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EVIDENCE OF CYSTEINE OXIDASE IN RAT MUSCLE

Chandra H. Misra* and Shri K. Mishra

Washington University School of Medicine
Department of Psychiatry
St. Louis, Mo. 63110, U.S.A.
and
The University of Mississippi Medical Center
Department of Medicine
Division of Neurology
Jackson, Miss. 39216, U.S.A.

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The presence of taurine, a metabolite of cysteine, in rat muscle has already been shown (1, 2). As early as 1950, Awapara (3) had also shown the formation in rat muscles of taurine and alanine from cysteine. The initial step in the cysteine catabolism which yields sulfite, sulfate, pyruvate and taurine in mammalian tissues is the conversion of cysteine to its product cysteine sulfinate (4). The presence of cysteine oxidase (cysteine dioxygenase, EC 1.13.11.20), an enzyme which oxidizes cysteine into cysteine sulfinic acid, has been documented in rat liver (5, 6) and in rat brain (7); however, no one has shown the presence of this enzyme in rat muscle. The present study was done to see whether cysteine oxidase, a rate-limiting enzyme for cysteine catabolism in liver (5), is present in rat muscle.

Rats were anesthetized with ether, and hind leg muscle fibers (8) were removed and chilled in 0.9% saline and then blotted, minced and weighed. The muscle tissue was then homogenized with a Teflon pestle tissue grinder at 4° in 0.05 M sodium phosphate buffer pH 6.5 (containing 0.32 M sucrose and 0.05 mM Fe^{2+}) in a ratio of 1:9 (w/v). Muscle homogenates were centrifuged at 800 g for 10 min at 4° to remove large cellular debris.

Cysteine oxidase was measured according to our previous studies (7), using a phosphate buffer pH 6.5 instead of pH 6.8, as the maximum activity of the muscle cysteine oxidase was found at pH 6.5. The assay mixture consisted of 15.5 μmoles L-[^{35}S]cysteine (0.2 to 0.4 μCi), 0.05 μmole Fe $(NH_4)_2(SO_4)_2 \cdot 6\ H_2O$, 1 μmole hydroxylamine-HCl, 10 μmoles phosphate buffer (pH 6.5), 0.4 μmole NAD^+ and 0.1 of Triton-X-100 (for solubilizing the enzyme) and enzyme preparation to make 200 μl. The reaction was started by adding substrate and incubation was carried out at 37° for 30 min and then stopped by the addition of 100 μl of 10% trichloroacetic acid. After removing the precipitated protein, the

* To whom all inquiries should be directed.

supernatant was passed through a mini-column (0.5 cm x 3.0 cm) of Dowex-50W-H^+ (200-400). The cysteine sulfinate retained (7) was eluted by passing 2.0 ml of water through the column. Either 1.0 ml of this eluate or the total eluate was counted in 10.0 ml scintiverse (Fisher Scientific Co.). In the case of total eluate, an extra 1.0 ml water was added to the counting vial to form a transparent gel.

The result (Table 1) indicated that cysteine oxidase activity in muscle is lower

TABLE 1. CYSTEINE OXIDASE ACTIVITY IN RAT MUSCLES*

Tissue	Protein	Specific activity of cysteine oxidase+
Soleus muscle	4.14 ± 0.19	0.50 ± 0.03
Quadriceps muscle	4.70 ± 0.36	0.44 ± 0.03
Plantaris muscle	4.70 ± 0.30	0.61 ± 0.05

*Each result represents the mean ± standard error of five separate experiments.

+Specific activity is expressed in μmoles cysteine sulfinate formed/hr/mg of protein.

than in brain (0.70) as reported by Misra and Olney (7), but is three times higher than in liver (0.18) as reported by Yamaguchi et al. (5).

From the present study it seems that in muscles cysteine oxidase may be a factor in the regulation of the level and the metabolism of endogenous cysteine, a precursor for the synthesis of alanine through pyruvate in rat muscles as suggested by Garber et al. (9).

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