

It is, however, still our opinion that no satisfactory device for infra-red double-beam microspectrophotometry has yet been made^{1,2}. In our microscope too much energy is lost at the entrance. Had this not been the case, an excessive heating of the sample would have been unavoidable.

The remedy should be to reverse the beam path by interchanging the energy source and the detector of the spectrometer. Thus the sample would be placed in a monochromatic beam causing little heat. We have, however, not been able to solve this problem practically. It seems a task for the makers.

1. Cf. Ford, M. A., Price, W. C., Seeds, W. E. and Wilkenson, G. R. *J. Opt. Soc. Am.* **48** (1958) 249, for a discussion of this subject.
2. Cf., however, Ref. 1, p. 250.

The Nucleophilic Reactivity of Biological Thiols with Respect to Thiol-Disulphide Exchange Reactions *

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The biological significance of thiols can to a considerable extent be explained by the nucleophilic reactivity of their thiol group. It has generally been felt that in most of the nucleophilic exchange reactions of thiols the mercaptide ion is the active molecular species. Recently this mechanism has been proven in the case of thiol-disulphide exchange reactions^{1,2}. At pH 7.4 the concentrations of the ionized forms of the physiological thiols are not inconsiderable as their p*K* values prove to be much lower than previously realized^{3,4}.

In the present investigation the relative nucleophilic reactivity of a series of biologically important thiols has been studied. The initial rate of interaction of the thiols (cysteine, glutathione, homocysteine, cysteamine, diethylcysteamine, and penicillamine) with cystine at pH 7.4 and 37°C was measured using

³⁵S-labeled cystine followed by paper electrophoretic separation of the reaction products⁵. The unexpected observation was made that the observed initial reaction rates (*k*_{SH}) were approximately equal in spite of the very large differences in the concentrations of the ionized thiols (p*K* varied from 7.7 to 9.75). The rate constants for the respective ionized thiols (*k*_{S⁻}) were found to obey the Brønsted equation with the following parameters:

$$\log k_{S^-} = 0.73 \text{ p}K_{SH} - 1.88$$

The above *k* values (expressed in liter × moles⁻¹ × min⁻¹) refer to the interaction of the thiols with the one of the sulphur atoms of cystine. Since cystine contains two symmetrical sulphur groups which are equivalent in these exchange reactions, the observed rates were actually twice the one given in the equation. The above presentation was preferred for thermodynamic reasons.

Several thiols tested did not obey the above equation but reacted at distinctly slower rates. This was the case with certain N-acyl derivatives (N-acetylcysteamine, aletheine, and coenzyme A). Compounds where the ionized thiol is part of a resonating system (thiocyanide, *o*-aminothiophenol, ergothionine, thiohistidine, etc.) did not interact at all with cystine.

Thiol-disulphide exchange reactions play an important part in the enzymatic reduction of disulphides⁶ and similar reactions may be envisaged to occur in the establishment and the splitting of disulphide cross linkages in proteins. The fact that a series of thiols obey the above equation strongly indicates that in such reactions the p*K* value of the attacking thiol is of minor significance with regard to the reaction rates. This finding may be interpreted to mean that with increasing p*K*_{SH} value the decrease in the concentration of the ionized thiol at a given pH is effectively counteracted by a concurrent increase in the nucleophilic reactivity of the ionized thiol.

1. Eldjarn, L. and Pihl, A. *J. Biol. Chem.* **225** (1957) 499.
2. Fava, A., Iliceto, A. and Camera, E. *J. Am. Chem. Soc.* **79** (1957) 833.
3. Calvin, M. in *Glutathione*, Academic Press, Inc. New York 1954, p. 3.
4. Benesch, R. E. and Benesch, R. *J. Am. Chem. Soc.* **77** (1955) 5877.
5. Eldjarn, L. and Pihl, A. *J. Am. Chem. Soc.* **79** (1957) 4589.
6. Pihl, A., Eldjarn, L. and Bremer, J. *J. Biol. Chem.* **227** (1957) 339.

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