ACETYL COA CARBOXYLASE, I. REQUIREMENT FOR TWO PROTEIN FRACTIONS*

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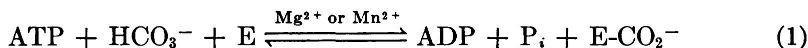
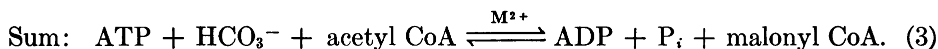
Acetyl CoA carboxylase has been studied in avian liver,[1-5] yeast,[6] rat adipose tissue,[7,8] and rat liver.[9,10] On the basis of these studies, the following mechanism involving two partial reactions has been proposed for this enzyme[1,4,10-12]



$$ATP + HCO_3^- + E \xrightleftharpoons{Mg^{2+} \text{ or } Mn^{2+}} ADP + P_i + E\text{-}CO_2^- \qquad (1)$$

$$E\text{-}CO_2^- + \text{acetyl CoA} \rightleftharpoons E + \text{malonyl CoA} \qquad (2)$$



$$\text{Sum:} \quad ATP + HCO_3^- + \text{acetyl CoA} \xrightleftharpoons{M^{2+}} ADP + P_i + \text{malonyl CoA.} \qquad (3)$$

E is a biotin enzyme which is carboxylated in reaction (1). In reaction (2) the carboxyl group is transferred to acetyl CoA to form malonyl CoA.

Liver acetyl CoA carboxylase has been isolated as a homogeneous protein.[2-5] The preparation with the highest enzymatic specific activity contains 1 mole of biotin per 409,000 gm of protein.[4] Ryder *et al.*[4] have reported preliminary investigations which indicate that the protomeric unit is composed of four polypeptide chains of approximately 100,000 mol wt. Thus it is apparent that this carboxylase is composed of nonidentical subunits, only one of them containing biotin, and this is consistent with previous studies of other biotin enzymes.[11,13] Studies of the mechanism of biotin enzyme reactions have been hampered by the irreversible inactivation of the enzyme brought about by those treatments which dissociate the protein into its subunits.

This communication describes the acetyl CoA carboxylase of *Escherichia coli*. Routine protein fractionation procedures lead to the separation of two protein fractions required for the over-all reaction. One of these, E_a, contains biotin and is carboxylated to form E_a-CO_2^- as in reaction (1). The other fraction, E_b, catalyzes carboxyl transfer from E_a-CO_2^- to acetyl CoA to form malonyl CoA.

Materials.—*E. coli* B, 1/4 log cells were obtained from Grain Processing Corporation. *Lactobacillus plantarum* 8014 was obtained from the American Type Culture Collection. Sodium C^{14}-bicarbonate was obtained from New England Nuclear. ATP and CoA were purchased from P-L Laboratories. Avidin was obtained from Nutritional Biochemical Company and alumina Cγ from Sigma. Acetyl CoA was synthesized as described.[14]

Methods.—*Preparation of enzyme fractions:* Cell-free extracts of *E. coli* B were prepared by homogenization in a Manton-Gaulin submicron disperser. After removal of cellular debris by centrifugation, nucleic acids were precipitated with $MnCl_2$ and the enzyme was subsequently precipitated between 25 and 45% ammonium sulfate saturation. Attempts at further purification using alumina Cγ gel led to the complete loss of enzymatic activity which could, however, be recovered by recombination of two fractions. A summary of this separation is shown in Table 1 where it is seen that one fraction, E_b, was not adsorbed to the gel and remained in the supernatant, whereas E_a was adsorbed to the gel an l was recovered by elution with potassium phosphate, pH 7.7, between 0.1

TABLE 1. *Resolution of acetyl CoA carboxylase into two fractions.*

Preparation	Total activity—E_a* (μmoles/min)	SA—E_a* (μmoles/min/mg protein)	Total activity—E_b† (μmoles/min)	SA—E_b† (μmoles/min/mg protein)
25–45% A.S.	2.060	0.001	1.736	0.00087
Al-Cγ supernatant	0	0	1.680	0.00093
Al-Cγ 0.1 M KPO_4 eluate	0.0427	0.0125	0	0
0.2 M KPO_4 "	0.232	0.0767	0	0
0.3 M KPO_4 "	0.212	0.0978	0	0

