JOURNAL OF BACTERIOLOGY, Oct. 1997, p. 6061–6065 Vol. 179, No. 19
0021-9193/97/$04.00+0

Vitamin K_2 (Menaquinone) Biosynthesis in *Escherichia coli*: Evidence for the Presence of an Essential Histidine Residue in *o*-Succinylbenzoyl Coenzyme A Synthetase

DIPAK K. BHATTACHARYYA, O. KWON, AND R. MEGANATHAN*

Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115

Received 4 April 1997/Accepted 22 July 1997

***o*-Succinylbenzoyl coenzyme A (OSB-CoA) synthetase, when treated with diethylpyrocarbonate (DEP), showed a time-dependent loss of enzyme activity. The inactivation follows pseudo-first-order kinetics with a second-order rate constant of $9.2 \times 10^{-4} \pm 1.4 \times 10^{-4}$ μM^{-1} min^{-1}. The difference spectrum of the modified enzyme versus the native enzyme showed an increase in A_{242} that is characteristic of *N*-carbethoxyhistidine and was reversed by treatment with hydroxylamine. Inactivation due to nonspecific secondary structural changes in the protein and modification of tyrosine, lysine, or cysteine residues was ruled out. Kinetics of enzyme inactivation and the stoichiometry of histidine modification indicate that of the eight histidine residues modified per subunit of the enzyme, a single residue is responsible for the enzyme activity. A plot of the log reciprocal of the half-time of inactivation against the log DEP concentration further suggests that one histidine residue is involved in the catalysis. Further, the enzyme was partially protected from inactivation by either *o*-succinylbenzoic acid (OSB), ATP, or ATP plus Mg^{2+} while inactivation was completely prevented by the presence of the combination of OSB, ATP, and Mg^{2+}. Thus, it appears that a histidine residue located at or near the active site of the enzyme is essential for activity. When His^{341} present in the previously identified ATP binding motif was mutated to Ala, the enzyme lost 65% of its activity and the K_m for ATP increased 5.4-fold. Thus, His^{341} of OSB-CoA synthetase plays an important role in catalysis since it is probably involved in the binding of ATP to the enzyme.**

Vitamin K_2 (menaquinone [MK]) is derived from the shikimate pathway (2) and plays an essential role in several anaerobic electron transport systems of *Escherichia coli*. The pathway for its biosynthesis has been reviewed recently (15) and is known to include at least seven enzymatically controlled reactions. One of the major reactions in the biosynthetic pathway is the two-step formation of the bicyclic aromatic compound 1,4-dihydroxy-2-naphthoic acid (DHNA) from the benzenoid compound *o*-succinylbenzoic acid (OSB), the first aromatic compound identified in the pathway (3, 4, 10). This cyclization of OSB to DHNA is dependent upon the formation of *o*-succinylbenzoyl coenzyme A (OSB-CoA) as an intermediate (16). Two enzymes, OSB-CoA synthetase and DHNA synthase, are required for this conversion. Formation of OSB-CoA by the enzyme OSB-CoA synthetase requires the presence of ATP and CoA in addition to OSB. During the reaction, ATP is converted to AMP and PP_i (16). The reactions, genes, and enzymes involved in the formation of OSB-CoA and its subsequent conversion to DHNA are summarized in Fig. 1.

The gene encoding OSB-CoA synthetase (*menE*) has been cloned from *E. coli* (22), and its complete nucleotide sequence has been determined (21). Recently, this enzyme has been overexpressed, purified, and biochemically characterized (12). OSB-CoA synthetase is a homotetrameric protein with a native molecular mass of 185 kDa (12).

In this report, we present evidence obtained by chemical modification of the enzyme with the histidine-specific reagent diethylpyrocarbonate (DEP) (5, 6, 18, 19, 23) that a single histidine residue is essential for catalytic activity. Further, substrate protection assays established that this histidine residue is located at or near the active site of the enzyme.

* Corresponding author. Phone: (815) 753-7803. Fax: (815) 753-0461. E-mail: rmeganathan@niu.edu.

MATERIALS AND METHODS

Materials. DEP, hydroxylamine, ATP, CoA-SH, pyrophosphatase, ammonium molybdate, and imidazole were from Sigma Chemical Co., St. Louis, Mo. The mutagenesis kit and restriction enzymes used were products of Promega, Madison, Wis. All other reagents were of the highest purity commercially available.

Preparation and assay of the enzyme. The native and mutant forms of OSB-CoA synthetase were overexpressed, cell extracts were prepared, and the enzymes were purified to homogeneity by using the methods described previously (12). The protein content of the enzyme during various stages of purification was determined by the method of Bradford (7). For the assay of the enzyme, the pyrophosphate formed in the reaction was determined after its conversion to inorganic phosphate by coupling with pyrophosphatase (12).

