ACETYL COENZYME A SYNTHESIS THROUGH PYROPHOSPHORYL SPLIT OF ADENOSINE TRIPHOSPHATE

by

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Two different enzymic pathways exist for the activation of acetate. One pathway is initiated by phosphorylation of acetate[1]:

$$\text{acetate} + \text{ATP} \rightleftharpoons \text{acetyl phosphate} + \text{ADP}^{***} \quad (1)$$

followed by acetyl transfer through transacetylase[2,3] to coenzyme A (CoA):

$$\text{acetyl phosphate} + \text{CoA} \rightleftharpoons \text{acetyl CoA} + \text{phosphate} \quad (2)$$

This relatively well understood mechanism is present in most micro-organisms[4,5], but not in animal tissues[6]. In tissues, yeast and probably also in some micro-organisms, another enzymic pathway leads through a complex reaction between ATP, CoA and acetate to acetyl CoA[7,8]. Inorganic pyrophosphate was recently found to be a product of this reaction[9,10]. It appears that a pyrophosphate split of ATP is involved in this energy transfer, which is formulated as follows:

$$\text{ATP} + \text{CoA} + \text{acetate} \rightleftharpoons \text{acetyl CoA} + \text{AMP} + \text{pyrophosphate} \quad (3)$$

Earlier experiments seemed to support a primary reaction between ATP and CoA, yielding presumably CoA-pyrophosphate. A more exhaustive study, however, has left us undecided with regard to the finer mechanism of reaction[3], which we assume to represent a double transfer system of some kind§. Some details of our experiments on this problem are presented here.

ENZYME PREPARATIONS AND ASSAY SYSTEM

Pigeon liver enzyme was prepared as described by KAPLAN AND LIPMANN[11] and by CHOU AND LIPMANN[12].

In most experiments yeast enzyme was used. Most brands of commercial Bakers' yeast were found to be suitable; the best preparations were obtained with "National" brand yeast.

The system extracts well from quick-frozen yeast; 1 part of yeast is mixed with 1 part of ether and 1.5 parts of dry ice, and stirred well. After 30 minutes the liquid is poured off, the yeast spread out in a thin layer on a cloth, and air is blown over it by a fan under a well-ventilated hood. An additional 1.5 parts of dry ice are mixed with the yeast and ventilation continued until practically all the ether is removed. The preparation is then generally stored in a deep freeze. On thawing, the yeast

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*** The following abbreviations have been used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; CoA, coenzyme A; PP, inorganic pyrophosphate; and P_i, orthophosphate.

§ Cf. addendum at end of paper (p. 147).

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autolyzes rather quickly. To promote this further, 33 ml of *M* dipotassium phosphate are mixed with 1,000 g of yeast and the mixture stirred overnight in the cold room at 0–5°. The yeast autolysate is centrifuged in a cold centrifuge. The supernatant solution does not store well and it is desirable to carry it immediately through the first ammonium sulfate fractionation step.

Sonic preparations. One part of fresh Bakers' yeast is mixed to a paste with 1 part of 0.1 *M* cold dipotassium phosphate and exposed to vibration in a 10 kilocycle magnetostrictive oscillator (Raytheon Manufacturing Company) for 40 minutes. The mixture is centrifuged in the cold in a Servall centrifuge at top speed (12,000 r.p.m.) for 30 minutes. The supernatant solution, amounting to slightly more than half the original volume, may be frozen and kept for a considerable length of time in the deep freeze.

This extract has the same specific activity as fraction 1 in Table I but is only half as concentrated. It behaves on fractionation like the ether extract and, after fractionation step 3, the two preparations become very similar in all respects.

Protamine precipitation. The slightly turbid supernatant solution is treated with protamine. The precipitate, containing very little active material, is discarded. For every mg of nucleic acid, 0.5 mg of protamine is added, using a 2% protamine sulfate solution. The protamine precipitate is centrifuged at 0° in a cooled centrifuge.

The nucleic acid content is determined turbidimetrically in the following manner: to 0.05 ml of the solution to be analyzed are added 4.5 ml of 0.05 *M* phosphate buffer, pH 6.1, and 0.5 ml of 2% protamine sulfate solution. The tubes are mixed and the turbidity is measured on a Klett colorimeter using filter No. 54. The reading is compared with a standard curve prepared with 0.1 to 0.6 mg of yeast nucleate adjusted to pH 6.