* Assayed in the presence of excess E_b. † Assayed in the presence of excess E_a.

and 0.3 M. In these and subsequent experiments, E_a activity was measured in the presence of excess E_b, and E_b activity was measured in the presence of excess E_a. E_a was further purified by ammonium sulfate fractionation to a specific activity of 0.16 units/mg protein. E_b was further purified by chromatography on hydroxylapatite and O-(diethylaminoethyl) cellulose (DEAE-cellulose) to a specific activity of 0.35 units/mg protein. Details of these purification procedures will appear in a subsequent publication.

Assay of enzymes: Acetyl CoA carboxylase assay: The assay of acetyl CoA carboxylase is based on the acetyl CoA-dependent formation of acid-stable radioactivity derived from $HC^{14}O_3^-$. The reaction mixtures contained 55 mM imidazole-HCl buffer, pH 6.7; 0.44 mM $MnCl_2$; 0.44 mM adenosine 5′-triphosphate (ATP); 0.3 mM acetyl CoA; 14 mM $KHC^{14}O_3$ (0.2–1.0 μcurie/μmole); and enzymes in a total volume of 0.09 ml. After incubation 5–15 min at 33°, the reactions were terminated by the addition of 0.01 ml 2 N HCl. Fifty-μliter aliquots were transferred to individual 2.2-cm Whatman no. 1 filter paper disks which were dried with the aid of a heat lamp. The disks were placed in scintillation vials containing 0.5 ml H_2O and, after the addition of 10 ml of Bray's solution,[15] counted in a Packard model 3375 liquid scintillation counter. To measure the individual enzymatic components, E_a and E_b, a limiting amount of one fraction (0–0.001 unit) was assayed in the presence of an excess (0.005 unit) of the other.

One unit of enzyme is defined as the amount of enzyme catalyzing the formation of 1.0 μmole of malonyl CoA per minute under these conditions.

Formation of E-$C^{14}O_2^-$: Incubation mixtures for the formation of E-$C^{14}O_2^-$ contained the following components: 55 mM imidazole-HCl buffer, pH 7.5; 0.2–1.5 mg E_a or E_b; 0.5 mM $MnCl_2$; 0.5 mM ATP; and 1.0 mM $NaHC^{14}O_3$ (20 mc/mmole) in a volume of 0.25 ml. After incubation for 2 min at 25°, the reaction mixtures were filtered through a 9 × 20-cm Sephadex G-50 column equilibrated with 0.01 M Tris-HCl buffer, pH 8.5, at 2°. The column was eluted with the same buffer. This filtration separated E-$C^{14}O_2^-$ which had formed from unreacted $HC^{14}O_3^-$. The fractions which contained protein were pooled and an aliquot was counted to quantitate the E-$C^{14}O_2^-$.

Transfer of "$C^{14}O_2^-$" from E_a-$C^{14}O_2^-$ to acetyl CoA: Reaction mixtures contained E_a-$C^{14}O_2^-$ (200–2500 cpm), 55 mM imidazole-HCl, pH 7.5; 0.15 mM acetyl CoA; and E_b as indicated in a total volume of 90 μliters. After 1 min at 25°, the reactions were terminated by the addition of 10 μliters of 2 N HCl. Acid-stable radioactivity was determined as described above for the assay of acetyl CoA carboxylase.

Determination of protein and biotin: Protein was determined by a microbiuret method.[16] Biotin content was determined microbiologically with *Lactobacillus plantarum*[17] after hydrolysis of protein samples in 3.6 N H_2SO_4 for 1 hr at 120°.

