BILIRUBIN GLUCURONIDATION IN FISH. I. URIDINE DIPHOSPHATE GLUCURONATE GLUCURONYL TRANSFERASE

Jayanta Roy Chowdhury, Namita Roy Chowdhury and I.M. Arias, Liver Research Center, Albert Einstein College of Medicine, Bronx, N.Y. and Mount Desert Island Biological Laboratory, Salisbury Cove, Maine

Although fish liver can excrete both conjugated and unconjugated bilirubin, most bilirubin in fish bile is bilirubin glucuronide (Jansen and Arias, 1977, Comp. Biochem. Physiol. 50(B), 255-258). UDP-glucuronate glucuronyl transferase (EC 2.4.1.17) activity has been demonstrated in elasmobranch liver. Using sodium cholate as the activating detergent, the temperature optimum for the glucuronyl transferase reaction has been reported to be 22°C and 30°C in dogfish and skate, respectively.

In the present study, we examined the optimum reaction conditions for UDP-glucuronyl transferase activity in two elasmobranchs (spiny dogfish, Squalus acanthias and small skate, Raja erinacea) and a teleost (winter flounder, Pseudopleuronectes americanus). Thermolability of the enzyme activity in presence of the non-ionic detergent Triton X-100 was compared to its thermolability in presence of sodium cholate, and in absence of any detergent.

Spiny dogfish, small skate and winter flounder were caught in the Frenchman's Bay, Me. Dogfish (100 g), skate (30 g) or flounder (12 g) liver was perfused with ice-cold 0.25 M sucrose-Tris/HCl buffer (0.005 M, pH 8.0), minced and homogenized with 3 ml/g liver of the same buffer. The homogenate was centrifuged at 9000 x g for 15 min. The top fatty layer was discarded, and the pink lower layer of the supernatant was centrifuged at 105,000 x g for 1 h. The pellet was designated as the "microsomal fraction" and was resuspended in homogenization buffer (5 ml in cases of dogfish and skate, 2.5 ml in case of flounder), and stored at -20°C.

For determination of UDP glucuronyl transferase activity the incubation mixture consisted of 0.1 mol bilirubin, 4.4 mol UDP glucuronic acid, 4.0 mol $MgCl_2$, 0.2 ml microsomal preparation and either Triton X-100 or sodium cholate in a final volume of 1.2 ml. After initial determination of optimal pH and Triton X-100 concentration, experiments were performed at pH 8.0 with 2% Triton X-100 (final concentration). For testing thermolability of the glucuronyl transferase activity, no detergent, sodium cholate (0.12%) or Triton X-100 (2%) was used. Blanks contained no UDPglucuronic acid, and were kept in ice. Following incubation at 37°C for 20 min, the reaction was stopped by adding 4 ml ice-cold glycine buffer (0.1 M, pH 2.7). Ethyl anthranilate diazo reagent (Van Roy and Heirwegh, 1968. Biochem. J. 107:507-508), 2 ml was added, and the mixture was incubated at 25°C for 30 min. The diazo reaction was stopped by addition of 1 ml 10% ascorbic acid, azopigments were extracted in 2 ml methy-n-propyl ketone/butyl acetate (17/3, v/v) and rates of glucuronidation were determined from difference of absorbance at 530 nm between reaction and blank, assuming E_{530} for conjugated bilirubin $44.4 \times 10^3 . M^{-1} . cm^{-1}$. The reaction products were analyzed by thin-layer chromatography of the azopigments, and were found to be bilirubin monoglucuronide.

Formation of bilirubin glucuronide was linear for protein concentration in microsomes and time of incubation during first 30 min of incubation. Triton X-100 at an optimum concentration (2%) enhanced the reaction rate 2-4 fold. The pH optimum for the reaction was 8.0 to 8.2. The optimum reaction temperature in all three fish was 37°C when either Triton X-100 (Fig. 1) or no detergent was used to activate the enzyme activity. Glucuronyl transferase activity was labile at 37°C in sodium cholate, but not in Triton X-100. This may explain the difference between the optimum reaction temperature observed in the presented study and that reported previously (Jansen and Arias, 1977. Comp. Biochem. Physiol. 50(B), 255-258

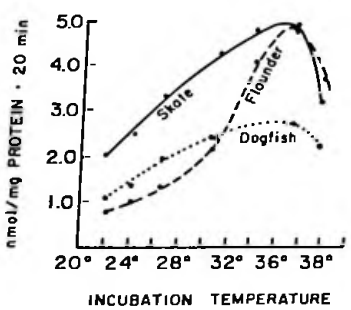

Figure 1. Temperature dependence of UDPglucuronate glucuronyl transferase activity of skate (—), flounder (---) and dogfish (. . .) liver microsomes. Abscissa represents incubation temperature, and ordinate represents specific enzyme activity.