First ammonium sulfate precipitation. To 1,000 ml of clear supernatant solution (pH about 6), 350 g of solid ammonium sulfate are added (55% saturation). The active material is precipitated and centrifuged off; the supernatant solution is discarded. The precipitate is dissolved with 66 ml of 0.05 *M* potassium bicarbonate solution. The resulting solution is 20% saturated with ammonium sulfate.

Second ammonium sulfate precipitation. Enough saturated ammonium sulfate solution is added to bring the above enzyme preparation from 20% to 35% saturation with respect to ammonium sulfate. The precipitate is discarded and the supernatant solution is brought to 45% saturation with saturated ammonium sulfate solution. The precipitate is dissolved in 0.05 *M* bicarbonate solution and is generally used in this form without further treatment. The enzyme is stable in ammonium sulfate for a considerable length of time when stored in the deep freeze, while dialyzed enzyme preparations are much less stable even when frozen. Therefore, in most cases, dialysis is omitted. However, for certain experiments, it is desirable to remove the ammonium sulfate. For this purpose, the solution may be dialyzed with agitation at 0° against a solution of 0.05 *M* potassium bicarbonate in 0.5% potassium chloride for 2 to 3 hours. Typical results of the above fractionation procedure are illustrated in Table I.

TABLE I

ACTIVITY OF VARIOUS YEAST FRACTIONS

Fraction	*Description*	*Volume ml*	*Units per ml*	*Protein per ml* mg*	*Specific activity units per mg protein*	*Total units*	*Recovery %*
1	Original extract	1020	66	66	1.0	67,400	100
2	Protamine supernate	1000	66	33	2.0	66,000	98
3	Ammonium sulfate precipitate 0–55%	132	495	97	5.1	65,200	97
4	Ammonium sulfate precipitate 35–45%	32	1063	95	11.2	34,000	51

* Turbidimetric[2].

Assay system; enzyme units. In general, enzyme activity was determined by the hydroxamic acid method as described by CHOU[12]. CHOU observed that, for the assay of the isolated ATP-CoA-acetate donor system, contained in the 40% acetone precipitate from pigeon liver extract (A-40 fraction), high concentrations of hydroxylamine were necessary. He also found that, in the pigeon liver fraction precipitating between 40% and 60% acetone (A-60 fraction) which contained the acceptor enzyme for aromatic amines, there was present another enzyme which catalyzed the reaction of acetyl CoA with hydroxylamine. The presence of the latter enzyme was demonstrated by the fact that, if the A-40 and A-60 fractions were combined, hydroxamic acid formation was maximal at low concentrations of hydroxylamine. The cruder yeast preparations seem also to contain an enzyme which catalyzes the hydroxylamine reaction. As shown in CHOU's experiments, the effect of the hydroxylamine

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acceptor enzyme is minimized by the use of higher concentrations of hydroxylamine [12]. The hydroxamic acid assay has occasionally been checked using a procedure which measures arylamine acetylation, namely: by combining the A-60 fraction of CHOU with the yeast enzyme. The combined assay has the advantage of greater sensitivity. As shown in Fig. 1b, it is linear over a wider concentration range than is the hydroxamic acid assay (Fig. 1a) which, at higher enzyme concentration, becomes less reliable.

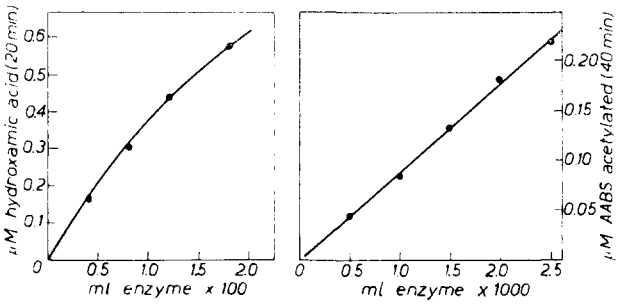

Fig. 1a. 1b. Comparison of hydroxamic acid and mixed enzyme assay.