Results.—Requirements for malonyl CoA formation: As indicated in the demonstrated experiments in Table 1, two protein fractions, E_a and E_b, are required for the carboxylation of acetyl CoA. Table 2 shows that $MnCl_2$, ATP, and acetyl CoA are required in addition to E_a and E_b for the formation of malonyl CoA, since omission of any one of these components abolished or greatly decreased the reaction. Propionyl CoA could not substitute for acetyl CoA. Both

TABLE 2. *Requirements for malonyl CoA formation.*

	Malonyl-CoA (mµmoles)		Malonyl-CoA (mµmoles)
Complete system	3.7	Complete system	
− E_a	0	− Acetyl CoA +	
− E_b	0	propionyl CoA (4.5×10^{-4} *M*)	0
− $MnCl_2$	0	Complete (boiled E_a)	0
− ATP	0.28	Complete (boiled E_b)	0
− Acetyl CoA	0		

The components of the complete system and the determination of malonyl-CoA are described in *Methods* except that 0.005 units each of E_a and E_b were used. Reaction mixtures were incubated for 10 min.

E_a and E_b are heat-labile since they were inactivated by boiling for one minute. The requirement for $MnCl_2$ is relatively specific. Of a series of salts tested, only $MgCl_2$ and $CoCl_2$ could replace $MnCl_2$, and these activated the reaction only to about 10 per cent of maximum when optimal concentrations were tested. The requirements for acetyl CoA carboxylation are thus similar to those previously reported except that two protein fractions are required. Carboxylase activity is proportional over a limited range to the concentration of E_a when assayed with an excess of E_b and to E_b when assayed with an excess of E_a (see Fig. 1).

The products of the acetyl CoA carboxylase reaction were isolated and identified as follows: malonyl CoA by DEAE-cellulose chromatography, thin-layer chromatography, and paper chromatography;[18] adenosine 5′-diphosphate (ADP) and P_i by thin-layer chromatography on cellulose and on PEI-cellulose.[19] Stoichiometry studies established that the reaction yields equivalent amounts of malonyl CoA, ADP, and P_i.

Biotin content of E_a and formation of E_a-CO_2^-: Avidin, the specific biotin-binding protein, was utilized to determine whether E_a or E_b contains biotin. As shown in Table 3, avidin inhibited the reaction 100 per cent when it was incubated with E_a and E_b in experiment 1. Incubation of the avidin with an excess of biotin prior to the addition of E_a and E_b prevented this inhibition

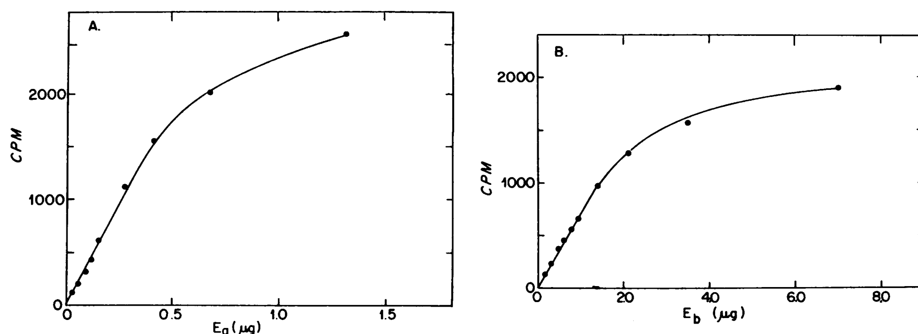


FIG. 1.—Effect of varying levels of E_a and E_b on the carboxylation of acetyl CoA. The assays were carried out as described in *Methods*. The specific activity of the $HC^{14}O_3^-$ used was 1 mc per mole. In (*A*) the effect of different levels of E_a in the presence of excess E_b (0.005 units) was measured. In (*B*), the effect of different levels of E_b in the presence of excess E_a (0.005 units) was measured. The duration of the assays was 15 min.

