Equilibrium Constants of Thiol-Disulphide Systems. The Relative Oxidation-Reduction Potential of Cysteine-Glutathione *

Alexander Pihl and
Lorentz Eldjarn

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

Previously we have presented evidence that the accessible thiols and disulphides of the body exist in a state of dynamic equilibrium, governed by the appropriate equilibrium constants and by the total SH/SS-ratio ^{1,2}. Clearly, information on the relevant equilibrium constants and reaction rates is necessary for the understanding of the role and mode of action of thiols and disulphides in biochemical processes.

* Supported by grants from the Norwegian Cancer Society.

A method for the analysis of thiol-disulphide equilibria has previously been presented ^{1,2}. Recently, by a modification of this procedure, we have succeeded in measuring directly the concentrations of three of the five constituents of thiol-disulphide equilibria, permitting the straight-forward calculation of the appropriate equilibrium constants. In the improved method, which is described in detail elsewhere ³, the ³⁵S-labeled constituents of the systems tested are separated by electrophoresis at pH 2 in mercuric acetate treated paper.

The procedure has been used for the determination of the equilibrium constants, at pH 7.4 and 37 °C, of systems containing cystine or oxidized glutathione and various sulphur containing X-ray protective thiols. The data are summarized in Table 1. Here is also given the approximate time needed to obtain equilibrium under these conditions.

The *K*-values found, imply that in these systems high amounts of mixed disulphides are present at equilibrium. The data confirm our previous findings ^{4,5} that sulphur-containing agents possessing high ability to protect animals against ionizing radiation, exhibit a pronounced tendency to form mixed disulphides with biological thiols and disulphides.

Table 1. The equilibrium constants *K*₂ and *K*₃ of various thiol-disulphide systems *.

ASH	BSSB						** E'_0 CSSC/ CSH
	Cystine			Oxidized glutathione			
	Time needed to reach equi- librium min	K_2	K_3	Time needed to reach equi- librium min	K_2	K_3	
CH ₃ CONHCH ₂ CH ₂ SH	10	5.00	0.62	12	2.86	0.28	0.0180
CINH ₃ CH ₂ CH ₂ SH	1	4.76	0.75	1.5	5.09	0.34	0.0099
Cl (CH ₃) ₂ NHCH ₂ CH ₂ SH	2	2.30	0.36	2	1.56	0.32	0.0068
Cl(C ₂ H ₅) ₂ NHCH ₂ CH ₂ SH	2.5	2.79	0.36	3	1.92	0.29	0.0079
Cl $\begin{matrix} \diagup \text{CH}_2 - \text{CH}_2 \diagdown \\ \text{CH}_2 - \text{CH}_2 \end{matrix}$ NHCH ₂ CH ₂ SH	3	2.81	0.31	2	1.89	0.25	0.0081
Mean							0.0101

* The measurements were carried out at pH 7.4 and 37 °C.

** *E*'₀ = standard oxidation-reduction potential, at pH 7.4 and 37 °C, relative to that of glutathione (*E*'₀ GSSC/GSH = 0 volts), with sign opposite to that used by Latimer ⁶.

From the equilibrium constants the relative oxidation-reduction potential of the 15 thiol-disulphide couples involved in the 10 systems studied, can be calculated. Of particular interest is the fact that the above equilibrium constants can be used for the calculation of the oxidation-reduction potential of cysteine, relative to that of glutathione. It is clear from Table 1 that the calculations, based on 5 independent sets of data, show good agreement. The results seem to present a solution to this controversial question.

1. Eldjarn, L. and Pihl, A. *Acta Chem. Scand.* **10** (1956) 1054.
2. Eldjarn, L. and Pihl, A. *J. Biol. Chem.* **225** (1957) 499.
3. Eldjarn, L. and Pihl, A. *J. Am. Chem. Soc.* *In press.*
4. Eldjarn, L. and Pihl, A. *Proc. of the 4th Internat. Conf. on Radiobiology*, Oliver and Boyd, London 1956, p. 249.
5. Pihl, A. and Eldjarn, L. *Proc. of the 5th Internat. Conf. on Radiobiology*, Oliver and Boyd, London. *In press.*
6. Latimer, W.M. In *Oxidation Potentials*. Prentice Hall, New York 1952.

Enzyme Kinetics in Univalent Buffers

J. S. McKINLEY MCKEE

Biochemical Institute, University of Oslo, Blindern, Oslo

Many ions have different effects on the kinetics of the fumarase-catalyzed reaction¹:

With buffer ions specific effects arise due to the binding of the different buffer ions. Kinetic studies in phosphate buffers² proved unsatisfactory due to the presence of HPO_4^{--} and H_2PO_4^- ions, each with its own effect on the enzyme and the relative concentration of which varied with pH. As the kinetic constants are unaffected by the buffer cation, while being very sensitive to the anion concentration and type, univalent buffers of the uncharged base type (e. g. imidazole, tris, amidol-chloride, fluoride or perchlorate) have been used. Thus specific buffer effects can be

studied as there is a single anion, the concentration of which can be held constant over a wide pH range.

The experiments have consisted of determining the pH variation of the maximum velocities (V_M , V_F) and Michaelis constants (K_M , K_F) for the forward and reverse reactions. These were obtained from Liveweaver-Burk plots. It was important to use substrate concentrations (range $4K-K/4$) which gave neither substrate activation or inhibition³, as otherwise erroneous kinetic constants resulted. The enzyme solutions were prepared from crystalline fumarase, and the kinetics were followed on the 80—100 % transmission expanded scale of a Beckman DUR spectrophotometer². The Michaelis constants for malate and fumarate in 5 mM chloride, perchlorate or acetate buffers rise markedly above pH 8.0. With malate, the K_M values are approximately constant below pH 7.0, while with fumarate this represents a minimum. Plots of V_M , V_F , V_M/K_M , and $V_F/4.4K_F$ vs. pH are symmetrical and bell shaped. For each buffer the data were consistent with the ionisation of two groups in the enzymatic site, having different ionisation constants in the free enzyme and the fumarase and L-malate complexes. In the various buffers the pK values vary markedly. Good agreement was found between the experimental points and theoretical curves calculated from pH independent kinetic constants and the pK values². In interpreting the data the secondary ionisations of the substrates have to be taken into account at the lowest pH values. A comparison of the kinetic constants in phosphate (approximate values) with those in the univalent buffers shows that while neither buffer type or concentration markedly affect the pH independent maximum velocity or turnover number, yet the corresponding Michaelis constants are much greater in phosphate, presumably due to the divalent ions present. As both substrates give the same ionisation constants for two essential groups in the free enzyme (V_M/K_M and $V_F/K_{eq}K_F$ superimposable) it seems that it is the same enzymatic site that is involved in the proton transfer reactions resulting in hydration or dehydration.

The author wishes to thank professor R. A. Alberty for many helpful discussions.

1. Massey, V. *Biochem. J.* **53** (1953) 67.
2. Alberty, R. A., Massey, V., Frieden, C. and Fuhlbrigge, A. R. *J. Am. Chem. Soc.* **76** (1954) 2485.
3. Alberty, R. A. *J. Cellular Comp. Physiol.* **47** (1956) 245.