The combined assay system was worked out by BESSMAN[13], who will report on this procedure separately. This assay is similar to the CoA assay described by HANDSCHUMACHER *et al*[14], using however, instead of their aminoazobenzene, aminoazobenzene sulfonate as acetyl acceptor. This compound has the advantage of greater solubility.

Hydroxamic acid assay[15]. One ml assay mixture contains: 25 units CoA, 10 μM ATP*, 10 μM potassium acetate, 100 μM potassium phosphate buffer, 200 μM hydroxylamine, (neutralized with KOH to pH 7.4), 50 μM KF, 10 μM $MgCl_2$, and 10 μM glutathione. The tubes are incubated at 37° for 20 minutes, at which time are added 1.5 ml of a ferric chloride reagent which contains 10% $FeCl_3$ and 3.3% trichloroacetic acid in 0.66 N HCl. The acethydroxamate formed is measured in the Klett-Summerson colorimeter with a No. 54 filter using 1 ml of H_2O and 1.5 ml of the $FeCl_3$ reagent to set the zero point. In all cases a blank tube which contains no CoA is incubated along with the tube containing CoA. The blank reading is subtracted from the reading obtained when CoA is present With increasing purification the blank reading becomes negligible. One unit enzyme activity is defined as that amount of enzyme which gives a reading of 100 (0.4 μM of hydroxamic acid) under these conditions.

Mixed assay. One ml of assay mixture contains: 100 μM potassium phosphate, 50 μM KF, 10 μM potassium acetate, 25 units CoA, 10 μM ATP, 10 units A-60 enzyme[13], 10 μM glutathione, 10 μM $MgCl_2$, and 0.6 μM aminoazobenzene sulfonate. The pH of the phosphate-acetate-fluoride mixture is set at 7.4. The tubes are incubated 40 minutes at 37°. At this time 0.2 ml of the incubation mixture is added to 3.0 ml of 10% TCA in 50% ethanol and centrifuged. The amount of dye acetylated is measured by the reduction in colour observed in the Klett-Summerson colorimeter with a No. 50 filter. The zero time reading is about 290. The colour difference should be less than 150 Klett units.

THE COMPONENTS OF THE ENZYMIC SYSTEM

CHOU had already found that magnesium was a component of the ATP-acetate reaction[12]. Magnesium dependence is shown in Fig. 2. It may be mentioned here that we routinely used all salts in the form of potassium salts in view of the general experience that this type of reaction seems to go better in a potassium-containing medium. We have, however, not studied the effect of monovalent ions in detail.

In Fig. 3, a pH-activity curve is shown, indicating an optimum between 7.2 and 7.8.

CoA reduction. When hydroxylamine was used to trap the acetyl CoA formed, glutathione was used to reduce the coenzyme. In stoichiometric balance experiments hydrogen sulfide was generally used except when a sulfhydryl balance was desirable. In this instance the CoA was reduced with potassium borohydride (Metal Hydrides, Inc.)

*The ATP used was purchased as the disodium salt from the Pabst Brewing Company. For a majority of the experiments, the CoA preparations were prepared from *Streptomyces fradiae* cultures by Dr. J. D. GREGORY. These products were 65 to 80% pure. For some of the experiments, the CoA was purchased from the Pabst Brewing Company. This product is about 75% pure.

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in the following manner: for each μM of CoA (310 units = 1 μM), 75 μM of KBH_4 in 0.002 M KOH and 10 μM of tris(hydroxymethyl)aminomethane buffer, pH 9, were added. The vessel was incubated for 15 minutes at 37°, at which time the contents were