TABLE 3. *Effects of avidin on E_a and E_b.*

Expt.	First incubation*	Second incubation	Third incubation	Per cent inhibition
1	Avidin + E_a + E_b	Biotin	—	100
2	Avidin + biotin	$E_a + E_b$	—	0
3	Avidin + E_a	Biotin	E_b	100
4	Avidin + E_b	Biotin	E_a	0

Each incubation was carried out for 1 min at 25°. After the third incubation, the remaining components of the system were added and each experiment was assayed for acetyl CoA carboxylase activity as described in *Methods*.

The components listed were present in the following amounts: avidin, 0.2 units; biotin, 0.1 mg; E_a, 0.002 unit; E_b, 0.002 unit.

* In addition to the components listed, the first incubation contained 5 μmoles of imidazole-HCl, pH 6.7, in a volume of 50 μliters.

(expt. 2). Experiments 3 and 4 establish that E_a is the avidin-sensitive site since the reaction was completely inhibited when E_a was incubated with avidin before the addition of biotin and E_b (expt. 3), whereas E_b was not affected by avidin (expt. 4).

The (+)-biotin contents of E_a and E_b were determined microbiologically.[17] As indicated in Table 4, the three preparations of E_a eluted from alumina $C\gamma$ contained biotin, the fraction with the highest enzymatic activity (E_a-3) having 2.32 mμmoles per milligram of protein. Although the protein is not homogeneous at this stage, preparation E_a-3 contains 1 mole of biotin per 430,000 gm of protein. The catalytic-center activity of the enzyme is approximately 42 moles of acetyl CoA carboxylated per minute per mole of biotin. Biotin was not detected in E_b, although much higher protein concentrations of the latter were assayed.

Both E_a and E_b were tested for ability to form $E\text{-}C^{14}O_2^-$ when incubated with ATP, $HC^{14}O_3^-$, and $MnCl_2$. As anticipated from the biotin experiments above, only E_a formed $E\text{-}C^{14}O_2^-$, and the amount of $E_a\text{-}C^{14}O_2^-$ that was formed was equivalent to the biotin concentration of the preparation (Table 4). As demonstrated in Table 5, the formation of $E_a\text{-}C^{14}O_2^-$ was dependent upon ATP, $MnCl_2$, and E_a but did not require the presence of E_b. Avidin inhibited formation of E_a-

TABLE 4. *Biotin content and formation of $E\text{-}C^{14}O_2^-$.*

Preparation	Biotin content* (mμmoles/mg protein)	$E\text{-}C^{14}O_2$ formed† (mμmoles/mg protein)
E_a 1	0.412	0.482
2	1.82	1.88
3	2.32	2.42
E_b	0	0

E_a 1, 2, and 3 refers to the preparations obtained from alumina $C\gamma$ with 0.1, 0.2, and 0.3 *M* KPO_4 shown in Table 1. The preparation of E_b tested had an enzymatic specific activity of 0.06 units/mg protein.

* Average of microbiological assays at three protein concentrations.

† $E\text{-}C^{14}O_2^-$ formation tested as indicated under *Methods*.

TABLE 5. *Requirements for $E_a\text{-}CO_2^-$ formation.*

	$E_a\text{-}C^{14}O_2^-$ formed (mμmoles)
Complete	0.468
− ATP	0.042
− $MnCl_2$	0.019
− E_a	0
− E_b	0.470
Complete + avidin (0.2 units)*	0.264
Complete + avidin (0.5 units)*	0.041
− E_b + avidin (0.2 units)*	0.261

The preparation of $E_a\text{-}C^{14}O_2^-$ and its separation from $HC^{14}O_3^-$ is described in *Methods*. In this experiment, 0.2 mg of E_a and 1.0 mg of E_b were used.

* Experiments were preincubated with avidin for 1 min. Excess avidin was removed by the addition of 0.1 mg biotin before assaying.

