Studies on the Formation of Mixed Disulphides of Biological Importance

L. Eldjarn and A. Pihl

Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Oslo

Previously we have shown that cystamine interacts *in vivo* as well as *in vitro* with the free SH groups of proteins and other body constituents to form considerable amounts of mixed disulphides [1-3]. This raises the question as to the chemistry of mixed disulphide formation in general.

A study has now been made of the chemical kinetics of the reaction of cystamine and cysteamine with various thiols and disulphides. A method has been worked out for the direct determination of the mixed disulphide formation. Use is made of the fact that the disulphide-thiol interaction is negligible below pH 2. ^{35}S-labeled cystamine or cysteamine is incubated with the thiol or disulphide, the reaction stopped by acidification, the mixed disulphide separated by rapid paper electrophoresis at pH 2, and the amount of mixed di sulphide measured by direct counting on paper.

In general the reaction proceeds according to the equations:

$$\mathrm{XSH} + \mathrm{R{-}SS{-}R} \underset{v_2}{\overset{K_1\ v_1}{\rightleftharpoons}} \mathrm{XSSR} + \mathrm{RSH} \qquad K_1 \qquad \text{I}$$

$$\mathrm{XSSR} + \mathrm{XSH} \underset{v_4}{\overset{v_3}{\rightleftharpoons}} \mathrm{XSSX} + \mathrm{RSH} \qquad \text{II}$$

When XSH = glutathione and RSH = cysteamine a rapid and extensive formation of mixed disulphide takes place. The $K_4 = K_2/K_3$ has been found to be greater than 130. This high K_4 value makes difficult the accurate determination of K_2 and K_3 from equilibrium data. The equilibrium values for the mixed disulphide were found to be independent of temperature (0 to 37° C), and of pH (5 to 7.4).

The velocity constants k_{v_1} and k_{v_4} were measured separately. Their pH dependence showed that $-S^-$ is the reactive form of the thiol. With equimolar amounts of the reactants, equilibrium at pH 7.4 and 37° C is attained in less than 45 seconds and 3 minutes in reactions I and II respectively.

The striking fact emerging from the present experiments is that at equilibrium nearly 80 % of glutathione (and of cysteine) exist as the mixed disulphide when incubated, in the absence of oxygen, with equimolar amounts of cystamine at 37° C, pH 7.4. Recently Kolthoff *et al.*[4] have calculated, from cystine solubility data, the equilibrium constants for the reactions of cystine with reduced glutathione and thioglycolic acid respectively. It can be calculated from these constants that in equimolar concentrations and under conditions comparable to ours, 41 % of the glutathione exist as mixed disulphides at equilibrium.

The rapid reaction velocities demonstrated in the present study and the high equilibrium values for the mixed disulphides would seem to have considerable interest with regard to the chemistry of protein sulphur groups. We have previously studied the formation of mixed disulphides between protein SH groups and cystamine [1-3]. In recent experiments we have found that cysteamine, as expected, forms mixed disulphides with accessible protein SS groups (serum albumin, desoxyribonuclease, cytochrom c).

Our data strongly suggest that the accessible protein SH and SS groups exist in a dynamic equilibrium with the thiols and disulphides of the surrounding medium. Thus, depending on the conditions, one particular protein sulphur group may conceivably exist either as a free SH group, in a mixed disulphide with a smaller compound, in an intramolecular disulphide, or occasionally in a protein-SS-protein linkage. Accordingly, the number of titratable SH groups of a protein may depend on the exact conditions of its pretreatment with thiols and/or disulphides (pH, temperature, chemical nature, concentrations). This view might offer an explanation of the numerous controversies concerning the number of free SH groups of various proteins.

It is suggested, on the basis of the above evidence, that the accessible sulphur groups of a protein should be classified as a) single SH groups, b) adjacent sulphur groups capable of forming intramolecular disulphides. It seems probable that in the case of proteins, the formation of intermolecular disulphides is of minor significance.