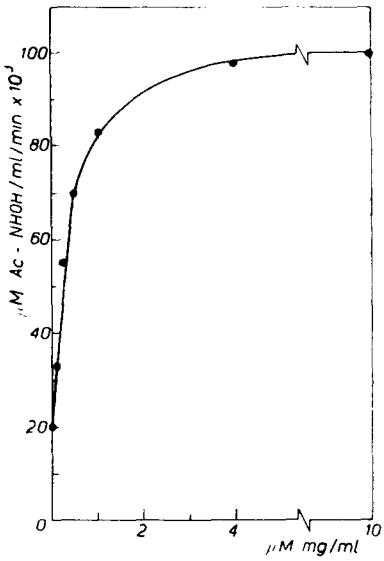

Fig. 2. Effect of magnesium concentration of the rate of acetate-ATP-CoA reaction. Each tube contained, in 1 ml. of reaction mixture: 25 units COA; 10 μM ATP; 10 μM potassium acetate; 200 μM tris (hydroxymethyl) - aminomethane buffer, pH 7.5; 50 μM KF; 10 μM glutathione; and 200 μM NH_2OH, neutralized to pH 7.4 with KOH; 0.01 ml (10 units) of yeast fraction 4 and $MgCl_2$ as indicated. Final pH was 7.4–7.2. The tubes were incubated at 37°, and 0.2 ml. samples were taken at 13, 30, and 60 minutes to determine the rate of the reaction.

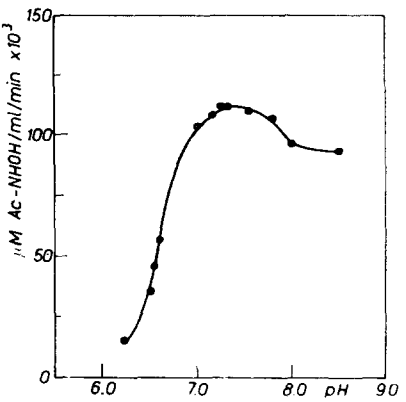

Fig. 3. Effect of pH on the rate of acetate-ATP-CoA reaction. Each vessel contained, in 1 ml reaction mixture: 25 units CoA; 10 μM ATP; 10 μM potassium acetate; 50 μM KF; 10 μM $MgCl_2$; 10 μM glutathione; 200 μM NH_2OH (pH adjusted with KOH to that of buffer); 50 μM each tris (hydroxymethyl)aminomethane and potassium phosphate buffer adjusted to the desired pH; and 0.01 ml (10 units) of yeast fraction 4. The tubes were incubated at 37°, and 0.2 ml samples were taken at 20, 40, and 60 minutes to determine the rate of the reaction.

neutralized with HCl to pH 7.2 and the volume adjusted with water to give a CoA solution of the desired concentration. Although solutions store fairly well on freezing, we have routinely prepared fresh solutions each day.

CoA concentration. It is a characteristic of the hydroxamic acid system that relatively high concentrations of CoA and hydroxylamine[12] are required for saturation. Under our conditions, as shown in Fig. 4, the maximum activity is approached at about 100 units of CoA. Since the reaction between acetyl mercapto CoA and hydroxylamine is non-enzymatic, this relatively high saturation concentration is not surprising.

The effect of inorganic phosphate. The addition of 50 to 100 μM of phosphate per ml generally increases the rate of the hydroxamic acid reaction considerably. For this reason phosphate was included in our assay system. Phosphate, however, is not indispensable. The mechanism of the phosphate effect is not clear.

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Identification of the reaction products. CHOU had frequently observed that the amount of phosphate liberated was far below the amount of hydroxamic acid formed. Recently these studies were resumed, particularly in view of LYNEN's identification of acetyl CoA as the mercaptoester of CoA[16]. Various observations then indicated that inorganic pyrophosphate rather than orthophosphate was being liberated. It was found that the liberation of inorganic phosphate could be almost completely suppressed by the addition of fluoride. Under these conditions the easily hydrolyzable phosphate did not change, but a substance appeared which gave, with the Fiske-Subbarow molybdate reagent, a slowly developing colour similar to that observed earlier by SACKS AND DAVENPORT with inorganic pyrophosphate[17]. In Table II an experiment is presented with CoA as acetyl acceptor. If fluoride is added, approximately equivalent amounts of pyrophosphate appear, as determined by the manganese precipitation method of KORNBERG[18] and also by a colorimetric method utilizing the above-mentioned colour increase with the molybdate reagent. This colour reaction has been developed into the convenient colorimetric method for determination of inorganic pyrophosphate, which will be described elsewhere.

