EFFECTS OF CHLOROSUCCINATE IN THE KIDNEY CORTEX*

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1. Introduction

L-Chlorosuccinate is a good substrate for succinic dehydrogenase; chlorofumarate is the product of the reaction [1]. The hydrogen atom on C-2 of L-2Cl-succinate is removed, as well as the hydrogen atom on C-3 trans† to this, the (3 S) hydrogen atom [2]. Thus, use of L-2Cl-succinate-2T and (3S)-L-2Cl-succinate-3T allows one to follow the fate of the hydrogen removed in the succinate dehydrogenase reaction.

D-Chlorosuccinate is a competitive inhibitor of succinate dehydrogenase [1]. L-Chlorosuccinate will also complete with succinate, but is oxidized at an appreciable rate [1]. Possible inhibitory effects of chlorofumarate have not yet been investigated. As might be expected, therefore, DL-Chlorosuccinate at concentrations above 0.05 mM produced significant inhibitions of the Krebs cycle and of gluconeogenesis in the kidney cortex.

2. Materials and methods

L-Aspartate-2T was made by incubating glucose-6P-1T (20 μmoles), Na Phosphate pH 7.4 (2000 μmoles), NH_4Cl (2000 μmoles), Na oxalacetate (500 μmoles), NAD + (50 μmoles) in a volume of 10 ml with 20 units of glucose-6P-dehydrogenase (from *L. mesenteroides*) and 10 units of glutamate dehydrogenase for 24 hr at room temp. (3S)-L-Aspartate-3T synthesis involved pre-incubation of DL-malate-2T (80 μmoles) and Na phosphate pH 7.4 (200 μmoles) in a 3 ml volume with 80 units of fumarase. Na phosphate pH 7.4 (1000 μmoles), NAD + (100 μmoles), Na glutamate (500 μmoles) and Na pyruvate (300 μmoles) were added together with 200 units malate dehydrogenase, 100 units lactate dehydrogenase and 50 units glutamate-oxalacetate transaminase in a final volume of 10 ml. Incubation was for 3 hr at room temp (longer incubations may decrease yields due to slow detritiation of oxalacetate-3T in equilibrium with L-aspartate-3T). The reaction mixtures from both syntheses were put on 1 cm × 5 cm Amberlite CG-120 (H+ form, 100–200 mesh) columns, which were washed with water. Aspartate-T was eluted with 20 ml of 2 N NH_4OH, taken to dryness, and put on 1 cm × 11 cm Amberlite CG-4B (acetate form, 200–400 mesh) columns. Aspartate-T was eluted with 1 N acetic acid.

L-aspartate was converted to L-Cl-succinate essentially according to Holmberg [3]. To L-aspartate-2T or (3S)-L-aspartate-3T (40 μmoles) in 3 ml of 8 N HCl in a 25° bath, 1 ml of 1 N $NaNO_2$ was added dropwise over a period of 10 min. The mixture stood 60 min, was made to 10 ml and put on tandem 1 cm × 5 cm Amberlite CG-120 (H+) and 1 cm × 30 cm Dowex-1 (acetate form, 100–200 mesh) columns, which were washed with water (no radioactivity appeared in the water wash). The Dowex-1 column was eluted with 100 ml of 1 N formic acid (about 5% of the original activity, probably malate, appeared here) and then with 6 N formic acid to give the tritiated chlorosuccinate. This was taken to dryness and chromatographed on Whatman 3 MM paper, using n-butanol/acetic acid/water (4/1/2, by volume). A single band with a high Rf was obtained. Authentic

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† In the reactive conformation in which the carboxyl groups are trans.

DL-chlorosuccinate (Fluka Inc., Switzerland) moved to the same region in this system.

Incubations with kidney cortex slices were carried out as described [4].