TABLE 6. *Requirements for carboxyl transfer from E_a-$C^{14}O_2^-$ to acetyl CoA.*

	E_a-$C^{14}O_2^-$ (mμmoles)	C^{14}-malonyl CoA formed (mμmoles)	Yield (%)
Complete	0.0055	0.0052	94.7
"	0.013	0.011	84.5
"	0.026	0.016	61.7
"	0.037	0.021	56.6
" (incubated 5 min)	0.037	0.029	78.4
" (omit E_b)	0.037	0	0
" (omit acetyl CoA)	0.037	0	0
" (omit acetyl CoA, add propionyl CoA)	0.037	0	0

The complete system contained 55 mM imidazole-HCl, pH 7.5; 0.15 mM acetyl CoA; 0.003 units E_b; and E_a-$C^{14}O_2^-$ in the amounts indicated in a volume of 90 μliters. Reactions were incubated 1 min except as indicated, then terminated with 10 μliters of 2 *M* HCl and malonyl CoA determined as described in *Methods*.

$C^{14}O^{2-}$. E_b had no detectable effect on avidin inhibition under these conditions.

Carboxyl transfer from E_a-$C^{14}O_2^-$ to acetyl CoA: The recognition that only the biotin enzyme, E_a, was required for the formation of E_a-$C^{14}O_2^-$ allowed independent testing of E_b in the second partial reaction (reaction 2), the carboxyl transfer to acetyl CoA. For these experiments E_a-$C^{14}O_2^-$ was prepared in larger quantities in the absence of E_b and stored after filtration through Sephadex G-50 at pH 8.5 and 2°. As indicated in Table 6, acid-stable radioactivity was formed from E_a-$C^{14}O_2^-$ when the latter was incubated for one minute with acetyl CoA and E_b. All acid-stable radioactivity was shown to co-chromatograph with malonyl CoA in thin-layer chromatography. With increasing amounts of E_a-$C^{14}O_2^-$, more C^{14}-malonyl CoA was formed, although the per cent conversion decreased. Incubation for five minutes rather than one minute increased the yield somewhat. Malonyl CoA formation was entirely dependent upon the presence of E_b and acetyl CoA. Propionyl CoA could not substitute for acetyl CoA.

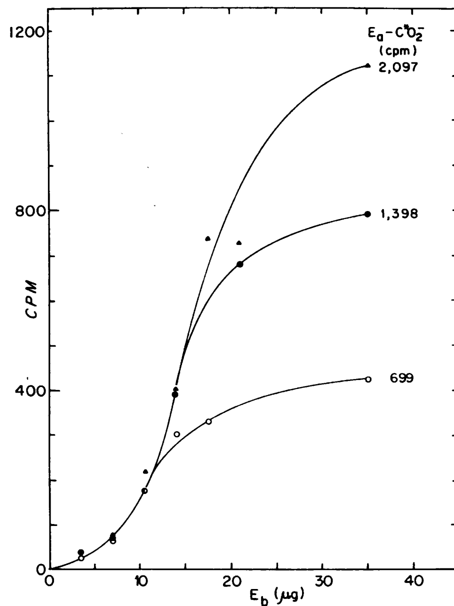


FIG. 2.—Effect of E_b on carboxyl transfer from E_a-$C^{14}O_2^-$ to acetyl CoA. The reaction mixtures contained in 90 μliters 5 μmoles of imidazole-HCl, pH 7.5; 15 mμmoles acetyl CoA; E_b as indicated; and E_a-$C^{14}O_2^-$ as follows; 699 cpm (○—○), 1,398 cpm (●—●), and 2,097 cpm (▲—▲). After 1 min at 25° the reactions were terminated by the addition of 10 μliters of 2 *M* HCl. Acid-stable radioactivity was then determined as described in *Methods*.

Further experiments have demonstrated the reversibility of the carboxyl-transfer reaction. The formation of E_a-$C^{14}O_2^-$ from 3-C^{14}-malon-

yl CoA was shown to require the presence of E_b.[18] These experiments all suggest that E_b is involved only in the carboxyl transfer between E_a-CO_2^- and malonyl CoA.