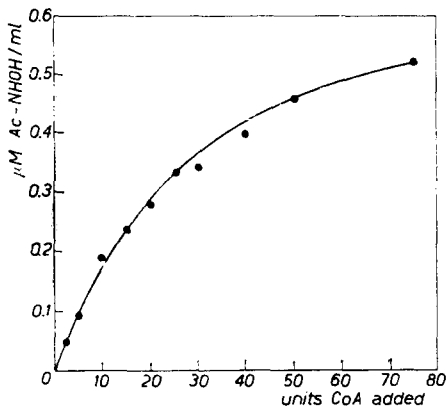

Fig. 4. CoA-concentration curve. Each tube contained, in 1 ml: 25 units CoA; 10 μM ATP; 10 μM potassium acetate; 50 μM KF; 100 μM potassium phosphate buffer (pH 7.4); 200 μM hydroxylamine neutralized with KOH to pH 7.4; 10 μM glutathione; 0.02 ml (1 unit) of yeast fraction 1. Tubes were incubated at 37° for 20 minutes at which time 1.5 ml of the 10% $FeCl_3$ reagent were added.

TABLE II

EFFECT OF FLUORIDE ON PYROPHOSPHATE FORMATION

Fluoride added μM per ml	*CoA* added μM per ml*	*Acetyl CoA μM per ml*	*PP μM per ml*	*Pi μM per ml*
0	0	0	0	7.4
0	2.9	2.72	0	13.2
50	0	0	0	1.75
50	2.9	2.88	3.10** 3.16***	2.10

* 1 μM CoA = 310 units.
** Determined by colour increase.
*** Determined by Mn precipitation method.
Each vessel contained in 1 ml: 12 μM ATP; 10 μM potassium acetate; 10 μM $MgCl_2$; 20 μM H_2S; 200 μM tris(hydroxymethyl) aminomethane buffer, pH 7.5 and 0.02 ml (20 units) of yeast enzyme fraction 4. Vessels were incubated at 37° for 30 minutes.

Without fluoride the excess of inorganic phosphate over the CoA-free blank corresponds to about twice the amount of acetyl CoA formed. As shown by KUNITZ[19], fluoride inhibits strongly the pyrophosphatase which is present in our yeast fractions. For this reason the presence of fluoride is necessary to preserve the pyrophosphate formed.

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In the experiment shown in Table III a determination of adenosine-5-phosphate (AMP) with the KALCKAR-SCHMIDT[20] procedure is included and, in addition, the acetylation of CoA is followed by determining the disappearance of SH-CoA[21] as well as the formation of acetyl-S-CoA. A similar balance with pigeon liver extract is presented in Table IV.

TABLE III

BALANCE FOR ACETYLATION OF COA

Free SH	*Acetyl CoA* *	*PP***	*AMP*
— 2.29	+ 1.66	+ 1.53	+ 2.28***

* Hydroxamic acid method.

** No sulfur bound PP could be demonstrated on treatment of the deproteinized samples with mercuric acetate.

*** Corrected for AMP formed in absence of CoA due to ATP-ase.

Concentration of factors per ml of reaction mixture: 0.1 ml (100 units) of yeast fraction 4; 5 μM Pabst CoA, reduced with potassium borohydride; 5 μM ATP; 20 μM potassium acetate; 10 μM $MgCl_2$; 50 μM KF; 100 μM tris (hydroxymethyl)aminomethane buffer, pH 7.5. The tubes were incubated at 37° for 15 minutes.

TABLE IV

CATALYTIC BALANCE WITH CRUDE PIGEON LIVER EXTRACT

CoA added units	*Time of incubation minutes*	*Hydroxamic acid μM*	*AMP μM*	*P–P μM*
	0	0	0	0.3
0	30	1.87	—	-
	120	4.25	6.9	1.85
	0	0	0	0
325	30	5.07	—	-
	120	16.6	16.3	16.2
				12.2*

* Assay by pyrophosphatase.

Each vessel contained, in a total volume of 3.2 ml: 47 μM ATP, of which 17 were added at 0 minutes and 30 μM at 30 minutes; 170 μM KF; 460 μM tris (hydroxymethyl)aminomethane buffer, pH 8.2; 75 μM $MgCl_2$; 870 μM NH_2OH, pH 6.5; 80 μM glutathione; 250 μM K acetate; and 0.96 ml crude pigeon liver enzyme. Vessels were incubated at 30° for 120 minutes.