Reaction of OSB-CoA synthetase with DEP. Just prior to use, fresh solutions of DEP were prepared by diluting the DEP stock solution with acetonitrile. The exact concentration of DEP in the acetonitrile solution was calculated from the increase in the A_{230}, when an aliquot of the dilution was added to 10 mM imidazole-HCl buffer, pH 7.5. The increase in A_{230} is due to the formation of *N*-carbethoxyimidazole. An extinction coefficient of 3×10^3 M^{-1} cm^{-1} was used in the calculations (17). The carbethoxylation of OSB-CoA synthetase was carried out by incubating the enzyme with DEP in 20 mM HEPES (pH 7.0) at 30°C. The modification reaction is specific in the pH range of 5.5 to 7.5 for the unprotonated histidine residues (14). The modification reactions were initiated by addition of DEP and terminated when the maximum A_{242} was reached. The stoichiometry of the formation of *N*-carbethoxyhistidine was calculated from the increase in absorbance by using an extinction coefficient of 3×10^3 M^{-1} cm^{-1}. The extent of inactivation of the enzyme was determined by assaying the residual activity in aliquots withdrawn from the reaction mixture at various time intervals.

Spectroscopic studies. The UV difference spectra of the carbethoxylated enzyme were recorded with a Perkin-Elmer Lambda 4A recording spectrophotometer.

CD spectral analysis. The circular dichroism (CD) spectra in the far UV region (260 to 190 nm) of both the native and DEP-modified forms of the enzyme were measured on a JASCO J-15 spectropolarimeter. Spectral analysis was performed with a solution containing 1 mg of enzyme per ml of 20 mM HEPES buffer (pH 7.2) in a cell with an optical path of 1 mm. The ellipticity values were corrected for the solvent and background noise.

Site-directed mutagenesis for replacement of His^{341} with Ala. For replacement of the histidine residue in position 341 of the enzyme with alanine, site-directed mutagenesis was performed on the previously described plasmid pAE73 (12). An oligonucleotide primer spanning the target histidine codon was used for the mutagenesis. The sequence of the *menE* gene (5′CGCGGTGAGATGCAT AATGGCAAGCTG3′) was altered by changing the histidine codon CAT (un-

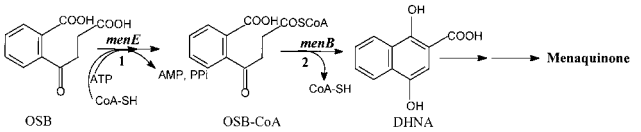

FIG. 1. Formation of OSB-CoA and its conversion to DHNA and MK. 1, OSB-CoA synthetase encoded by the *menE* gene; 2, DHNA synthase encoded by the *menB* gene. The DHNA is subsequently prenylated and methylated, resulting in the formation of MK.

derlined) to the alanine codon GCC. Site-directed mutagenesis was performed by using the Altered Sites II in vitro mutagenesis kit as described previously (12). The presence of the desired mutation was verified by restriction analysis using the enzymes *Nsi*I and *Eae*I as follows. Plasmid pAE73 containing the targeted His codon has the *Nsi*I recognition site ATGCAT. However, after mutagenesis, *Nsi*I can no longer restrict at this site. Further, after mutagenesis and introduction of the Ala codon, the resulting sequence, ATGGCC, creates the restriction site for the enzyme *Eae*I, whose recognition sequence is PyGGCCPu. The mutated *menE* gene was then subcloned into the overexpression vector pT7-7 and expressed in *E. coli* BL21(DE3) as described before (12).

RESULTS

Time course of inactivation of OSB-CoA synthetase by DEP. When an acetonitrile solution of DEP was added to OSB-CoA synthetase and incubated at pH 7.0 and 30°C, the enzyme lost its catalytic activity. As a control, when the enzyme was incubated with the same concentration of acetonitrile under identical conditions as the experimental tube, no loss of activity was observed. Since DEP is hydrolyzed in aqueous solution, when calculating the kinetics of inactivation, this hydrolysis must be taken into consideration. Hence, the rate of inactivation of the enzyme can be expressed by the following equation (13):