Effect of E_b on carboxyl-transfer reaction: Since the carboxyl-transfer reaction is a complex one involving two proteins, one of them (E_a-CO_2^-) acting at least in part as a substrate, it is not surprising that the effect of the addition of E_b to E_a-$C^{14}O_2^-$ is not a simple relationship. The experiments of Figure 2 show the effect of increasing E_b concentrations at three different concentrations of E_a-$C^{14}O_2^-$. With each E_a-$C^{14}O_2^-$ concentration tested there was a sigmoid relationship between E_b and the rate of malonyl CoA formation. The kinetic experiments in Figure 3 demonstrate that the sigmoid relationships in Figure 2 were not due to a time lag in initiation of the reaction at different E_b concentrations. Carboxyl transfer began immediately at both 7- and 14-μg concentrations of E_b. It is again apparent that doubling the concentration of E_b increased the initial rate of the carboxyl-transfer reaction more than tenfold. Although the same amounts of E_a-$C^{14}O_2^-$ were used with both E_b concentrations, the amount of carboxyl

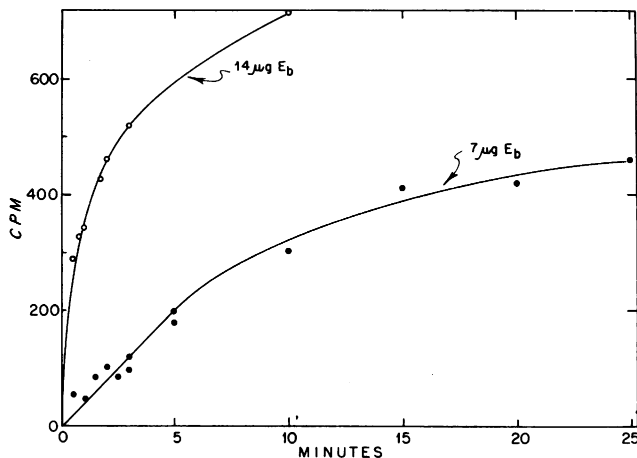


FIG. 3.—Kinetics of carboxyl transfer. Malonyl CoA formation was determined as described in Fig. 2 at the times indicated. Each reaction mixture contained 1,200 cpm of E_a-CO_2^- and either 7 μg (●—●) or 14 μg (○—○) of E_b (0.070 units/mg).

transferred with 7 μg of E_b never reached that amount transferred with 14 μg, probably due to E_a-$C^{14}O_2^-$ decomposition under these conditions.

Avidin inhibition of carboxyl transfer from E_a-$C^{14}O_2^-$ to acetyl CoA: Avidin has been shown to inhibit both the over-all synthesis of malonyl CoA in the presence of E_a and E_b and the formation of E_a-$C^{14}O_2^-$ from E_a, $HC^{14}O_3^-$, and ATP. In both kinds of experiments the avidin-sensitive site was localized to E_a which contains biotin. The presence of E_b had no detectable effect on the inhibition of these reactions by avidin. The reactivity of E_a-$C^{14}O_2^-$ with avidin, however, is greatly influenced by the presence of E_b. Thus, as indicated in Figure 4, the extent of inhibition by avidin of carboxyl transfer was greatly en-

hanced by the presence of E_b at the levels of avidin tested. When E_a-$C^{14}O_2^-$ was incubated with 0.04 units of avidin, malonyl CoA formation was only 10 per cent inhibited; whereas when E_b was present during the initial incubation of E_a-$C^{14}O_2^-$ with avidin, malonyl CoA formation was 87 per cent inhibited. This inhibition was specifically due to added avidin since prior treatment of the avidin with biotin completely prevented its inhibitory effect. Avidin had no direct