3. Results

Kidney cortex segments were incubated with a gluconeogenic substrate plus tracer levels of succinate-2,3-T, L-2Cl-succinate-2T and (3S)-L-2Cl-succinate-3T and the yields of tritium in glucose and water were determined (table 1). While tritium from succinate-2,3-T appears in glucose, tritium from the L-chlorosuccinates labels only water. Thus the tritium labeling in glucose from succinate-2,3-T must be the result of the metabolism of the L-malate-2,3-T produced. These findings are similar to those previously described by Hoberman and Presky [5] in studies with liver mitochondria.

When kidney cortex segments were incubated with lactate and a range of concentrations of DL-chlorosuccinate, inhibitory effects on gluconeogenesis and the Krebs cycle could be seen (table 2). In the experiment with 0 and 2 mM DL-chlorosuccinate, organic acids were separated on an anion exchange column. Chlorosuccinate produced about a 50% increase in the radioactivity in succinate and citrate, but the major increase (> 300%) was in the malate fraction. The mechanism of inhibition in the intact cell remains to be established. In the experiments with tritiated substrates (table 1), these were added at concentrations of less than 0.05 mM and only minor effects on gluconeogenesis were found. In the kidney cortex, at equal concentrations, chlorosuccinate is a considerably more potent inhibitor than is malonate.

Table 2
Effect of DL-chlorosuccinate on gluconeogenesis and on $^{14}CO_2$ production from acetate-1-^{14}C.

DL-Chloro succinate concentration (mM)	Glucose formed (μmoles/125 mg wet wt/2 hr)	^{14}C yield in CO_2 (% injected ^{14}C)
0	4.21	45.1
0.05	3.93	49.8
0.10	3.13	48.6
0.20	3.00	52.0
0.50	2.27	51.4
1.0	1.49	41.7
2.0	1.14	39.1
5.0	0.67	31.0

The substrates were 10 mM L-lactate and 10 mM acetate. Incubation conditions were as in table 1. 2×10^6 cpm of acetate-1-^{14}C were used in each flask.

Table 1
Fate of tritium from succinate-2,3-T compared with L-2Cl-succinate-2T and (3S)-L-2Cl-succinate-3T.

Unlabeled substrate	Labeled substrate	Tritium yield (% injected activity) Water	Glucose	Specific yield in glucose
L-Lactate (20 mM) plus Butyrate (20 mM)	Succinate-2,3-T	72.8	8.7	10.7
	L-2Cl-succinate-2T	67.0	0	0
Pyruvate (20 mM) plus Acetoacetate (20 mM)	Succinate-2,3-T	84.8	4.3	4.8
	L-2Cl-succinate-2T	97.2	0	0
	(3S)-L-2Cl-succinate-3T	84.1	0	0
L-Lactate (10 mM) plus Acetate (10 mM)	Succinate-2,3-T	73.5	7.6	9.4
	L-2Cl-succinate-2T	83.3	0	0
	(3S)-L-2Cl-succinate-3T	69.5	0	0

125 mg of kidney cortex slices were incubated for 2 hr at 37° under 100% oxygen with the unlabeled substrates shown, together with tracer levels (< 0.1 μmole) of the tritiated substrate (approx. 4×10^6 cpm per flask). Specific yield is defined as the percent of the total utilized substrate activity in the given product.

4. Discussion

Succinate 2,3-T has been used in experiments in which tritium yields in products have been interpreted to be the result of the succinic dehydrogenase reaction producing tritiated reduced flavin which then by "reverse oxidative phosphorylation" produces NADT with subsequent incorporation of tritium from this into products [6]. We show here that the hydrogen atoms involved in the succinic dehydrogenase reaction in the intact kidney cortex cell exchange with water. The exact locus of the exchange is not known, but it could occur at the flavin level or possibly at cytochrome *b* or ubiquinone [5]. On the other hand our data in the previous paper [4] suggest that mitochondrial NADT does not rapidly exchange with water. These divergent results suggest that, in the intact cell, the reactions of the respiratory chain between NADH and the succinic dehydrogenase flavoprotein are not close to equilibrium. Our results are more in accord with the concept that the respiratory chain is under kinetic control [7, 8] rather than thermodynamic control [9].

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