TRITIUM AS A TRACER FOR MITOCHONDRIAL AND CYTOSOLIC REDUCING HYDROGEN IN THE KIDNEY CORTEX*

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1. Introduction

Tritium labeled substrates have been used to follow the coupling of redox systems in the cytosol of various tissues and cells [1–3]. Coupling of mitochondrial redox systems has been less widely studied with tritium labeled compounds, perhaps because of the belief that exchange reactions with water in the mitochondria are so rapid that water will be the only labeled product. In this paper we have investigated the fate of tritium which labels mitochondrial NADH and cytosolic NADH in several enzyme reactions in the kidney cortex. Tritium from citrate-2T†, isocitrate-2T, L-glutamate-2T and D-β-hydroxybutyrate-3T, which labels the mitochondrial NADH pool, appears in glucose, probably as a result of coupling of the mitochondrial and cytosolic NADH pools via the malate dehydrogenase isoenzymes in both compartments [4, 5].

2. Materials and methods

Kidney cortex segments were prepared essentially by the method of Guder et al. [6] using a 30 mesh sieve. 125 mg segments were incubated in 2 ml of the phosphate-salts buffer of Krebs et al. [7] for 2 hr at 37° under 100% oxygen with a suitable glucogenic substrate together with 10 mM acetate and tracer levels of various tritiated substrates. The medium was made up to 10 ml and 8 ml of this was put through tandem 1 cm × 5 cm columns of Amberlite CG-120 (H+ form, 100–200 mesh) and Dowex-1 (acetate form, 100–200 mesh). The columns were washed with water until 20 ml was collected. Duplicate 1 ml aliquots of this were counted in a scintillation counter, one of the aliquots being dried (twice, with addition of water). The non-dried aliquot gives a measure of the yield of tritium in water and glucose, and the dried aliquot represents the yield in glucose. When L-α-glycerol-P-2T was the substrate, the effluent from the tandem columns was taken to dryness on a rotary vacuum evaporator and then was treated with hexokinase and ATP plus $MgCl_2$. Glucose-6-P was trapped using a similar tandem column method, and eluted from the Dowex-1 column with 4N formic acid. This procedure is necessary since some dephosphorylation of the α-glycerol-P occurs.

Succinate-2,3-T and glycerol-2T were obtained from New England Nuclear. L-lactate-2T and L-malate-2T were prepared as described elsewhere [5]. DL-β-hydroxybutyrate-3T was synthesized according to Lowenstein [8]. The L isomer was prepared from this by removing the D isomer with D-β-hydroxybutyrate dehydrogenase. D-β-hydroxybutyrate-3T was made from glucose-6P-1T, glucose-6P-dehydrogenase from *L. mesenteroides*, NAD^+, NH_4^+, α-ketoglutarate and glutamate dehydrogenase. Threo-D_s-isocitrate-2T was made with glucose-6P, $NADP^+$(T), glucose-6P-dehydrogenase, α-ketoglutarate, Mg^{2+}, bicarbonate and isocitrate dehydrogenase. L-α-glycerol-P-2T was syn-

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† We use the convention which numbers the citric and isocitric acids in the same fashion. Thus acetyl CoA furnishes C-4 and C-5 of citrate in the mammalian citrate synthase reaction.

Table 1
Yield of tritium in glucose and water, and percent of tritium on C-6 and C-4 of glucose.

Unlabelled substrates	Tritium labelled substrates	AOA[a] Conc. (mM)	Tritium yield (% of I.D.) Water	Glucose	Specific yield in glucose (%)	% T on C-6 of glucose	% T on C-4 of glucose[b]
L-Malate (10 mM)	L-Glutamate-2T	0	93	0	0	–	–
	L-Glutamate-2T	0.2	46	0.2	0.4	–	–
	L-Glutamate-2T	2.0	33	0.6	1.8	–	–
	L-Norvaline-2T	0	71	0	0	–	–
	L-Norvaline-2T	1.0	5.2	0.11	2.1	–	–
Fructose (10 mM)	D-β-HO-Butyrate-3T	1.0	69	0.4	0.6	–	–
	L-β-HO-Butyrate-3T	1.0	51	1.2	2.3	–	–
	L-Glutamate-2T	1.0	72	0.9	1.2	–	–
	Citrate-2T	1.0	81	4.0	4.7	–	–
	L-Malate-2T	1.0	81	7.6	8.6	–	–
	L-Lactate-2T	1.0	74	7.9	9.6	–	–
L-Lactate (20 mM)	L-β-HO-Butyrate-3T	0	26	0.6	2.2	19	81
	Citrate-2T	0	64	1.4	2.2	38	62
	Succinate-2,3-T	0	73	4.9	6.3	43	57
	Fumarate-2,3-T	0	75	6.3	7.7	44	56
	L-Malate-2T	0	78	6.2	7.3	46	54
	L-Lactate-2T	0	35	3.0	8.1	23	77
L-Lactate (10 mM)	Citrate-2T	0	70	1.8	2.5	38	62
	Succinate-2,3-T	0	71	6.0	7.7	41	59
	L-Malate-2T	0	71	6.8	8.7	40	60
	L-Lactate-2T	0	56	5.5	9.0	21	79
	L-α-Glycerol-P-2T	0	54	8.1	13.1	8	92
Pyruvate (10 mM)	Citrate-2T	0	58	1.9	3.2	38	62
	Succinate-2,3-T	0	73	7.6	9.4	39	61
	L-Malate-2T	0	72	8.0	10.0	38	62
	L-Lactate-2T	0	50	5.7	10.3	19	81
	L-α-Glycerol-P-2T	0	47	9.5	16.8	6	94
L-Lactate (10 mM)	D-Isocitrate-2T	0	30	0.8	2.6	–	–
Pyruvate (10 mM)	D-Isocitrate-2T	0	52	1.3	2.5	–	–