TABLE V

CONVERSION OF ACETYL COA, PYROPHOSPHATE,

AND ADENOSINE-5-PHOSPHATE TO ADENOSINE TRIPHOSPHATE

	ATP μM	*AMP μM*	*Acetyl phosphate μM*
Complete system	+ 6.5	— 7.7	— 5.0*
Replace pyrophosphate with orthophosphate	+ 0.3	— 0.5	0
Omit CoA	+ 0	— 0.2	— 0.7

Each vessel contained: 25 μM lithium acetyl phosphate; 0.25 μM CoA; 100 μM potassium pyrophosphate; 10 μM potassium adenylate; 10 μM $MgCl_2$; 10 μM cysteine; and 50 μM KF; 0.1 ml transacetylase, *Clostridium kluyveri*; 0.3 ml yeast enzyme preparation. The pH was 7.1. The vessels were incubated for 30 minutes at 37°.

* These values are corrected for spontaneous breakdown of acetyl phosphate.

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Reversibility. The conversion of the energy-rich bond of acetyl CoA into the energy-rich pyrophosphoryl bond of ATP was demonstrated by using acetyl phosphate and phosphotransacetylase as acetyl feeder system with catalytic amounts of CoA. The experiment of Table V shows that the addition of pyrophosphate and AMP is necessary for this reaction.

Furthermore, the reversibility of the reaction can be shown spectrophotometrically using the mercaptoester absorption at 232 μM as described by STADTMAN[22] for determination of acetyl CoA. As shown in Fig. 5, on mixing the enzyme, ATP, CoA and acetate, the absorption reaches steady values in about 10 minutes. If at this time pyrophosphate is added, a rather rapid decrease of the acetyl CoA absorption is observed, the degree of which depends on the pyrophosphate concentration. The reason for the slow secondary decline in the absorption of acetyl CoA with higher pyrophosphate concentration is not understood at the present time.

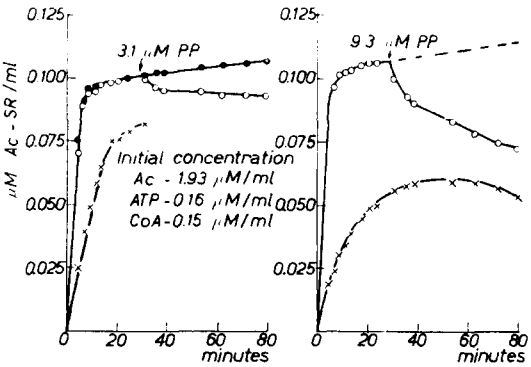

Fig. 5. Reversibility of ATP-CoA-acetate reaction by addition of pyrophosphate. Each cell contained, per ml: 0.15 μM CoA-SH (reduced with potassium borohydride); 0.16 μM ATP; 1.93 μM potassium acetate; 35 μM KF; 2 μM $MgCl_2$; 40 μM tris (hydroxymethyl) aminomethane buffer, pH 7.5; and 0.001 ml (1 unit) of yeast enzyme fraction 4.
Left Figure: Solid circles = control cell to which no pyrophosphate is added. Open circles = cell which contains the same reactants as the control tube except that 3.1 μM potassium pyrophosphate buffer, pH 7.5, are added at the time indicated by the arrow. Crosses = cells which contain the same reactants as the control cell except that 3.1 μM potassium pyrophosphate buffer, pH 7.5, are added at the start.
Right Figure: Curves as above except that 9.3 μM of the pyrophosphate buffer, pH 7.5, were added to the open circle and cross curves rather than 3.1 μM.

Attempts to clarify the finer mechanism of the reaction. In a previous report experiments were presented which seemed to indicate a primary reaction between pyrophosphate and acetyl CoA and were interpreted as an indication of the formation of a CoA-pyrophosphate. However, it has been difficult to reproduce these experiments with more highly purified acetyl CoA preparations. It is now suspected that the disappearance of acetyl CoA under these conditions may be explained through contamination with small amounts of AMP. If large amounts of pyrophosphate are added, very little AMP is needed for the reverse reaction. Attempts also were made to obtain a reaction between ATP and CoA. Frequently in such experiments a small disappearance of ATP may be observed without addition of acetate. However, it was found very difficult to exclude a contamination with small amounts of acetate, to which this system is quite sensitive.

