Monatshefte für Chemie 122, 319–321 (1991)

Monatshefte für Chemie
Chemical Monthly

Printed in Austria

Short Communication

Reduction of a Bilindione-10-Thiol-Adduct as a Model of the Reduction Step of the Biliverdin Reductase System

Heinz Falk* and **Helmut Marko**

Institut für Chemie, Johannes-Kepler-Universität, A-4040 Linz, Austria

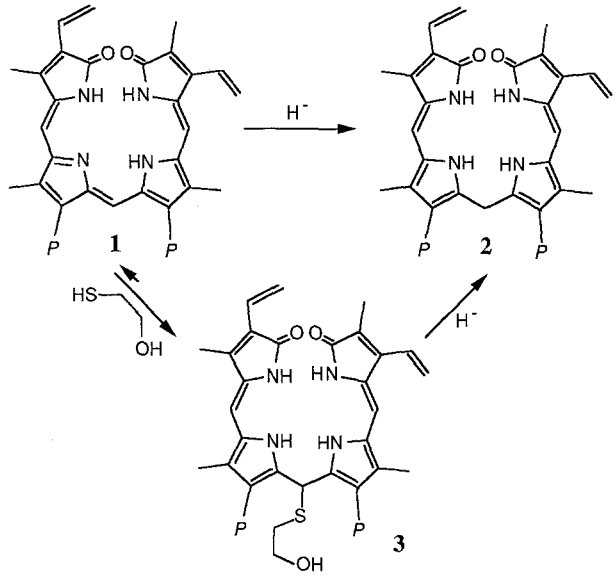
Summary. Biliverdin dimethyl ester and its 10-thioethanol addition product are reduced by $LiBH_4$ with similar reaction velocities as derived from NMR experiments. This reaction serves as a model of the biliverdin reductase system. According to this model the addition of the enzyme thiol group to position 10 of biliverdin primarily serves as the binding and orienting mode. It is obviously not intended as such to accelerate the velocity of the reduction step.

Keywords. Biliverdin dimethylester; Biliverdin reductase; Reduction; NMR kinetics.

Die Reduktion eines Bilindion-10-thioladduktes als Modellreaktion für den Reduktionsschritt des Biliverdinreduktase-Systems (Kurze Mitt.)

Zusammenfassung. Biliverdindimethylester und sein 10-Thioethanoladdukt werden, wie durch NMR-Experimente gezeigt werden konnte, durch $LiBH_4$ mit vergleichbaren Geschwindigkeiten reduziert. Diese Reaktion dient als ein Modell für das Biliverdinreduktasesystem. Entsprechend diesem Modell dient die Addition der Enzym-Thiolgruppe in Position 10 des Biliverdins in erster Linie der Bindung und Orientierung und ist als solche nicht in Hinblick auf eine Reaktionsbeschleunigung der Reduktion konzipiert.

Ten years ago we investigated a reversible and fast addition of donors (alcohols, thiols, amines, cyanide, bisulfite, and C–H acidic compounds) to position 10 of bilindiones [1]. This equilibrium was extensively used to synthesize the photodiastereomers of bilindiones and 2,3-dihydrobilindiones [2]. The formation of a rubinoid intermediate (the adduct) that is susceptible to photodiastereomerization proved to be the key step in this process [1]. Recently it was demonstrated by Frydman [3] that the addition of a biliverdin reductase thiol group to biliverdin is the initial step in the reaction sequence leading to bilirubin [4]. Binding of this substrate provides a proper orientation for the following reduction step using the biological hydride donors *NAD*H or *NADP*H. Thus it eventually turned out that this, at the outset, rather artificial addition reaction is indeed a fundamental reaction of bile pigment metabolism. Therefore it seemed to be of interest to document the possibility of reducing a bilindione-10-thiol adduct by means of hydride ions, and to compare the reaction velocities of hydride reduction of a verdinoid pigment with and without thiol addition.