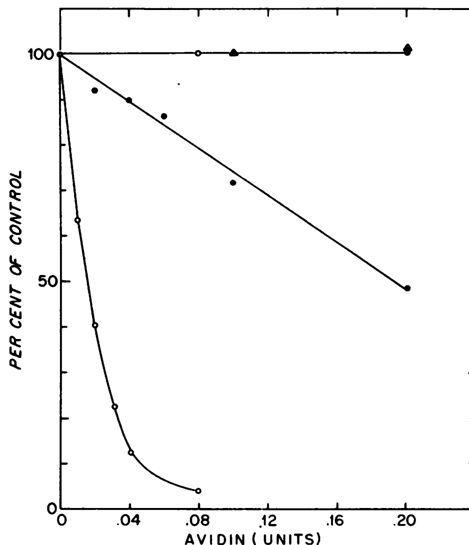
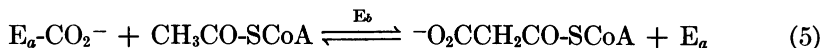


FIG. 4.—Effect of avidin on the carboxyl transfer reaction. In the middle curve (●—●), E_a-$C^{14}O_2^-$ (800 cpm) was incubated with 5 μmoles of imidazole-HCl, pH 7.5, and the indicated units of avidin in a volume of 50 μliters. After 1 min at 25°, 0.1 mg of biotin was added and the mixture was incubated an additional minute. Then 0.002 units of E_b and 15 mμmoles of acetyl CoA were added in a final volume of 90 μliters and the reaction was incubated 1 min. Malonyl CoA formation was determined as described in Fig. 2 and *Methods*. Lower curve (O—O) is the same as the middle curve except that both E_a-$C^{14}O_2^-$ and E_b were incubated with avidin before the addition of biotin. Upper curve (▲—▲) is the same as the middle curve except only E_b was incubated with avidin. E_a-$C^{14}O_2^-$ was added after biotin. Also included on the upper curve are the controls for the middle (—●—) and lower (—O—) curves. In these experiments avidin was incubated with biotin for 1 min before the addition of E_a-$C^{14}O_2^-$ and E_a.

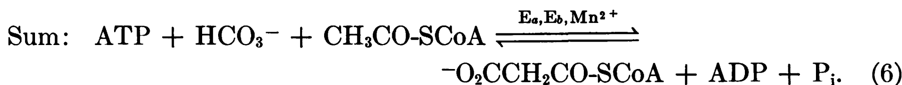
effect on E_b since incubation of E_b with avidin did not decrease the reactivity of E_b when it was subsequently tested. These experiments suggest that the biotin of E_a-$C^{14}O_2^-$ in the presence of E_b is more available for binding by avidin.

Discussion.—The results of these experiments support the following sequence for the carboxylation of acetyl CoA by the *E. coli* enzyme system:

$$\mathrm{ATP} + \mathrm{HCO_3^-} + \mathrm{E}_a \xrightleftharpoons{\mathrm{Mn^{2+}}} \mathrm{E}_a\text{-}\mathrm{CO_2^-} + \mathrm{ADP} + \mathrm{P_i} \quad (4)$$



$$\mathrm{E}_a\text{-}\mathrm{CO_2^-} + \mathrm{CH_3CO\text{-}SCoA} \xrightleftharpoons{\mathrm{E}_b} {}^-\mathrm{O_2CCH_2CO\text{-}SCoA} + \mathrm{E}_a \quad (5)$$



$$\text{Sum:}\quad \mathrm{ATP} + \mathrm{HCO_3^-} + \mathrm{CH_3CO\text{-}SCoA} \xrightleftharpoons{\mathrm{E}_a,\mathrm{E}_b,\mathrm{Mn^{2+}}} {}^-\mathrm{O_2CCH_2CO\text{-}SCoA} + \mathrm{ADP} + \mathrm{P_i}. \quad (6)$$

Over-all acetyl CoA carboxylation requires ATP, $MnCl_2$, and the enzymes E_a and E_b. The role of E_a in reaction (4) is established by the demonstration that this enzyme is inhibited by avidin, that it contains bound biotin, and that it forms E_a-CO_2^- from HCO_3^- and ATP in amounts equivalent to the biotin concentration of the enzyme preparation. E_b is not inhibited by avidin, does not contain biotin, does not form E_b-CO_2^- and is not directly involved in reaction

(4). It functions in reaction (5), the reversible carboxyl transfer from E_a-CO_2^- to acetyl CoA to form malonyl CoA. The formation of malonyl CoA from E_a-CO_2^- and acetyl CoA is absolutely dependent on the presence of E_b. Neither ATP nor $MnCl_2$ is required for this reaction.

