OXYGEN-18 STUDY OF THE ENZYMATIC OXIDATION OF CYSTEAMINE TO HYPOTAURINE[1]

D. Cavallini and R. Scandurra
Department of Biological Chemistry of the University of Rome
and
F. Monacelli
Department of General Chemistry of the University of Rome, Rome, Italy

Received June 8, 1966

Enzymatic oxidation of cysteamine to hypotaurine (I) has been reported to occur in the presence of catalytic amounts of sulfide or sulfur which act as cofactor-like compounds (Cavallini, Scandurra and De Marco, 1963).

(I) $$\begin{matrix} CH_2\text{-}SH \\ | \\ CH_2\text{-}NH_2 \end{matrix} + O_2 \longrightarrow \begin{matrix} CH_2\text{-}SO_2H \\ | \\ CH_2\text{-}NH_2 \end{matrix}$$

Cysteamine Hypotaurine

The enzyme responsible for this oxidation has been extracted from horse kidney and recently obtained in a pure form by Cavallini, De Marco, Scandurra, Dupré and Graziani (1966). No clear indication is available up to now as to the role of sulfur or sulfide, and the mechanism of the reaction is still obscure.

Methylene blue and other artificial electron acceptors may be used in catalytic amounts by the enzyme in place of sulfur or sulfide (Cavallini, Scandurra and De Marco, 1965). Although

1- This investigation has been supported with grants of the Impresa di Enzimologia del Consiglio Nazionale delle Ricerche.

indirect evidence has been obtained indicating that the artificial electron acceptors are unable to replace molecular oxygen in the enzymatic oxidation of cysteamine to hypotaurine (Scandurra, Duprè and Cavallini, 1965), it was thought of interest to establish whether the oxygen incorporated into hypotaurine was derived from molecular oxygen or from oxygen of water. This investigation was necessary in order to characterize this new enzyme as an oxygenase.

The enzyme was extracted from horse kidney and purified up to the step 5 of the procedure described recently (Cavallini et al., 1966). The reaction mixture contained: 12 units of enzyme; 300 μmoles of cysteamine; 50 μmoles of Na_2S; 1 ml of 1 M potassium phosphate buffer pH 7.4; water to a total volume of 10 ml. The gas phase was oxygen containing 4.9 per cent excess O_2^{18}. Enriched water was supplied by YEDA (Israel) and O_2^{18} was obtained by electrolysis of the labelled water using an apparatus similar to that described by Samuel (1962). In the experiment with enriched water, the gas phase contained natural tank oxygen.

After 2 hours of shaking at 38°, the incubation mixture was treated with 1 ml of 16 per cent calcium formate. The precipitate was removed by filtration through Whatman no. 42 filter paper. The clear solution was treated with 5 vols. of 95 per cent ethanol and 12 vols. of chloroform. Ethanol and chloroform were previously chilled at -2°. The mixture was centrifuged at 700 x g, 0° for 15 min. in a swinging bucket head centrifuge. The aqueous layer was carefully removed and again centrifuged in the cold at 10,000 x g. The final solution was passed through a bed of 1.1 x 18 cm of Dowex 2 x 10 (200-400 mesh) neutralized with formic acid and equilibrated with water. The effluent containing hypotaurine was passed through a bed of 1.1 x 18 cm of Dowex 50 x 4 (200-400 mesh) neutralized with ammonia and equilibrated with water. The effluent was freeze-dried, and contaminating ammonium formate was removed by sublimation of the residue at 80°. Purity of hypotaurine was checked by ion-exchange chromatography

of an aliquot of the final product and was found to be in the range of 95 per cent. About 10 mg of purified hypotaurine were placed in a break-seal combustion tube with 150 mg of a 1/1 mixture of carefully dried $HgCl_2$ and $Hg(CN)_2$ and sealed under vacuum. The sealed tube was heated for 2 hours at 400°. The CO_2 produced was purified with 5,6-benzoquinoline (Rittenberg and Ponticorvo, 1956) and analyzed for the mass 46/44 ratio in a mass spectrometer (Italelettronica SP 21 F).

The experimental data indicate the incorporation of 76 per cent of the theoretical amount of 2 atoms of oxygen (Table I).

Table I

Incorporation of O^{18} into enzymatically produced hypotaurine

Medium	Atoms per cent excess in the medium	Substrate	Atoms per cent excess in hypotaurine	Per cent of incorporation
O_2^{18}	4.90	cysteamine	3.73	76
H_2O^{18}	4.30	cysteamine	0.03	0.7
O_2^{18}	4.90	hypotaurine	0.04	0.8

It is difficult to reconcile this result with a possible mechanism accounting for the use of less than 2 atoms of oxygen. It is more likely, however, that technical difficulties have been responsible for this results. Although the procedure for the extraction of hypotaurine has been devised in order to be as little damaging as possible, it is likely that some exchange of sulfinic oxygen with water oxygen or the oxygen of the reagents has occurred. If this interpretation is correct we may reasonably assume that both the oxygen atoms of hypotaurine derive from molecular oxygen. Furthermore the result of the experiment reported in Table I, where hypotaurine has been added as substrate, in-

dicates that the incorporated oxygen has not appreciably arisen by exchange of sulfinic oxygen with O^{18} first introduced into an oxidation product of sulfide, or by enzymatic exchange reactions.

In the light of the above results, the enzyme oxidizing cysteamine to hypotaurine is a new oxygenase. Since its specificity towards cysteamine is very high (Cavallini et al., 1966), we propose for this enzyme the name of cysteamine oxygenase in the place of the name persulfurase given in previous papers.

Acknowledgments

We are indebted with Dr. D. Samuel and Dr. O. Hayaishi for criticism and suggestions regarding the isotope analysis.

References

Cavallini,D., De Marco,C., Scandurra,R., Duprè,S., and Graziani, M.T., J. Biol.Chem., (1966) in press.

Cavallini,D., Scandurra,R., and De Marco,C., J.Biol.Chem., 238, 2999 (1963).

Cavallini,D., Scandurra,R., and De Marco,C., Biochem. J., 96, 781 (1965).

Rittenberg,D., and Ponticorvo,L., J. Appl. Rad.Isotopes, 1,208, (1965).

Samuel,D., in O. Hayaishi, Oxygenases, Academic Press, N.Y. p. 31, (1962).

Scandurra,R., Duprè,S., and Cavallini,D., Arch.Sci.Biol., 49, 161, (1965).