Radioaktivität des extrahierten TNP-Amins im Flüssigkeitsscintillationsspektrometer (Tricarb, Packard) gemessen.

Inkubiert man mit DL-5-Hydroxy[3-^{14}C]tryptophan und DOPA gleichzeitig, so tritt eine gegenseitige Hemmung der Dekarboxylierung ein (Tabelle I), es werden beide Aminosäuren umgesetzt. Da beide TNP-Amine nahezu den gleichen molaren Extinktionskoeffizienten bei 340 mμ haben, kann aus der Messung der Absorption bei 340 mμ und der Radioaktivität des Benzolextraktes der Anteil des TNP-5-Hydroxytryptamins und TNP-Dopamins errechnet werden. Die Summe der prozentualen Aktivitäten (maximale Aktivität = 100%) ergibt stets ungefähr 100% (Spalte 8, Tabelle I), während die absolute Menge der gebildeten Amine ausgedrückt in Mol, unterschiedlich ist (Spalte 7, Tabelle I).

Es tritt hier also der besondere Fall ein, dass der Hemmstoff eines Enzyms gleichzeitig auch als Substrat desselbenProteins fungieren kann. Es erhebt sich die Frage, ob diesem Umstand physiologische Bedeutung zukommt.

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Citrate synthase interaction with polyphosphate derivatives

The competitive inhibition of citrate synthase (EC 4.1.3.7) from pig heart[1,2], yeast[1], beef heart, beef liver and *Escherichia coli*[3] by ATP with respect to CoASAc has been reported. Citrate synthase isolated from rat liver mitochondria[4] and lemon are also inhibited by ATP. Citrate synthase from *E. coli* is inhibited by NADH[6]. Triphospho- and diphosphonucleotides and palmitoyl-CoA exhibit an inhibition on pig heart citrate synthase which is reduced at low concentrations of divalent metal ions (Mn^{2+}, Mg^{2+} and Ca^{2+}) suggesting that the effect of the metal ions is due to the chelation with the polyphosphate moiety of the inhibition molecules[2]. The inhibition of pig heart citrate synthase by divalent metal ions (Mn^{2+}, Mg^{2+} and Ca^{2+}) has also been reported[2]. This inhibition is competitive with respect to CoASAc. Results reported in this communication indicate the CoASAc molecule binds to citrate by its polyphosphate group.

Abbreviation: DTNB, 5,5′-dithio-bis-(2-nitrobenzoic acid).

Citrate synthase was assayed spectrophotometrically by following –SH appearance of CoA (ref. 7) with the use of 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB). Citrate synthase was also assayed spectrophotometrically in a system coupled to malate dehydrogenase[8]. For both assay procedures, the reaction mixture was inincubated for 2–3 min at 25° and then the reaction was initiated by the addition of citrate synthase (1.0 cm light path, 1.0 ml final volume). Rates of reactions were followed on the Gilford Instrument model 2000 absorbance recorder (Beckman monochromator) for 1–2 min. Under our experimental conditions, the absorbance increased lineary with time for at least the first 2 min of the reaction. The rates of increase of absorbance (ΔA/min) between 15 sec and 1 min after the start of the reaction, are used to calculate the enzyme activity. The initial rates (v_0) are expressed as μmoles of substrate utilized per min (25.0° ± 0.2°). Crystalline pig heart citrate synthase and malate dehydrogenase used were commercial preparation (Boehringer). Pig liver and pig kidney citrate synthase were prepared according to the method of SRERE AND KOSICKI[9]. The purification was carried out to the second ammonium sulfate fractionation. Protein concentrations were determined spectrophotometrically[10]. Enzymes were diluted with a solution of serum albumin (1 mg per ml of 0.02 M Tris acetate buffer of pH 8.2). Preparation of CoASAc was based on the procedure developed by SIMON AND SHEMIN[11]. Solutions of oxaloacetic acid, malic acid, DTNB, and nucleotides were neutralized with solid $KHCO_3$ before use.

Further studies show that CoA, PP_i, NAD^+, $NADP^+$ and NADPH exhibit a competitive inhibition on pig heart citrate synthase with CoASAc as shown by Lineweaver–Burk plots. The inhibitor constants are listed in Table I. Mg^{2+} at low concentrations reduces the inhibition (Table II). The observed inhibitory effect of the above compounds is not due to an ionic strength effect in the systems studies. KCl, at concentrations approximately three times those of the inhibitors, does not show the same inhibitory effect. P_i (potassium phosphate buffer at pH 8.2) at the same

TABLE I

COMPETITIVE INHIBITION OF PIG HEART CITRATE SYNTHASE

Expt. 1. Malate dehydrogenase coupled assay. Each cuvette contained 0.2 M Tris acetate buffer (pH 8.2), 5 mM malate, 0.27 mM NAD^+, 3 μg malate dehydrogenase, varying concentrations of CoASAc (K_m $1.2 \cdot 10^{-5}$ M), 0.125 mM CoA or 10 mM PP_i and 1.3 μg of pig heart citrate synthase. *Expt. 2.* DTNB assay. Each cuvette contained 0.2 M Tris acetate buffer (pH 8.2), 0.2 M oxaloacetate, 0.5 mM DTNB, 5 mM nucleotides, varying concentrations of CoASAc (K_m $1.1 \cdot 10^{-5}$ M), 0.1 μg of citrate synthase. The K_i for ATP^+ was calculated from pig heart synthase[2] and for ATP^{++} was calculated from pig kidney citrate synthase (CoASAc K_m, $1.8 \cdot 10^{-5}$ M).

