that this shunt pathway functions in fish. Additional research will tell us the relative contribution to carbohydrate metabolism made by these two alternate routes.

The tricarboxylic acid cycle is of prime importance not only to carbohydrate metabolism but to fat and protein metabolism as well, since it provides a means for the various products of metabolism to be oxidized to carbon dioxide and water. This unique and versatile cycle unites glycolysis, fatty acid oxidation, and amino acid breakdown to one final oxidative mechanism. It occurs in a large number of organisms, including animals, microorganisms, and higher plants. In our research, it has been studied in more detail than has glycolysis. A majority of the enzymes known to be involved in this cycle have been found to be present in fish. In some cases, the products formed by the enzyme reactions have been measured, and the effects of various activators and inhibitors have been studied. The enzymes of the tricarboxylic acid cycle investigated to date are condensing enzyme, aconitase, isocitric dehydrogenase, succinoxidase, fumarase, and malic dehydrogenase. The evidence obtained so far in this study indicates that this cycle in fish is similar to that in other animals.

FATTY ACID OXIDATION: Considerable attention has been paid in the past to the fact that many fish contain relatively large amounts of highly unsaturated fatty acids. The significance of these unique fatty acids is well recognized from the commercial standpoint, owing to their desirability, for example, in fish-oil fractions for some purposes; or to their undesirability, for example, in contributing to rancidity in fresh or manufactured fish products and heating in fish meals. Unfortunately, little or no research has been undertaken to elucidate, at the enzyme level in fish, the biochemical basis of the synthesis and oxidation of these fatty acids.

Our initial experiments in this area have been patterned after the research of investigators who used mammalian tissue. In general, this involved preparing a cell-free fish-liver homogenate, which was fractionated by differential centrifugation into various particulate fractions. The enzyme-containing fraction (mitochondria) then was added to a system containing fatty acid and various cofactors and activators, and the consumption of oxygen was measured manometrically. Mitochondria were used as an enzyme source because most of the activity is localized in these particles; additionally, their use enables the development of a more defined system, since soluble substances are washed away, and furthermore, any information gained is at the more basic cellular component level.

The multienzyme system causing the oxidation of fatty acids in fish tissue has been studied in detail. The requirements for the system have been defined, its properties outlined, and the effect of numerous inhibitors and activators on the system observed. The ability of the system to utilize a considerable number of fatty acids has been ascertained. The fatty acids used as substrates include butyric,

octanoic, palmitic, linoleic, linolenic, arachidonic, and a fish-oil fatty acid fraction. In general, the behavior of this system resembles that observed in mammals, but there are some differences in detail.

CURRENT RESEARCH

Current research in this phase of the program is in two areas: (1) tricarboxylic acid cycle and (2) fatty acid oxidation and synthesis. The nature of the research in both these areas is primarily an extension of the work outlined above.

In the case of the tricarboxylic acid cycle, a continuation of the study will include assays for those members of the cycle not yet demonstrated in fish, as well as detailed fundamental studies of specific enzymes or reactions of particular importance. In this connection it may be pointed out that one of the enzymes of this cycle, succinoxidase, is of great interest, since it is one of the more stable enzymes and would be a surviving enzyme in refrigerated or frozen fish.

In the near future, the investigation of oxidation of the highly unsaturated fatty acids will be intensified, because these are of such great importance in fish. We propose an investigation on the synthesis of these unsaturated fatty acids by fish. This particular problem has great general interest as well, since the mechanism of biosynthesis of highly unsaturated fatty acids in any organism is not well defined.

DISCUSSION

The information gathered to date on this portion of our investigation has been purposely presented in summary fashion. Much data have been accumulated; to present even a portion of the information in detail, however, would make this report too unwieldy to serve its purpose. The detailed results of these studies therefore will be presented for publication to a biochemical journal.

As was suggested in the introduction to this paper, the most immediate application of these studies may well have to do with enzymic reactions that occur after death of the fish and that affect the desired final product. As a hypothetical example, use might be made of some surviving enzyme, for example, succinoxidase, to act as an oxygen scavenger in a packaged frozen product and thus remove traces of oxygen that might otherwise have undesirable effects on the color or flavor of the product.

It cannot be stressed too strongly that in order to develop any application, it is necessary to understand the enzymes of fish at a basic biochemical level. Our fundamental studies of certain of these enzymes are designed to provide such an understanding and thereby to lay the basis for sound applied research.

LITERATURE CITED

- STANSBY, M. E., and BROWN, W. D.
1958. Review of Progress on the Program on Oxidative Deterioration in Fish and Fishery Products. (See p. 24 of this issue.)



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