succinate can be explained most simply as the result of succinate decarboxylation and this interpretation is used in the present papers. It is, however, recognized that experiments with purified enzymes may show that the process involves successive oxidations of four-carbon compounds and reductions of three-carbon compounds.

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THE MECHANISM OF PROPIONIC ACID FORMATION BY SUCCINATE DECARBOXYLATION. II. THE FORMATION AND DECARBOXYLATION OF SUCCINYL-CoA

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Communicated by C. B. van Niel, June 5, 1953

The effects of arsenate, phosphate, Na^+, and Li^+, described in the preceding paper,[1] suggest that succinyl-CoA is enzymatically produced from succinyl phosphate and CoA in extracts of *Micrococcus lactilyticus*. The CoA requirement for succinate decarboxylation thus indicated that succinyl-CoA might be the actual substrate for the decarboxylase. Evidence is presented here for the decarboxylation of succinyl-CoA and the mechanism of its regeneration in such extracts.

Methods.—Sulfanilamide acylation was tested by the method of Kaplan and Lipmann,[2] sulfanilamide being determined according to Bratton and Marshall.[3]

Pyrophosphate formation was investigated under the conditions described by Lipmann, *et al.*;[4] pyrophosphate was estimated by acid hydrolysis,[5] and enzymatically with yeast pyrophosphates,[6] orthophosphate by the method of Sumner.[7] Pyrophosphatase-free extracts were obtained by treatment of the crude extracts with the calcium phosphate gel which does not absorb pyrophosphates,[8] and elution of the gel with buffer at pH 5.7.

Succinoxidase was purified by the method of Keilin and Hartree.[9] Acetyl phosphate was synthesized from isopropenyl acetate and phosphoric acid;[10] succinyl-CoA and propionyl-CoA, according to Simon and Shemin.[11]

Results.—Decarboxylation of succinyl-CoA: Extracts depleted of ATP and CoA by treatments with adsorbents are unable to decarboxylate succinate.[1] However, the addition of succinyl-CoA to such extracts results in a rapid evolution of carbon dioxide (table 1, exp. 1). The fact that succinyl-CoA decarboxylation proceeds at a much greater rate than that of succinate in the presence of free CoA precludes the possibility of attributing the results to a preliminary decomposition of succinyl-CoA by a deacylase.

TABLE 1

CARBON DIOXIDE PRODUCTION BY DEPLETED EXTRACTS FROM SUCCINYL-COA AND SUCCINATE WITH VARIOUS ADDITIONS

ADDITIONS	EXP. 1 10 μM SUCCINATE	EXP. 2 50 μM SUCCINATE	EXP. 3 50 μM SUCCINATE
None	36	61	20
10 μM ATP	35	70	22
18 units CoA	58	87	38
360 units CoA[a]	82	110	..
10 μM ATP, 18 units CoA	143	180	141
10 μM succinyl-CoA[c]	232[b]	..	..
1 μM propionyl-CoA	..	244	..
4 μM acetyl phosphate	..	..	19
4 μM acetyl phosphate, 18 units CoA	..	..	181

Values given in μl/10 mg. extract protein/hour.
[a] Amount of CoA used in preparing propionyl-CoA.
[b] Succinate omitted.
[c] Bicarbonate-free preparation.

The decarboxylation of succinyl-CoA may be represented either as a process yielding directly carbon dioxide, propionate, and free CoA, or as a reaction in which one of the end-products is released as a CoA compound. The latter possibility was examined by incubating succinate, ATP, CoA, and an amount of hydroxylamine insufficient to cause a complete inhibition of succinate decarboxylation, with an extract capable of activating succinate but virtually incapable of activating free propionate. Chromatography of the reaction mixture disclosed the presence of both succin- and propionhydroxamic acids, and comparison of the quantity of the latter

formed from succinate with that produced in a similar reaction mixture containing free propionate instead of succinate showed that the amount produced from succinate was by far the greater (table 2). Of special significance is the close correspondence between the quantities of propionhydroxamic acid recovered and carbon dioxide liberated during the decomposition of succinate. Under the experimental conditions used, the action of CoA transphorase, described below, cannot be detected. These results strongly suggest that propionyl-CoA is a direct product of the decarboxylation of succinyl-CoA.

TABLE 2

QUANTITIES OF PROPIONHYDROXAMIC ACID FORMED BY A DEPLETED EXTRACT BY THE ACTIVATION OF PROPIONATE AND THE DECARBOXYLATION OF SUCCINATE

INCUBATION PERIOD, MIN.	5 μM PROPIONATE AS SUBSTRATE μM PROPIONHYDROXAMIC ACID	50 μM SUCCINATE AS SUBSTRATE μM PROPIONHYDROXAMIC ACID	50 μM SUCCINATE AS SUBSTRATE μM CO_2
30	0.03	0.55	0.58
45	0.06	0.77	0.82
60	0.20	0.88	0.93

Reaction mixture contained: 800 μM hydroxylamine, 50 μM phosphate buffer pH 7.3, 10 μM glutathione, 10 μM $MgCl_2$, 100 μM NaF, 10 μM ATP, 36 units CoA; incubated at 30°C. under pure N_2. Values expressed in μM/10 mg. extract protein/hour.