	Inhibitor	*K_i (M)*
Expt. 1	CoA	$1.3 \cdot 10^{-4}$
	PP_i	$1.3 \cdot 10^{-2}$
Expt. 2	NADPH	$2.8 \cdot 10^{-3}$
	NADH	$4.6 \cdot 10^{-3}$
	NADP	$9.6 \cdot 10^{-3}$
	NAD	$9.3 \cdot 10^{-3}$
	ATP^+	$6.7 \cdot 10^{-4}$
	ATP^{++}	$2.2 \cdot 10^{-3}$

concentrations as those of the inhibitors studied, gives no measureable inhibitory effect on pig heart citrate synthase. An inhibitory effect of P_i at much higher concentrations (40 mM) is due to the effect of ionic strength.

We confirmed the observations with the malate dehydrogenase assay that pig heart citrate synthase is inhibited by higher concentrations of Mg^{2+} and also by ATP and that the ATP inhibition is reduced at low concentrations of Mg^{2+} (Table II).

Citrate synthase from pig kidney and pig liver is also inhibited by high concentrations of Mg^{2+} and by ATP (Table I) and the ATP inhibition is reduced at low concentrations of Mg^{2+}. Both Mg^{2+} and ATP inhibition of citrate synthase from pig kidney and pig liver were found to be competitive with CoASAc.

TABLE II

EFFECT OF Mg^{2+} ON THE INHIBITION OF PIG HEART CITRATE SYNTHASE

Expt. 1. Malate dehydrogenase assay. Each cuvette contained 0.2 M Tris acetate buffer (pH 8.2), 5 mM malate, 0.27 mM NAD^+, 33.2 μM CoASAc, 3 μg malate dehydrogenase, $MgCl_2$ and inhibitors as indicated, 0.13 μg. *Expt. 2.* DTNB assay. Each cuvette contained 0.2 M Tris acetate buffer (pH 8.2), 0.2 M oxaloacetate, 0.5 mM DTNB, 28.1 μM CoASAc, $MgCl_2$ and PP_i as indicated, 0.12 μg citrate synthase. *Expt. 3.* DTNB assay. Each cuvette contained 0.2 M Tris acetate buffer (pH 8.2), 0.2 mM oxaloacetate, 0.5 mM DTNB, 25.8 μM CoASAc, $MgCl_2$ and inhibitors as indicated, 0.1 μg citrate synthase.

Expt.	*Inhibitors*	*Rate v_0 (μmoles/min · 10^3)*							
	Concn. of Mg^{2+} (mM)	*0*	*1.5*	*2.0*	*2.5*	*3.0*	*5*	*7.5*	*10*
1	None	8.1	8.0	8.0	7.9	7.8	7.6	6.8	6.0
	3 mM ATP	5.6	6.7	6.9	7.0	7.5	6.9	6.1	5.5
	0.13 mM CoA	5.8	7.7	7.2	6.0	5.7	5.3	—	5.1
2	None	8.3	8.3	8.2	8.2	8.1	8.1	6.5	5.8
	5 mM PP_i	7.1	7.3	7.4	7.5	7.9	8.0	6.2	—
3	None	6.0	6.0	6.0	6.0	5.9	5.2	4.6	4.5
	2.5 mM NADH	4.8	5.6	5.9	5.7	5.3	4.7	4.4	—
	2.5 mM NADPH	4.6	5.5	5.8	5.6	5.1	4.6	4.4	4.3

An examination of the acetyl-CoA molecule and all the inhibitors studied *i.e.*, ATP, GTP, ADP, GDP, ITP, IDP, NAD^+, NADH, $NADP^+$, NADPH, propionyl-CoA[12], glycolyl-CoA[12], palmitoyl-CoA[13–16], and CoA shows that there is a great similarity in their molecular structures. Everyone of the inhibitors, as well as acetyl-CoA, contains a polyphosphate chain and an aromatic purine residue. This leads us to think that either the polyphosphate chain or the purine residue or both are responsible for the inhibition. The fact that palmitoyl-CoA after hydrolysis[16] and palmitic acid (J. W. CALLAHAN, personal communication) do not inhibit the enzyme, and that acetylpantetheine does not act as a substrate or an inhibitor in the reaction, supports this proposal. Our observation that AMP and 3′,5′-AMP give no measurable inhibition[2] eliminates the probability that the inhibition observed is due to the purine residue. Our observations that PP_i acts as a competitive inhibitor with respect to CoASAc and that P_i causes no inhibition suggest that there is definitely a binding between the enzyme and the polyphosphate chain of the CoASAc molecule

as well as that of the inhibitors. An inhibitor with a polyphosphate moiety similar to that of CoASAc will bind to the enzyme competitively with the substrate CoASAc at the site (or sites) on the enzyme where the polyphosphate chain of CoASAc is normally bound thus causing the observed competitive inhibition.

Divalent metal ions (Mn^{2+}, Mg^{2+} and Ca^{2+}) at low concentrations, reduce the inhibition caused by triphosphonucleotides and palmitoyl-CoA on pig heart citrate synthase[2]. Mg^{2+} at low concentrations exerts a similar effect on the inhibition caused by NAD^+, NADH, $NADP^+$, NADPH, CoA and P_i. The metal ions are thought to reduce the inhibition by chelating with the inhibitors at the polyphosphate site, thus making them unsuitable as inhibitors. Our observations that Mg^{2+} reduces the inhibition of triphospho- and diphosphonucleotides, palmitoyl-CoA, CoA and pyrophosphate on glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ oxidoreductase, EC 1.1.1.49) give further support to this proposal[17]. These results indicate that the binding between the polyphosphate chain of the acetyl-CoA molecule and citrate synthase is essential for the enzymic reaction.

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