Transsulfurase Activity In Digestive Glands Of Gastropods

Hans G. Borei and Norman Meadow
University of Pennsylvania

An improved assay procedure for the enzyme has been worked out, which more closely corresponds to the optimal substrate conditions. The extreme cyanide sensitivity of the enzyme calls for conditions favoring a complex-binding to the sulfur source. Also the buffer capacity of the test has been increased. Introduction of formaldehyde for stopping the reaction, also stabilizes and enhances the extinction at 460 mu of the iron-complex used for optical assay.

The use of a detergent, cetyltrimethylammonium bromide, in low concentrations considerably facilitates the extraction of the enzyme from the mitochondria, increasing the yield by 50%. --- The pH optimum of the enzyme activity was found to be different in different animals: *Pecten* 8.6 - 8.8, *Littorina* 9.0 - 9.1, *Mytilus* above 10.0. --- Freezing of the enzyme homogenate (*Littorina*) in presence of thiosulfate and storage at low temperature, increases the enzyme yield progressively over the next 5-7 days up to a maximum of 3 times the yield of the untreated preparation. Lyophilization achieves a similar mitochondrial leakage, with an even more spectacular increase in enzyme yield in presence of thiosulfate. The lyophilized preparations lose their activity only slowly; 20 days after preparation the activity is still maximal. The *Littorina* preparations are entirely dependent on the protective function of added thiosulfate; *Buccinum* and *Thais* do not need it.

Aided by a research grant from NIH.

Properties Of Thiosulfate Transsulfurase From Marine Invertebrates

Hans G. Borei
University of Pennsylvania

The pH-optima of thiosulfate transsulfurase from midgut glands of various marine molusks show this enzyme to occur in two main variants, one with a broad optimum between pH 8.8 - 9.2, the other with a sharp activity peak around pH 10.0 - 10.2. *Littorina* and *Pecten* belong to the former group, whose characteristics are very similar to those of mammalian liver transsulfurase. In the latter group are found *Mytilus* and *Anodonta*. This group shows only ca. 50% activity at the pH of the optimum of the first group (pH 9), whereas the first group is still less active at pH 10. No other principal enzymatic differences are found between the two groups. It is thought that the found difference in pH-dependency rather reflects structural properties of the total enzyme molecule than specific differences in the active site.

The highly active thiosulfate transsulfurase of the hepatic caeca of

Asterias belongs to the group with optimum at pH 10.0 -10.2. Considering the *Mytilus*-diet of these animals, experiments have been started to determine whether the enzyme is of external origin from the food, or is part of the mitochondrial complex of the starfish cells.

The activity of the enzyme is characterized by the formation of an enzyme-substrate complex with the thiosulfate. At optimal cyanide level. 50mM., the *Littorina* enzyme gives a $K = 6 \times 10^{-3}$ at 25 °C. The association of the enzyme with its other substrate, the cyanide, seems more complicated. The activity at low cyanide level suggests a K_m below 10^{-3}, but the picture at higher cyanide levels is complicated by an inhibitory effect. Indeed, the enzyme is very sensitive towards cyanide; in the absence of thiosulfate, low cyanide concentrations render the enzyme irreversibly inactive. For protection and activity the optimal thiosulfate concentration is 100 - 120 mM. Under normal activity-measurement conditions there is a competitive balance between the protective effect of thiosulfate and the inactivation through cyanide. However, v_{max} is identical for both substrates if measured at optimal concentration of the other substrate.

Respiration Of The Avian Salt Gland In Media Of Various Ionic Composition

Arieh Borut
Duke University and Hebrew University, Jerusalem, Israel

Respiration measurements of tissue slices from the salt gland of herring gulls was undertaken in order to investigate the correlation between active ion transport and oxygen consumption. The Qo_2 (ul 0_2 per mg dry weight of tissue per hr.) of the salt gland in Krebs phosphate media (pH 7.4) and oxygen gas phase was about 9.2, declining slowly after an initial period of 30 to 40 minutes of constant respiration. Addition of glucose to the media had no effect. Metacholine, if added to the medium, increased the Qo_2 by 55%. When metacholine was added while a respiration experiment was in progress there was an immediate increase of the respiration by at least 20%.

The salt gland slices showed remarkably little response to changes in the NaCl concentration in the media. Increase by 50% of the NaCl concentration, with or without metacholine, gave no increase in respiration, and doubling the amount caused inhibition. In substituted Krebs solutions the strongest effect was shown by Na-succinate. When Na-succinate substituted all the NaCl in the medium, the Qo_2 was 20 to 24, or more than twice the Qo_2 in the Krebs phosphate solution. This increase was probably due to a large amount of succinic dehydrogenase in the glands. Other substitutes such as Na-lactate or NaBr showed about the same activity as normal Krebs medium, Qo_2 being around 11. Media with isotonic sucrose, and without Na, or without Cl, showed lower activity than normal Krebs medium (Qo_2 about 7.5 for Na less media, and 6.4 for Cl less, values close to the respiration of strophanthidin inhibited slices -