TABLE 3

ACYLATION OF SULFANILAMIDE BY A DEPLETED EXTRACT

μM ATP ADDED	UNITS CoA ADDED	SULFANILAMIDE ACYLATED, μG./10 MG. EXTRACT PROTEIN/HOUR
0	0	0
0	18	2
10	0	2
10	18	10
10	36	12
20	18	22

Reaction mixture contained: 50μM TRIS buffer pH 8.0, 0.2 ml. pigeon liver preparation, 50 μM succinate, 100 μg. sulfanilamide, 10 μM glutathione, 100 μM NaF, 10 μM $MgCl_2$; incubated at 30°C. under pure N_2.

Reversibility of the decarboxylation reaction was tested by incubating an extract with propionate and bicarbonate. The formation of succinate was revealed by the reduction of 2,6-dichlorophenol indophenol under the influence of partially purified succinoxidase. Addition of malonate inhibited dye reduction, as did omission of bicarbonate from the mixture. These observations eliminate oxidation of propionate or other substances present in the extract as the cause of the reduction. On the other hand, addition of ATP and CoA greatly increased the reduction; this was not the case when succinate was used as substrate.

Formation of CoA compounds in extracts: Because the evidence for succinyl-CoA production by phosphotrans-succinylase[1] is indirect, an attempt was made to determine whether acylation of sulfanilamide, considered specific for acyl-CoA derivatives, can occur in extracts provided with ATP, CoA, succinate, sulfanilamide, and "acylating enzyme."[2]

The results of these experiments show unequivocally that sulfanilamide acylation occurs (table 3), though only to a small extent. If succinyl-CoA is the acylating agent, this is readily understandable since this substance is also rapidly decarboxylated; the acylating enzyme must therefore compete with decarboxylase for its substrate. In agreement with this explanation is the fact that dialyzed extracts, with reduced decarboxylase activity, cause a larger sulfanilamide acylation. Moreover, in a reaction mixture incubated at pH 8.0, considerably above the pH optimum for decarboxylation, more sulfanilamide disappears, although this may be partly the result of more favorable conditions for the action of the acylating enzyme.

Both ATP and CoA are essential for sulfanilamide acylation; a small amount of CoA suffices for maximum effect, but a substantial increase in sulfanilamide disappearance is caused by augmenting the ATP supply. Regeneration of CoA as a consequence of the transfer of the acyl moiety of an acyl-CoA compound to sulfanilamide readily accounts for the former; the ATP effect is discussed in more detail below.

Mechanism of succinyl-CoA formation: Small amounts of ATP and CoA in extracts of *M. lactilyticus* permit the decomposition of large quantities of succinate to propionate and carbon dioxide.[1] If this process involved the decarboxylation of succinyl-CoA with the liberation of free CoA, the need for only catalytic amounts of the coenzyme would be understandable. However, the situation with respect to ATP is different; succinyl-CoA formation exclusively by way of succinyl phosphate, or by some other mechanism involving both ATP and CoA, should require a quantity of ATP equivalent to that of succinate decomposed, since it is difficult to conceive of a regeneration of ATP from ADP, or AMP, and inorganic phosphate coupled with a simple decarboxylation.

Because traces of ATP suffice for an extensive succinate decomposition, a mechanism of succinyl-CoA formation independent of ATP appeared probable. Such a mechanism would be provided by a decarboxylation of succinyl-CoA with the liberation of an acyl-CoA compound which could react with free succinate to regenerate succinyl-CoA through the mediation of a CoA transphorase. A reaction of this type has been postulated to account for the activation of propionate[12] and formate[13] by extracts of *Clostridium kluyveri* in the presence of acetyl phosphate and CoA.

Formation of propionyl-CoA by the decarboxylation of succinyl-CoA seemed probable; hence the occurrence of a transphorase reaction between

propionyl-CoA and succinate was investigated. Depleted extracts, able to decarboxylate succinyl-CoA but not free succinate, were incubated with succinate and a small amount of propionyl-CoA. As seen from Table 1, exp. 2, rapid decarboxylation occurred, and the rate of carbon dioxide evolution exceeded that from succinate in extracts supplemented with ATP and CoA.

In view of these results it seemed possible that other CoA compounds, e.g., acetyl-CoA, would likewise serve as CoA donor. Acetyl-CoA was not added as such, but generated in the extract from acetyl phosphate and CoA through the action of phosphotransacetylase whose presence had been established by tests on the arsenolysis of acetyl phosphate and acetylation of sulfanilamide. Table 1, exp. 3, shows that depleted extracts can decarboxylate succinate if supplemented with acetyl phosphate and CoA.