$$\ln A/Ao = -K_{obs}[DEP_0]^n/n\ (1 - e^{-nk't}/k') \qquad (1)$$

where A and Ao are the activities at time t in the presence and absence of DEP, respectively, $[DEP_0]$ is the initial concentration of DEP, K_{obs} is the pseudo-first-order rate constant of inactivation of the enzyme by DEP, k' is the pseudo-first-order rate constant of hydrolysis of DEP in buffer, and n is the number of residues involved in enzyme activity (11). If $n = 1$, the reaction becomes a simple bimolecular reaction. The value of k' for DEP in 20 mM HEPES (pH 7.0) at 30 ± 2°C was found to be 1.44×10^{-1} min^{-1}. The inactivation of OSB-CoA synthetase follows pseudo-first-order kinetics with respect to the DEP concentration (Fig. 2A). The data were analyzed by simultaneous nonlinear least-squares regression using K_{obs} and n as adjustable parameters, and the value of n was calculated to be 0.87 ± 0.03. The K_{obs} values obtained from the slope of the curves in Fig. 2A exhibited a straight line when plotted against the DEP concentration (Fig. 2B). This observation suggests that the reaction between the enzyme and DEP is a simple bimolecular reaction without the formation of a reversible complex (9). Further, the second-order rate constant calculated from Fig. 2B was found to be $4.2 \times 10^{-4} \pm 1 \times 10^{-4}$ μM^{-1} min^{-1}. In calculating the rate constant, if the spontaneous hydrolysis of DEP in buffer is taken into consideration, the value becomes $9.2 \times 10^{-4} \pm 1.4 \times 10^{-4}$ μM^{-1} min^{-1}.

UV difference spectra of DEP-modified versus native OSB-CoA synthetase. The optical difference spectrum of DEP-modified OSB-CoA synthetase versus the native enzyme at different time points is shown in Fig. 3. The peak at 242 nm is characteristic of the formation of *N*-carbethoxyhistidine. Further, the absence of a decrease in absorption at 278 nm, which is characteristic of the formation of *o*-carbethoxytyrosine, excludes the possibility of tyrosine modification.

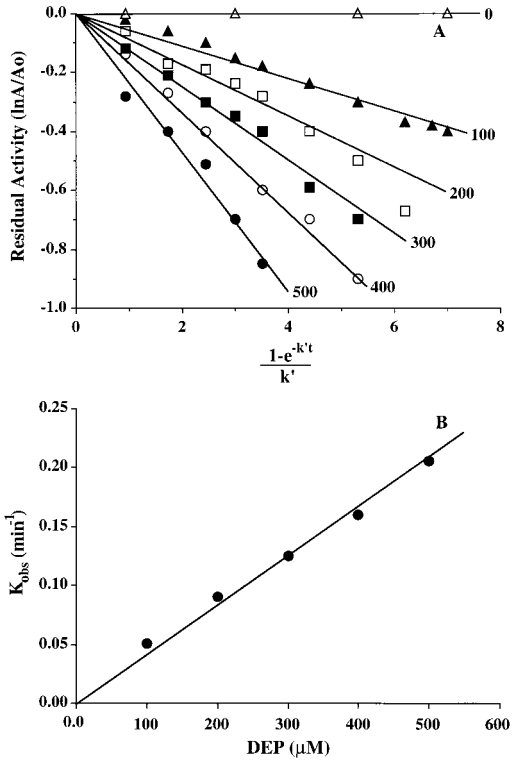

FIG. 2. Kinetics of inactivation of OSB-CoA synthetase by DEP. (A) Determination of the pseudo-first-order rate of inactivation. The enzyme at a concentration of 1 μM was incubated with different concentrations of DEP (the values shown are micromolar concentrations) at 30°C in 20 mM HEPES buffer, pH 7.0, in a total volume of 300 μl. At the indicated times, 25-μl aliquots were withdrawn and assayed for enzyme activity in 3.0 ml of 0.1 M HEPES buffer, pH 8.0, as described before (12) and the data were plotted according to equation 1. The curves were best fitted by least-squares analysis. (B) Dependence of the pseudo-first-order rate constants for inactivation on the concentration of DEP. The slopes of the straight lines obtained in panel A were plotted against the concentration of DEP.

Structural integrity of DEP-modified OSB-CoA synthetase. The CD spectrum of the DEP-modified enzyme was compared with that of the native enzyme. No significant changes were observed between the spectra before and after modification of the enzyme with DEP (data not shown).