We conclude, therefore, that a separate reaction either between ATP and CoA or between acetyl CoA and pyrophosphate has not been proven convincingly. Furthermore, attempts to divide the system into two enzyme fractions have not given encouraging results (see also Addendum).

Addendum (added in proof)

Further information on the finer mechanism was recently obtained by JONES,

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LIPMANN, HILZ AND LYNEN[23] through the use of isotopes. The isotope experiments are interpreted to indicate enzyme-bound intermediaries. ATP (Ad·P ~ PP) equilibrates rapidly through yeast enzyme with inorganic ^{32}P-pyrophosphate in the absence of other components of the system. This seems to implicate an enzyme-adenosine monophosphate (Ad·P) compound:

$$\text{Enzyme} + \text{Ad·P} \sim \text{PP} \rightleftharpoons \text{Enzyme} \sim \text{P·Ad} + \text{PP} \quad (4)$$

A subsequent replacement of Ad·P in enzyme ~ P·Ad by CoA is indicated by a reduction of the rate of Ad·P ~ PP ⇌ PP-exchange through the addition of CoA:

$$\text{Enzyme} \sim \text{P·Ad} + \text{CoA·SH} \rightleftharpoons \text{Enzyme} \sim \text{S·CoA} + \text{Ad·P} \quad (5)$$

Finally, the same enzyme was found to equilibrate acetyl CoA with free acetate rather easily, although less rapidly than Ad·P ~ PP and pyrophosphate. Such an exchange indicates enzyme ~ S·CoA to react eventually with acetate to yield the final product, acetyl ~ S·CoA:

$$\text{Enzyme} \sim \text{S·CoA} + \text{acetate} \rightleftharpoons \text{Enzyme} + \text{acetyl} \sim \text{S·CoA} \quad (6)$$

It is felt that this interpretation furnishes a reasonably good approximation to the final understanding of the mechanism.

SUMMARY

The formation of acetyl CoA by interaction of ATP, CoA and acetate has been studied with yeast and pigeon liver preparations.

Pyrophosphate and AMP have been found to be the products of the reaction, with the formulation as follows:

$$\text{ATP} + \text{CoA} + \text{acetate} \rightleftharpoons \text{acetyl CoA} + \text{AMP} + \text{pyrophosphate}$$

Magnesium is a component of the enzyme system.

The reaction is reversible as shown by the conversion of acetyl CoA, pyrophosphate and AMP, to ATP and free CoA. The reversion specifically requires pyrophosphate.

Enrichment of the yeast enzyme is described.

RÉSUMÉ

Les auteurs ont étudié la formation d'acétyl CoA par interaction entre l'ATP, le CoA et l'acétate chez des levures et dans des préparations de foie de pigeon.

La réaction conduit au pyrophosphate et à l'AMP. Elle peut se formuler ainsi:

$$\text{ATP} + \text{CoA} + \text{acétate} \rightleftharpoons \text{acétyl CoA} + \text{AMP} + \text{pyrophosphate}$$

Le magnesium est un constituant du système enzymatique.

La réaction est réversible ainsi que le montre la transformation de l'acétyl CoA, du pyrophosphate et de l'AMP en ATP et CoA libre. Le renversement de la réaction exige spécifiquement du pyrophosphate.

Une purification partielle de l'enzyme de la levure est décrite.

ZUSAMMENFASSUNG

Die Bildung des Acetyl-CoA bei der Reaktion mit ATP, CoA und Acetat wurde mit Hefe und Taubenleberpräparaten untersucht.

Es wurde gefunden, dass Pyrophosphat und AMP die Produkte der Reaktion sind, die sich wie folgt formulieren lässt:

$$\text{ATP} + \text{CoA} + \text{Acetat} \rightleftharpoons \text{Acetyl CoA} + \text{AMP} + \text{Pyrophosphat}$$

Magnesium ist ein Bestandteil des Enzymsystems.

Die Reaktion is reversibel, wie durch die Umwandlung von Acetyl-CoA, Pyrophosphat und AMP in ATP und freies CoA gezeigt wurde. Die Umkehr erfordert die Gegenwart von Pyrophosphat.

Die Anreicherung von Hefeenzym wird beschrieben.

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