1. Eldjarn, L., Pihl, A. and Shapiro, B. *Internat. Conf. on the Peaceful Uses of Atomic Energy* (1955). *In press.*

2. Eldjarn, L. and Pihl, A. In Radiobiology Symposium, Cambridge, Oliver and Boyd, Edinburgh (1955).
3. Eldjarn, L. and Pihl, A. *J. Biol. Chem.* (1956) *In press.*
4. Kolthoff, I. N., Stricks, W. and Kapoor, R. C. *J. Am. Chem. Soc.* **77** (1955) 4733.

Influence of Thyroid Hormone on the Formation of Bile Acids

Sten Eriksson

Department of Physiological Chemistry, University of Lund, Lund, Sweden

It has long been recognized that an inverse relationship exists between plasma cholesterol level and thyroid activity [1]. As the bile acids have been shown [2,3] to be the main metabolic end-products of cholesterol in the rat we have studied the influence of thyroid activity on the excretion of bile acids in bile fistula rats. Thompson and Vars [4] studying the same problem found lower values of cholic acid in the hyperthyroid and hypothyroid rats than in the euthyroid animals. In their experiments they did not determine taurochenodesoxycholic acid, the second bile acid of quantitative importance in the rat [5]. In the present work taurocholic and taurochenodesoxycholic acids have been determined with the technique used in a previous work from this laboratory in which the quantitative excretion of bile acids in normal bile fistula rats were studied [6]. The rats were made hyper- and hypothyroid by including 0.4 % thyroid or 0.5 % propyltiouracil, respectively, in the diet. The bile was collected for five days following operation. In Table 1 are given the values obtained for the excretion of taurocholic and taurochenodesoxycholic acids during the time of collection. In the hypothyroid group there is a large reduction of the total excretion of bile acids. In the hyperthyroid group the total excretion remains unchanged as compared to the control rats. In the hypothyroid group the excretion of taurochenodesoxycholic acid is less than 10 % of the total excretion, whereas the normal rats had about 20 %. On the other hand in the hyperthyroid group taurochenodesoxycholic acid constitutes between 70 and 80 % of the total excretion. Thus the excretion of bile acids in hyperthyroid rats is not decreased but the composition is changed in that taurocholic acid is markedly decreased but taurochenodesoxycholic acid increased.

Further data will be presented.

1. Peters, J. P. and Van Slyke, D. D. *Quantitative clinical chemistry*, 1, 2nd ed. Baltimore: Williams and Wilkins (1946).
2. Bergström, S. *Kgl. Fysiograf. Sällskap Lund Förh.* **22** (1952) No. 16.
3. Byers, S. O. and Biggs, M. W. *Arch. Biochem. Biophys.* **39** (1952) 301.
4. Thompson, J. C. and Vars, H. M. *Am. J. Physiol.* **179** (1954) 405.
5. Bergström, S. and Sjövall, J. *Acta Chem. Scand.* **8** (1954) 611.
6. Eriksson, S. *Acta Chem. Scand.* **10** (1956) 156.

Table 1. Excretion of Na-taurocholate (TC) and Na-taurochenodesoxycholate (TCD). The total excretion (TC+TCD) is given as mg per 100 g body weight. The rats weighed 180—250 g.

Hours After operation	Hypothyr. (4 rats)		Controls (4 rats)		Hyperthyr. (4 rats)	
	TC+TCD Mg	TCD % of total excretion	TC+TCD Mg	TCD % of total excretion	TC+TCD Mg	TCD % of total excretion
0—6	10.4	6.5	8.5	12.6	15.5	50.6
6—12	5.7	12.5	3.1	14.7	4.9	48.9
12—18	1.4	15.7	2.8	11.8	3.5	72.0
18—24	1.2	5.5	2.1	15.4	5.0	80.5
24—48	8.7	4.1	20.4	18.4	25.2	69.5
48—72	12.7	6.2	22.8	28.6	22.2	71.3
72—96	13.7	12.7	17.3	24.8	19.9	70.0
96—120	10.2	6.5	19.7	22.9	19.8	67.7