# BILIVERDIN REDUCTASE ACTIVITY IN THE LITTLE SKATE, RAJA ERINACEA

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In the pathway of heme degradation, cofactor-dependent biliverdin reductase is the enzyme responsible for catalyzing the conversion of biliverdin to bilirubin. Though humans and many other mammals secrete only bilirubin and its conjugates into their bile, Grossbard et al. (J. Comp. Physiol. 157:61-66, 1987) found that the elasmobranch Raja erinacea (little skate) produces a bile which has a bile pigment composition of approximately 50% biliverdin and 50% bilirubin and its conjugates. Furthermore, it was postulated that, since $H^3$ bilirubin injected intravenously was recovered mostly as $H^3$ bilirubin and its conjugates in the bile, the biliverdin found in the skate bile is unlikely to be the product of oxidized bilirubin and so must be excreted by an endogenous mechanism of its own. Perhaps the reason for the excretion of both biliverdin and bilirubin in the skate bile is that low biliverdin reductase activity is limiting the complete reduction of available biliverdin. The aim of this study was to determine if the finding of both substrate and product bile pigments in the skate gallbladder could be accounted for by decreased biliverdin reductase activity, and if so, to characterize some of the properties of the enzyme.

Fresh skate livers were homogenized in a 33% weight by volume 0.25 M sucrose solution or a 0.02 M potassium phosphate and 0.134 M potassium chloride buffer at pH 7.4. The cytosol was separated through a series of centrifugations performed at 4°C. This non-purified preparation was the sample tested for biliverdin reductase activity. The biliverdin reductase assay contained the following: biliverdin substrate (80% pure) from Sigma, 0.1 mM NADPH, 0.035% bovine serum albumin, and protein of the cytosol preparation in a 0.02 M potasssium phosphate buffer at pH 7.4. Activity was determined spectrophotometrically at 670 nm by measuring the disappearance of biliverdin over time at 25°C. The change in absorbance without NADPH was used as the blank. Enzyme activity was measured at varying substrate concentrations (12 $\mu$M - 160 $\mu$M), protein concentrations (1 mg - 3 mg), and buffer pH's (pH 6.9 - pH 7.7). Protein determinations were made by the Lowry method.

Fig.1, which depicts absorbance versus time for a typical assay, demonstrates that the reduction of biliverdin occurs in two stages. The initial decrease in absorbance occurs within a few minutes of the addition of the cytosol preparation to the assay mixture and does not require the cofactor NADPH. This dramatic decline in biliverdin is the result of non-enzymatic reducing factors present in the crude preparation of hepatic cell cytosol. A similar phenomenon has been noted in other oxidoreduction reactions in vitro (Bergmeyer, In Methods of Enzymatic Analysis, Vol.1, pp.106-107, Verlag Chemie, Weinheim, West Germany, 1983). Changes in the amount of preparation and in the pH do not have any effect on this initial part of the curve; increasing the substrate concentration actually causes a decrease in the rate of reduction.

Catalyzed by biliverdin reductase, the second stage of biliverdin reduction is much slower than the first, requires a cofactor (NADPH), and lasts more than 120 minutes if enough cofactor is present to sustain this reaction. Increasing the protein content in the assay results in a proportional increase in activity, as would be expected for an enzymatic reaction. Activity in this part of the curve is also pH dependent; it is highest at pH 7.2 to 7.6. Moreover, prolonged heating of the cytosol preparation destroys this activity. Using various substrate concentrations in the assay, a Lineweaver-Burk plot of biliverdin

reductase activity was constructed (Fig.2). This graph shows the Km of the enzyme in the skate to be 199 $\mu$M, much higher than the reported Km of 3-5 $\mu$M for mammals such as the rat (Kutty and Maines, J. Biol. Chem. 256:3956-3962, 1981).

Therefore, we submit that, provided substate concentrations are similar <u>in vivo</u> to the range of the Km's found, the enzyme activity of biliverdin reductase in the little skate is so low that it is the limiting step in the degradation of heme to bilirubin. The enzyme seems unable to convert all of the skate's biliverdin to bilirubin and thus, both bile pigments are excreted into the bile.

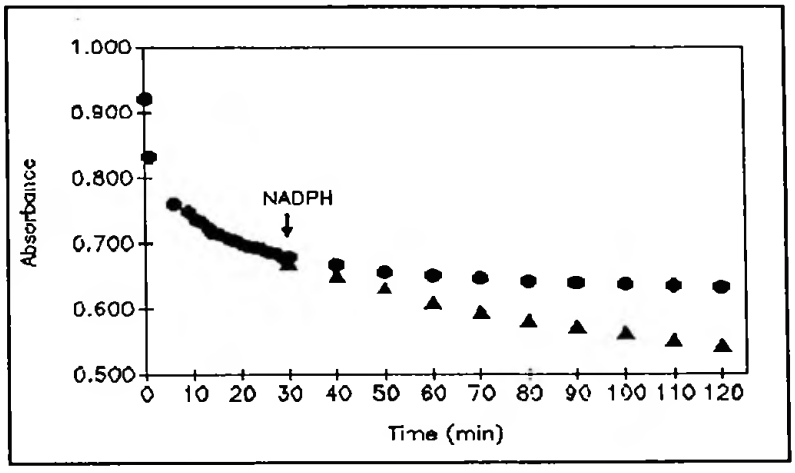


Fig. 1. Changes in absorbance at 670 nm as a function of time (min). The assay contained 80 $\mu$M biliverdin, 2.7 mg protein, 0.035% BSA and potassium phosphate buffer with (▲) and without (●) 0.1 mM NADPH. NADPH was added at t = 30 min.

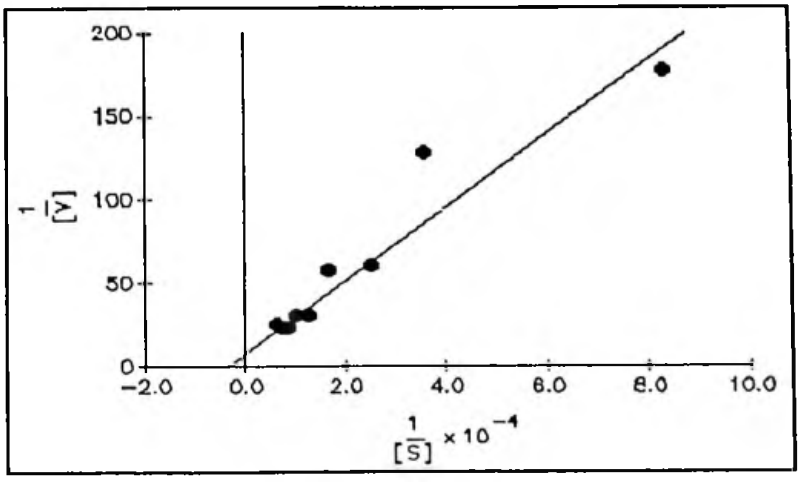


Fig. 2. Lineweaver-Burk plot of biliverdin reductase in the little skate. The inverse of specific activity (mg protein / enzyme units) is plotted against the inverse of various substrate concentrations (moles$^{-1}$). Each data point represents an average of experiments with up to 8 separate skates. One enzyme unit is defined as a change of 0.01 absorbance over one minute. The Km is the negative inverse of the x-intercept of this plot (199 $\mu$M); the Vmax of the plot is the inverse of the y-intercept (0.104 enzyme units / mg protein).

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