REDUCTION OF ACTIVE SULFATE (PAPS) BY DIHYDROLIPOIC ACID AS SUBSTRATE

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Previous work from this laboratory (Hilz et al., 1958, 1959) has shown that in yeast extracts a reduction of sulfate to H_2S takes place, which involves a first activation step of sulfate to PAPS, which then is reduced by TPNH (DPNH) to sulfite. Sulfite is further reduced by TPNH to hydrogen sulfide (Lezius and Lynen, 1958). Thiosulfate is not an intermediate in this reaction sequence, but acts as a competitive inhibitor in the sulfate activation step. A reduction of PAPS (to a lesser extent APS) to sulfite in yeast extracts was also reported by Wilson and Bandurski (1958, 1959). A similar mechanism acting on APS rather than on PAPS was found by Peck (1959, 1960) in Desulfovibrio desulfuricans.

Since a purified enzyme system from yeast bringing about the reduction of PAPS to sulfite with TPNH is strongly inhibited by arsenite and stimulated by reduced α-lipoic acid, we proposed a mechanism for sulfate reduction involving a thiolytic split of PAPS by dihydrolipoic acid (Hilz, Kittler and Knape, 1959).

By further purification of the enzyme system we are now able to show that TPNH is not required as a reducing

agent. Instead, reduced lipoic acid[1] or the amide thereof[2] in substrate amounts brings about sulfite formation from PAPS (Table 1). Other sulfhydryl compounds like cysteine

Table 1

additions		muM. HSO_3^- formed
none	---	0
TPNH	400 muM.	3
D,L-lip$(SH)_2$	38 "	18
"	386 "	82
"	3 860 "	98
"	500 "	83
D,L-lip$(SH)_2$-amide	500 "	137
cysteine	500 "	7
glutathion	500 "	10
BAL	500 "	2

144 mμM. PAPS, 50 μM. phosphate buffer pH=7.4, 3 μM.versene and 0.05 ml enzyme fraction Ac.II.75 in a total volume of 1.0 ml were incubated anaerobically for 60 min. at 37° and the samples then analysed for sulfite (Hilz, 1960).

or BAL are practically without effect in this system. We do not know as yet, if dihydrolipoic acid acts in a protein-bound form under in vivo conditions. On the basis of our findings we propose the following mechanism of sulfite formation:

$$PAP\text{-}O\text{-}SO_3^- + lip\langle^{SH}_{SH} \rightleftharpoons PAP\text{-}OH + lip\langle^{S\text{-}SO_3^-}_{S\text{-}H}$$

$$lip\langle^{S\text{-}SO_3^-}_{S\text{-}H} \rightleftharpoons lip\langle^{S}_{S} + HSO_3^-$$

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2) kindly supplied by Dr.L.J.Reed

Attempts to isolate the postulated intermediate, "lipothiosulfate", were not successfull so far, probably because of the much favored disulfide ring closure with relase of sulfite (cf. Fridovich and Handler).

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References

Fridovich,J. and Handler,P., J.Biol.Chem., 223, 321 (1956).
Hilz,H., IV.Intern.Congr.Biochem., Vienna 1958.
Hilz,H. and Kittler,M., Biochim.Biophys.Acta, 30, 650 (1958).
Hilz,H., Kittler,M. and Knape,G., Biochem.Z., 332,151 (1959).
Hilz,H., Habilitationsschrift, Hamburg 1960.
Lezius,A., Dissertation, München 1960.
Peck,H.D., Proc.Nat.Acad.Sci., 45, 701 (1959).
Peck,H.D., Fed.Proc., 19, 242 (1960).
Wilson,L.G. and Bandurski,R.S., J.Amer.Chem.Soc., 80, 5576 (1958).
Wilson,L.G., Asahi,T. and Bandurski,R.S., Fed.Proc., 19, 2 (1960).