[a] AOA = aminooxyacetate.
[b] By difference.

thesized with glycerol-2T, ATP, Mg^{2+} and glycerokinase.

Glucose-6P-dehydrogenase from *L. mesenteroides* was from P-L Laboratories; other enzymes were from Sigma. Glucose degradations were carried out by the periodate method of Bloom [11].

3. Results and discussion

Table 1 indicates that, under suitable conditions, tritium from substrates which form NADT in mitochondrial NAD-linked dehydrogenase reactions appears in glucose. Since transaminase reactions cause exchange of tritium on carbon 2 of glutamate with water, in order to follow the NADT produced in the glutamate dehydrogenase reaction it is necessary to inhibit the transaminases with a rather high concentration of

aminooxyacetate [10]. Under these conditions tritium from L-norvaline-2T, which it also a substrate of glutamate dehydrogenase, also appears in glucose.

Active malate dehydrogenases are present in the cytosol and mitochondria of the kidney cortex. We have shown previously that malate exchanges rapidly between the cytosol and the mitochondria [5]. Thus mixing and at least partial isotopic equilibration of the mitochondrial and cytosolic NADH pools should be considered likely. The amount of tritium appearing in glucose from citrate-2T ranges from 2–5% of the substrate utilized. In these and similar experiments mapping of the fluxes of carbon and reducing hydrogen [10] shows that the cytosolic NADH flux leading to glucose synthesis is about 10% of the total NADH turnover in the mitochondria plus cytosol. Thus if complete equilibration of the two NADH pools were assumed, one might expect about 10% of the tritium to appear in glucose. This figure, however, must be decreased by one-half since all the tritium on carbons 1 and 3 of dihydroxyacetone-P is lost to water in the aldolase and subsequent hexose-P isomerase reactions [11, 12]. Thus the maximal expected yield in glucose is approximately 5%. In view of the unlikelihood of complete equilibration via the malate dehydrogenases and malate exchange, and in view of possible cytosolic tritium exchange reactions (e.g. aldolase, triose-P isomerase exchange [3, 13]), it appears that tritium release to water via mitochondrial exchange reactions may be rather limited. Thus while water is the main labeled product, this is to be expected on the basis of the high net rate of mitochondrial NADH oxidation via the respiratory chain, and on the basis of the known loss of tritium to water occurring in the cytosol.

In the interpretation of the results it is necessary to know the specificity of the NAD-linked dehydrogenases involved in regard to whether they transfer hydrogen from the substrate to the A or B side of C-4 of the nicotinamide moiety (see footnote †). Since the route of tritium out of the mitochondria involves the A-type malate dehydrogenase, higher yields should be expected in glucose from mitochondrial substrates metabolized by A-type enzymes (e.g. isocitrate dehydrogenase) than from those metabolized by B-type enzymes (e.g. glutamate dehydrogenase). Conversely, since the cytosolic glyceraldehyde-P-dehydrogenase is of the B-type, higher yields in glucose would be expected from cytosolic substrates metabolized by B-type enzymes (e.g. α-glycerophosphate dehydrogenase) rather than A-type enzymes (e.g. lactate dehydrogenase).

Probably the major difference between the cytosolic and mitochondrial substrates is the greater percent labeling of C-6 of glucose with the latter class, reflecting greater fumarase randomization. All the tritium in glucose derived from the mitochondrial substrates must pass through L-malate-2T (which largely randomizes to L-malate-2,3-T via fumarate), while L-malate-2T is on a side pathway in the route of tritium from cytosolic substrates to glucose. The results obtained with L-β-hydroxybutyrate-3T are somewhat anomalous, with the low glucose yield being similar to that from a mitochondrial substrate, but the high C-4 labeling typical of a cytosolic substrate. This possibly might be the result of metabolism by both the mitochondrial β-hydroxyacetyl CoA dehydrogenase and the (cytosolic?) β-hydroxyacid dehydrogenase described by Smiley and Ashwell [14].

† In general, A or B specificity must be considered unless there is a single labeled product or an unbranched pathway, or unless active dehydrogenase exchange systems of A and B specific enzymes operate at rates greatly in excess of net fluxes.

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