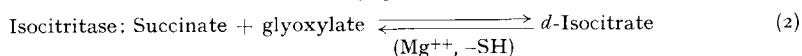
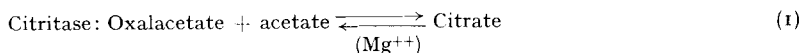


Citritase and isocitritase reactions: equilibria-energetics

Two new enzymes have been found which catalyze the aldol condensations indicated by equations 1 and 2:



Citritase^{1,2} is divalent metal dependent and isocitritase divalent metal and sulfhydryl dependent³.

Citritase, a substrate induced enzyme, has been studied in extracts of anaerobically grown *Streptococcus faecalis*¹, *Escherichia coli*² and *Aerobacter aerogenes*⁴. Isocitritase is widely distributed in aerobic^{5,6} and facultative bacteria^{6,9a} and in fungi⁷; its formation is favored by aerobic growth on organic acids and repressed by growth on carbohydrate⁶.

Both reactions are reversible, as demonstrated by exchange experiments with ¹⁴C labeled substrates⁸. In reaction 1, acetate-1-¹⁴C was incorporated into citrate in the presence of unlabeled oxalacetate, magnesium and the enzyme-citritase. Evidence for the reversal of the citritase reaction has also been obtained by WHEAT AND AJL⁹ in isotope exchange experiments. In reaction 2, succinate-2,3-¹⁴C was incorporated into isocitrate in the presence of unlabeled glyoxylate, magnesium, cysteine, and the enzyme-isocitritase. The reversibility of the isocitritase reaction was suggested by the initial experiments of CAMPBELL *et al.*¹⁰ with crude extracts of *Pseudomonas aeruginosa* and by OLSON's⁷ experiments with mold extracts. Evidence of its direct reversal, that is, isocitrate formation from succinate and glyoxylate has been obtained by following TPN reduction in the presence of added isocitric dehydrogenase.

In view of these evidences of reversibility, the equilibrium of each reaction was measured by determining substrate and product concentrations at equilibrium.

TABLE I
CITRITASE EQUILIBRIUM

Added (time 0)		Found, at equilibrium (20 min)			$K = \frac{(\text{citric})}{(\text{OAA})(\text{Ac})}$ l/M
Acetic $\mu\text{M/ml}^{***}$	OAA* $\mu\text{M/ml}$	Acetic $\mu\text{M/ml}$	OAA* $\mu\text{M/ml}$	Citric** $\mu\text{M/ml}$	
155	7.7	154	5.55	1.14	1.33
155	15.4	153	10.6	2.13	1.31
33.3	27.8	32.3	22.2	1.0	1.39
30	25	29	18.5	1.05	1.95
150	25	146	15.9	4.02	1.74
250	25	244.3	13.7	5.65	1.68
—	25	—	18.3	0.23	—
100	—	100	0	0	—
					Av = 1.56

Per ml: 50 μM Tris buffer, pH 7.6; 2 μM MnSO_4 ; 27° C; under N_2 ; 10 μM units citritase (from *S. faecalis**, Sp. Act. = 4); reaction stopped with 0.1 ml 10N H_2SO_4 .

* Aniline citrate method (N. L. EDSON, *Biochem. J.*, 29 (1935) 2682).

** Pentabromacetone method (S. NATELSON, J. B. PINCUS AND J. K. LUGOVOY, *J. Biol. Chem.*, 175 (1948) 745).

*** Reactants expressed in units measured, for moles/liter multiply by 10^{-3} .

For citritase, the equilibrium constant, 1.5 l/M (Table I) corresponds to a ΔF of —240 calories —i.e., favors synthesis. Fourteen measurements of the equilibrium from both directions gave at the extremes a variability of ± 160 calories, thereby confirming by direct measurement the ΔF = —70 calories calculated by BURTON¹¹ from the heats of combustion and equilibria data for the analogous CoA reactions. The present calculations from equilibria data of reaction 1 would appear to be subject to less error than BURTON's calculations, which suffer from lack of a precise heat of combustion value for acetate, or than those of KAPLAN¹² deduced from calculations of the free energy of formation of reactants.

TABLE II
 ISOCITRITASE EQUILIBRIUM

Added (time o) $\mu\text{M}/\text{ml}$		Found, at equilibrium (30 min)			$K = \frac{(d\text{-isocit.})}{(gly.) (succ.)}$ l/M
		Succinic* $\mu\text{M}/\text{ml}$	Glyoxylic** $\mu\text{M}/\text{ml}$	Isocitric*** $\mu\text{M}/\text{ml}$	
Succinic	Glyoxylic				
3.33	3.53	3.08	2.90	0.39	43.6
3.53	6.67	5.97	2.97	0.73	41.2
7.06	6.67	5.45	6.28	0.70	20.5
<i>d</i> -Isocitric					
	1.67	1.67	1.63	0.067	24.6
	3.33	3.10	3.03	0.36	38.4
	5.00	4.26	3.52	0.55	36.6

$$Av = 34.3$$

Per ml: 50 μM Tris buffer, pH 7.6; 2 μM MgCl_2 ; 1 μM cysteine; 27° C; under N_2 ; 10 units isocitritase (from *P. aeruginosa*³, Sp. Act. = 35); reaction stopped with 0.1 ml 100% trichloroacetic acid—or when succinate was to be determined by boiling three minutes.

* Succinoxidase method (DEITRICH *et al.*, *Arch. Biochem.*, 41 (1952) 118).

** 2,4-Dinitrophenylhydrazine method (T. E. FRIEDEMANN AND G. E. HAUGEN, *J. Biol. Chem.*, 147 (1943) 415).

*** Isocitric dehydrogenase method (S. OCHOA, in *The Enzymes* 2, 1017; edited by J. B. SUMNER AND K. MYRBÄCK, Academic Press, New York, 1951).

The isocitritase equilibrium constant, 32 l/M , (Table II) corresponds to $\Delta F = -2,100 \pm 200$ calories. These free energy data, favoring synthesis, may lack significance as an indication of the importance of reactions 1 and 2 in synthesis because of the difference in number of reactants on the two sides of the equations; *i.e.* first order for substrate in the cleavage direction, and second order for synthesis; thus, for the two reactions at 1 molar concentration of reactants, their equilibria would favor synthesis to the extent of 45% and 85%, respectively, whereas at 10^{-3} molar (1 $\mu\text{M}/\text{ml}$) only 1% and 3%, respectively, of the reactants would be converted to citrate and isocitritate.

In summary: The citritase and isocitritase reactions are exergonic in the direction of synthesis to the extent of approximately 200 and 2,000 calories, respectively. A large concentration effect is introduced: since the reaction is 2nd order in the direction of synthesis as contrasted to 1st order in cleavage. Water is not a reactant in these aldol condensations, and thus does not serve as a driving force as for the condensing enzyme reaction¹³.

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Received December 15th, 1955