The following experiment provides more direct evidence for succinyl-CoA formation from succinate, acetyl phosphate, and CoA. Depleted extracts, incubated with succinate, acetyl phosphate, and CoA, yield three hydroxamic acids upon the addition of hydroxylamine; these have been identified as acet-, propion-, and succinhydroxamic acids. The latter is formed by the above reaction mixture only if CoA is added, and since in the presence of both acetyl phosphate and CoA, succinate is decarboxylated, the formation of activated propionate could be expected. It should be noted that if hydroxylamine is added at the start of the incubation period, CoA transphorase action cannot be demonstrated and only acethydroxamic acid is found in the above reaction mixture.

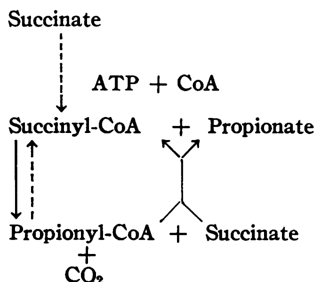
Incubation of depleted extracts with acetyl phosphate, CoA, and propionate, followed by the addition of hydroxylamine, yields propionhydroxamic acid, as well as acethydroxamic acid, showing that CoA transfer can also occur between acetyl-CoA and propionate. Activated formate is produced by substituting formate for propionate in the above reaction mixture. The quantity of succin- and propionhydroxamic acids found in these experiments is far greater than the available amount of CoA, suggesting that phosphate derivatives are formed from the CoA compounds by the action of phosphotrans-succinylase and -propionylase.

The effect of propionyl-CoA and acetyl-CoA on the decarboxylation of succinate, and the evidence for the formation of activated succinate from succinate and acetyl-CoA indicate that generation of succinyl-CoA by CoA transfer can occur. Hence a decarboxylation of succinyl-CoA with the production of an acyl-CoA compound provides a simple mechanism for the continued formation of succinyl-CoA independent of ATP. The experiment summarized in table 2 permits the conclusion that propionyl-CoA is this compound, rather than a one-carbon CoA compound.[14]

In the absence of acyl-CoA compounds, the initial formation of succinyl-CoA must, of course, proceed by other means. That succinate can be

decarboxylated in the presence of CoA and ATP shows that this can be accomplished through a reaction in which ATP takes part, either through the primary formation of succinyl phosphate,[1] or by a different type of reaction involving both ATP and CoA. Such a reaction could result in the concomitant formation of ADP and inorganic phosphate, as described for the activation of succinate by heart preparations,[15] or of AMP and pyrophosphate, as described for the activation of acetate by yeast preparations.[4] Since pyrophosphatase-free extracts of *M. lactilyticus*, incubated with succinate, ATP, CoA, and hydroxylamine[4] showed an increase in inorganic phosphate and gave negative tests for pyrophosphate, it must be concluded that an appreciable quantity of succinyl-CoA is not formed by the latter activation mechanism.

The results of the experiments to date indicate that the decomposition of succinate could be schematically represented by the following diagram:



Theoretically the formation of an infinitely small amount of succinyl-CoA suffices to initiate the decarboxylation. In the presence of hydroxylamine, or of sulfanilamide and an "acylating enzyme," the acyl-CoA compounds of the cycle are partly drained off. Thus the cycle is interrupted, and larger quantities of ATP are required for the primary synthesis of succinyl-CoA.

The diagram indicates that succinate formation by a reversal of the decarboxylation reaction would require carbon dioxide and propionyl-CoA. However, this synthesis of succinate may be considered to be analogous to that of acetoacetate from acetate. It has been established that the latter requires two "active" acetate units, in the form of acetyl-CoA.[16] Hence it appears reasonable to suggest that the synthesis of succinate, too, may be possible only if both propionyl-CoA and an activated one-carbon unit are available. This view receives support from the experiments of Delwiche, *et al.*[14] The effect of ATP and CoA on the synthesis of succinate by extracts of *M. lactilyticus* might be attributed to their involvement in the production of both these "active" building blocks.

Acknowledgment.—It is a pleasure to acknowledge the advice and encouragement of Dr. C. B. Van Niel in the course of this work.

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GENERALIZED MATHEMATICAL RELATIONSHIPS FOR POLYPEPTIDE CHAIN HELICES. THE COORDINATES OF THE Π *HELIX**

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Communicated by J. T. Edsall, June 4, 1953

The formulation of polypeptide chain configurations is concerned principally with intra-chain packing and interactions between atoms in neighboring residue units of the skeletal backbone chain $(\text{—C}\cdot\text{CO}\cdot\text{NH}\cdot\text{—})_n$. The folded or coiled configurations are held together by intra-chain (CO·HN) hydrogen bonds between residues. In helical configurations the residues are structurally equivalent; they all have identical intra-chain environments (exclusive of amino acid residue variations). Such configurations, therefore, effectively provide maximal intra-chain hydrogen bonding.

Detailed studies depend on the use of precise dimensions for the poly-