As a model system we used biliverdin dimethylester (**1**; *P* denote propionic methylester side chains) which was reduced by means of one hydride equivalent of $LiBH_4$ to bilirubin dimethylester (**2**) at room temperature (5 μmolar solution in deuterated dimethyl sulfoxide). The velocity of this reaction (disappearance of **1**) was monitored from the intensity of its 10-H ^{1}H-NMR signal at 6.95 ppm (Fig. 1). Likewise, formation of **2** also could be monitored in principle. However, due to the intermediate complexation of **2** with boranate or boranate reaction products, from which **2** is formed by hydrolysis, we preferred to monitor the disappearance of **1**. Upon addition of an equimolar amount of 2-thioethanol to a solution of **1** the addition product **3** is immediately formed (with respect to the NMR time scale) to an extent of > 95% as derived from the signals of its NMR spectrum. Gradual disappearance of **3** upon addition of $LiBH_4$ as monitored by the intensities of ^{1}H-NMR signals at 3.35, 5.70, or 6.10 ppm is shown in Fig. 1. According to this plot

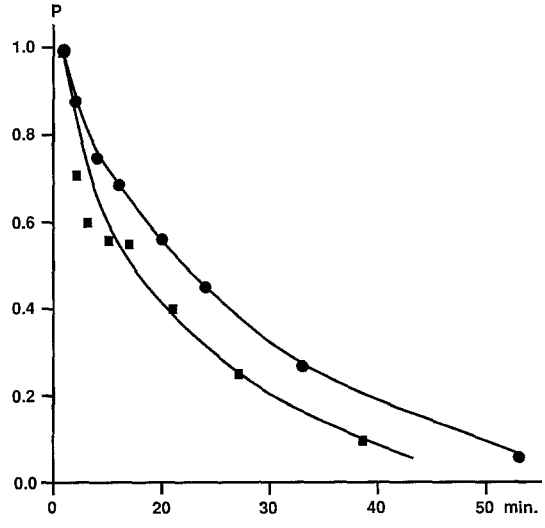


Fig. 1. Disappearance of **1** (squares) and **3** (circles) as monitored by the normalized intensities (*P*) of their ^{1}H-NMR signals

the velocities of the reduction step of the two reaction systems are within the same order of magnitude.

From these results we may conclude that in the biliverdin reductase system addition of an enzyme thiol group to position 10 of biliverdin serves primarily for binding the substrate to the active site. This addition also will provide the proper orientation with respect to an attack of the reducing agent. Judged from the similar velocities of the reduction steps in the homogeneous reaction systems of the model, the addition step is not intended to accelerate the reduction step. Such acceleration could be envisaged from the presence of a good leaving group (-S-R) for the attack of a hydride particle in a nulceophilic substitution as compared to the nucleophilic addition to a double bond. Only the entropic term provided by the resulting proper orientation of substrate and reagent will yield such an acceleration in the natural system.

Acknowledgements

The authors are grateful to Dipl.-Ing. W. Schmitzberger who studied the complexation of **2** with boranate.

References

[1] Falk H., Müller N., Schlederer T. (1980) Monatsh. Chem. **111**: 159

[2] E.g.: Falk H., Grubmayr K. (1977) Ang. Chem. **89**: 487; Falk H., Grubmayr K., Haslinger E., Schlederer T., Thirring K. (1978) Monatsh. Chem. **109**: 1451; Falk H., Grubmayr K., Kapl G., Müller N., Zrunek U. (1983) Monatsh. Chem. **114**: 753; Falk H. (1989) The Chemistry of Linear Oligopyrroles and Bile Pigments. Springer, Wien-New York

[3] Frydman R. B. (in press) Biochim. Biophys. Acta; private communication, summer 1990

[4] For a review on heme catabolism and biliverdin reductase see: Frydman R. B., Frydman B. (1987) Acc. Chem. Res. **20**: 250

Received September 18, 1990. Accepted October 9, 1990