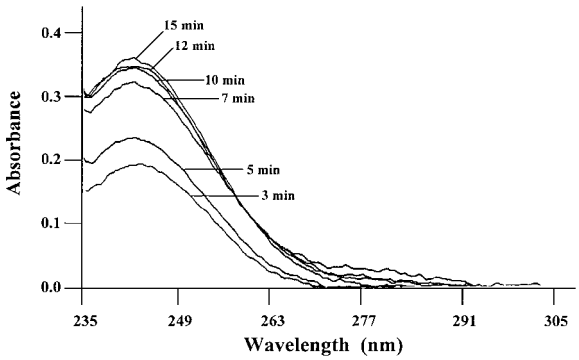

FIG. 3. UV difference spectra of the time-dependent modification of OSB-CoA synthetase with DEP. The enzyme (5 μM) was incubated with 200 μM DEP in 1.0 ml of 20 mM HEPES buffer, pH 7.0, and the spectra were recorded against a blank containing the untreated enzyme between the wavelengths of 235 and 305 nm at the indicated time intervals after the addition of DEP.

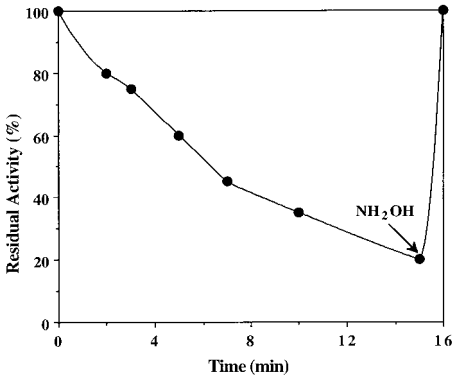

FIG. 4. Reactivation of DEP-inactivated OSB-CoA synthetase by hydroxylamine. The enzyme (1 μM) was incubated with 300 μM DEP in 20 mM HEPES buffer, pH 7.0, in a total volume of 500 μl, and 25 μl-aliquots were withdrawn and assayed at the indicated time points as described in the legend to Fig. 2. After 15 min, hydroxylamine was added to a final concentration of 800 mM and the mixture was incubated for 30 min. The reaction mixture was dialyzed against a large volume (2 liters) of 20 mM HEPES buffer, pH 7.0, for 24 h with buffer changes at 2-h intervals. The control enzyme solution was similarly treated with hydroxylamine. At the end of dialysis, the enzyme activity was determined in both the control and experimental samples.

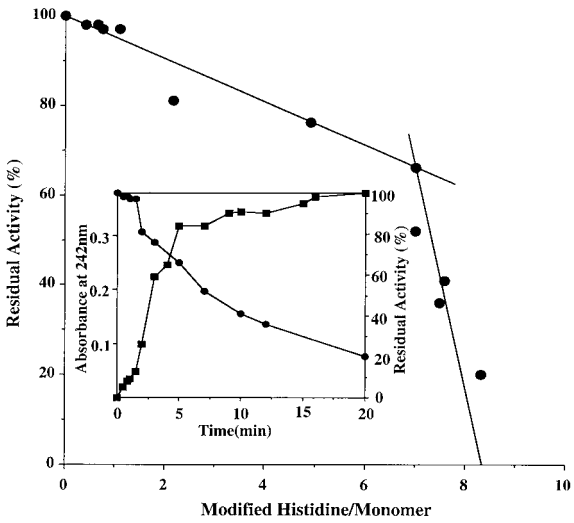

FIG. 5. Correlation between the number of histidine residues modified per monomer of OSB-CoA synthetase and the extent of residual activity. OSB-CoA synthetase (5 μM) was incubated with 300 μM DEP in 20 mM HEPES buffer at pH 7.0 in a total volume of 1.2 ml. The percent residual activity was plotted against the number of modified histidine residues per enzyme monomer, the line was extrapolated to the *x* axis, and the total number of modified histidine residues was determined. The insert shows the correlation between the extent of inactivation and the extent of modification. The amount of enzyme and the conditions were the same as those described above. The percent residual activity (●) and the A_{242} (■) were plotted as a function of time.

Reversibility of DEP inactivation of OSB-CoA synthetase with hydroxylamine. One of the characteristics of DEP-modified histidine residues is decarbethoxylation (reversal) upon the addition of hydroxylamine (18). To determine whether DEP-modified OSB-CoA synthetase was decarbethoxylated, the enzyme was treated with DEP and the increase in A_{242} was monitored. Maximum absorbance was attained in 15 min (Fig. 3). At this time, when hydroxylamine was added, there was an immediate decrease in optical density (data not shown).

To establish whether decarbethoxylation of the enzyme by hydroxylamine resulted in complete restoration of enzymatic activity, the enzyme was treated with 300 μM DEP and the activity was determined at timed intervals (Fig. 4). As shown by Fig. 4, the enzyme showed progressive loss of activity with time. After 15 min, when the enzyme had lost 80% of its original activity, hydroxylamine (800 mM) was added to decarbethoxylate the *N*-carbethoxyhistidine. The reaction mixture was dialyzed extensively, and the OSB-CoA synthetase activity was determined. As shown by Fig. 4, hydroxylamine treatment resulted in complete reactivation of the enzyme activity.