Thus *E. coli* acetyl CoA carboxylase is composed of two protein components which have different functions. One of these, E_a, is a biotin-protein, and this must represent the biotin subunit of other acetyl CoA carboxylases which have not yet been dissociated into active components. It is not known whether E_a and E_b occur together in a complex in *E. coli.* E_a has catalytic center activity of about 40 moles of acetyl CoA carboxylated per minute per mole of biotin, and this is roughly 100-fold lower than the activity of other biotin enzymes.[4, 11, 12] Although this enzyme is not stimulated by citrate,[18] the low activity might be due to the requirement for unknown allosteric activators. It could also be due to irreversible protein changes caused by the purification procedure. Such changes could prevent optimal association between E_a and E_b. That E_b does interact with E_a is suggested by the protein-activity relationships shown in Figures 2 and 3 and by the facilitation by E_b of the avidin inhibition of E_a-CO_2^- shown in Figure 4. The mechanism of action of E_b is still unknown. It may contain the acetyl CoA binding site, in which case it should be possible to demonstrate substrate binding to this protein. Such substrate binding studies and studies to delineate interactions between E_a and E_b are presently under investigation.

Summary.—Two protein fractions, which are required for the carboxylation of acetyl CoA, have been partially purified from extracts of *E. coli.* One fraction, E_a, contains biotin and forms E_a-CO_2^- in the presence of ATP and $MnCl_2$. The other fraction, E_b, is required for carboxyl transfer from E_a-CO_2^- to acetyl CoA, forming malonyl CoA.

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[1] Waite, M., and S. J. Wakil, *J. Biol. Chem.*, **237**, 2750 (1962).

[2] Gregolin, C., E. Ryder, A. K. Kleinschmidt, R. C. Warner, and M. D. Lane, these PROCEEDINGS, **56**, 148 (1966).

[3] *Ibid.*, p. 1751.

[4] Ryder, E., C. Gregolin, H. Chang, and M. D. Lane, these PROCEEDINGS, **57**, 1455 (1967).

[5] Numa, S., E. Ringelmann, and B. Riedel, *Biochem. Biophys. Res. Commun.*, **24**, 750 (1966).

[6] Matsuhashi, M., S. Matsuhashi, S. Numa, and F. Lynen, *Biochem. Z.*, **340**, 243 (1964).

[7] Martin, D. B., and P. R. Vagelos, *J. Biol. Chem.*, **237**, 1787 (1962).

[8] Vagelos, P. R., A. W. Alberts, and D. B. Martin, *J. Biol. Chem.*, **238** 533 (1963).

[9] Matsuhashi, M., S. Matsuhashi, and F. Lynen, *Biochem. Z.*, **340**, 263 (1964).

[10] Numa, S., E. Ringelmann, and F. Lynen, *Biochem. Z.*, **340**, 228 (1964).

[11] Kaziro, Y., and S. Ochoa, *Advan. Enzymol.*, **26**, 283 (1964).

[12] Lynen, F., *Biochem. J.*, **102**, 381 (1967).

[13] Scrutton, M. C., and M. F. Utter, *J. Biol. Chem.*, **240**, 1 (1965).

[14] Simon, E. J., and D. Shemin, *J. Am. Chem. Soc.*, **75**, 2520 (1953).

[15] Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).

[16] Munkres, K. D., and F. M. Richards, *Arch. Biochem. Biophys.*, **109**, 466 (1965).

[17] Wright, L. D., and H. R. Skeggs, *Proc. Soc. Exptl. Biol. Med.*, **56**, 95 (1944).

[18] Alberts, A. W., unpublished observations.

[19] Randerath, K., *Thin Layer Chromatography* (New York: Academic Press, 1963), p. 185.