SULFHYDRYL GROUPS OF CHOLINE ACETYLASE*

by

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Inhibitors of sulfhydryl groups have been found to block the enzymatic acetylation of choline in solutions prepared from rat or guinea pig brains (NACHMANSOHN AND MACHADO[1]). After LYNEN *et al.*[2] identified an SH group as a functional group of Coenzyme-A (which is necessary for the acetylation of choline[3]), the problem arose whether the effect should be attributed to the inhibition of the sulfhydryl group of the coenzyme, or whether the enzyme too has such a group essential for its activity.

TABLE I

EFFECT OF SH INHIBITORS ON CHOLINE ACETYLASE ACTIVITY

The test mixture contained the following components in μM per ml: choline chloride 20; acetyl-CoA 3.5; K-phosphate buffer pH 7.0 100; TEPP 0.13. Inhibitor as indicated. 0.01 ml enzyme, containing 150 μg protein, was added to the test mixture which had a final volume of 0.5 ml. When iodoacetate was the inhibitor the test mixture contained in addition 4 μM per ml EDTA; in case of *p*-chloromercuribenzoate and iodosobenzoate 2 μM/ml; no EDTA in case of $CuSO_4$. The acetylcholine formed was determined by biological assay[6]. The activity of the control was about 80 μg acetylcholine formed in 10 min and about 170 μg in 30 min. The enzyme, inhibitor, buffer, and EDTA were preincubated at 30° C for 30 min, and the complete system incubated at 30° C.

Compound	*M conc.*	*Inhibition per cent*	
		10' incub.	*30' incub.*
Iodoacetate	$5 \cdot 10^{-5}$	0	0
	$5 \cdot 10^{-4}$	35	27
	$5 \cdot 10^{-3}$	60	64
	$5 \cdot 10^{-4}$	36	20
	$1.5 \cdot 10^{-3}$	63	48
	$4.5 \cdot 10^{-3}$	78	64
p-Chloromercuribenzoate	$4 \cdot 10^{-5}$	96	94
	$2 \cdot 10^{-4}$	100	100
	$1 \cdot 10^{-3}$	100	100
	$4 \cdot 10^{-7}$	12	6
	$2 \cdot 10^{-6}$	40	18
	$1 \cdot 10^{-5}$	69	48
Iodosobenzoate	$1 \cdot 10^{-5}$	48	34
	$1 \cdot 10^{-4}$	63	50
	$1 \cdot 10^{-3}$	70	65
	$1 \cdot 10^{-6}$	16	12
	$1 \cdot 10^{-5}$	50	39
	$1 \cdot 10^{-4}$	69	62
$CuSO_4$	$1 \cdot 10^{-6}$	13	10
	$1 \cdot 10^{-5}$	80	83
	$1 \cdot 10^{-4}$	100	100
	$2.5 \cdot 10^{-6}$	38	30
	$5 \cdot 10^{-6}$	60	59
	$1 \cdot 10^{-5}$	84	86

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Experiments with purified choline acetylase[4] prepared from Squid head ganglia with a specific activity of about 50 μM acetylcholine formed per mg protein per hour indicate that SH groups are necessary for the action of this enzyme. In these experiments acetyl CoA (prepared by acetylating CoA (Pabst) with acetic anhydride)[5] and choline were used as substrates. The enzyme activity was found to be depressed by all sulfhydryl inhibitors tested. The results are summarized in the Table.

Addition of ethylene diamine tetraacetate (EDTA) to the reaction mixture in phosphate buffer increased the CoA liberated up to 40%; with tris(hydroxymethyl)aminomethane buffer recrystallized twice from alcohol the activity was increased 450%. The activity was the same with either buffer in the presence of EDTA. Apparently traces of metal inhibit the enzyme and are removed by complex formation with EDTA. In these experiments the enzyme activity was assayed by a modified nitroprusside test for liberated CoA[6].

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OXIDATIVE PHOSPHORYLATION BY HEART MUSCLE MITOCHONDRIA

by

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The yields of oxidative phosphorylation by heart muscle mitochondria for a number of substrates of the KREBS' cycle were reported in a recent communication[1]. The one-step oxidation of α-ketoglutarate to succinate was examined in particular detail in this investigation. On the basis of this study it was concluded that the P/O ratio for the oxidation of α-ketoglutarate to succinate was more than 3. This result was in agreement with that obtained by other investigators using tissue preparations different from heart[2-7]. At about the same time a report on the same subject appeared by SLATER AND HOLTON[8]. These authors used a method for the preparation of heart particles somewhat different than that employed by us[9], and obtained a P/O of below 3 for the oxidation of α-ketoglutarate in the presence of malonate. SLATER AND HOLTON[8] expressed the belief that the P/O values of above 3 for this step obtained with liver mitochondria by COPENHAVER AND LARDY[3] were in error because preincubation of the Warburg flasks for 5 minutes was inadequate to insure thermal equilibration, resulting in underestimation of the oxygen consumption during the test period. (It should be emphasized that COPENHAVER AND LARDY specifically stated that the 5 minute preincubation period was adequate for thermal equilibration under their conditions.) Since a 5 minute preincubation period was also used in our experiments with heart mitochondria, the same criticism might be applied to our results. It was demonstrated with heart muscle mitochondria that the rates of oxygen consumption, α-ketoglutarate disappearance, and phosphate uptake were approximately linear when measured at 5 minute intervals during an incubation period of 20 minutes (*cf.* Fig. 1[1]). This suggested that thermal equilibration was adequate in our experimental procedure. Nevertheless,

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