Correlation between the number of histidine residues modified and loss of catalytic activity. The number of histidine residues modified can be estimated from the change in A_{242} on treatment with DEP (20). Figure 5 (inset) shows the correlation between the extent of histidine modification and enzyme inactivation on treatment with DEP over an incubation period of 20 min. The increase in A_{242} nm represents the extent of modification, while the inactivation of the enzyme is expressed as the percent residual activity. After 3 min of incubation with DEP, when about 60% of the total histidine residues were modified, there was no significant loss of activity (76% of the initial activity was retained). At about 5 min, when the modification was 85%, the enzyme still retained 66% of its original activity. However, after 5 min, the last 15% of the histidine residues were modified, resulting in the loss of most of the remaining activity. Thus, on the basis of reactivity with DEP, two populations of histidine residues can be distinguished in OSB-CoA synthetase. There is a population of nonessential histidine residues that react at a faster rate than a second population of essential histidine residues; modification of the latter population results in inactivation of the enzyme. This argument is further strengthened when the number of histidine residues modified per monomer of the enzyme is plotted against the residual activity (Fig. 5). As shown by Fig. 5, about eight histidine residues are modified per monomer of the enzyme. It is further evident that when the first seven histidine residues are modified, the enzyme loses only 34% of its activity. However, when the eighth histidine residue is modified, the enzyme loses 80% of its original activity. By extrapolation of Fig. 5; it can be calculated that when 8.3 histidine residues are modified, the enzyme is completely inactivated, suggesting that the eighth histidine residue plays a critical role in enzymatic activity.

Determination of the number of essential histidine residues by kinetic analysis. The number of essential histidine residues in OSB-CoA synthetase was determined by kinetic analysis (13, 23). The inactivation process may be represented as follows:

$$\text{OSB-CoA synthetase} + n\text{DEP} \rightarrow \text{OSB-CoA synthetase-DEP}n \quad (2)$$

where OSB-CoA synthetase-DEP*n* is the inactivated enzyme and *n* represents the number of moles of DEP required to inactivate the essential histidine residues in OSB-CoA synthetase. The rate of inactivation may be expressed as follows:

$$-d[\text{OSB-CoA synthetase}]/dt = K[\text{OSB-CoA synthetase}][\text{DEP}]^n \quad (3)$$

If [DEP]>>[OSB-CoA synthetase], equation 3 can be integrated into the following form:

$$\log K_{obs} = n\log[\text{DEP}] + \log K \quad (4)$$

A plot of log K_{obs} against log [DEP] will have a slope equal to *n*. The order of the inactivation was determined experimentally by plotting K_{obs} as a function of the DEP concentration.

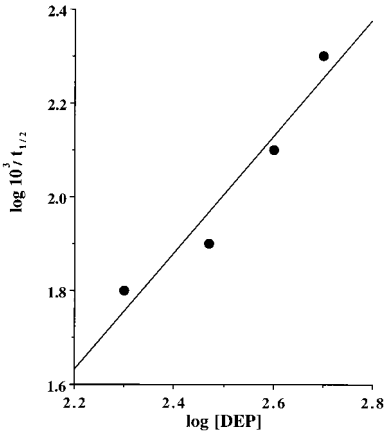

FIG. 6. The apparent order of the inactivation of OSB-CoA synthetase. The conditions were the same as those described in the legend to Fig. 2. The slope of this line, 1.2/mol of enzyme monomer, indicates that one histidine residue is essential for the enzyme activity.

For convenience, the K_{obs} value in equation 4 may be replaced by the reciprocal of the half-time of inactivation. This introduces a constant having no effect on the slope (13). Hence, the above equation may now be modified as follows:

$$\log 10^3/t_{1/2} = n\log [DEP] + \log K \quad (5)$$

The plot of log $10^3/t_{1/2}$ against log [DEP] (Fig. 6) results in a straight line with a slope of $n = 1.2$ for the enzyme monomer. This suggests that one histidine residue is essential for the catalytic activity of OSB-CoA synthetase.

Protection against DEP inactivation by OSB-CoA synthetase ligands. The effects of various OSB-CoA synthetase ligands on the inactivation of the enzyme by DEP are shown in Fig. 7. When the enzyme was incubated for 20 min with DEP, it lost 80% of the original activity. However, if the enzyme was preincubated with 3 mM OSB, 56% of the activity was retained. When 3 mM ATP or 3 mM ATP plus 3 mM Mg^{2+} was used, the enzyme retained 70% of the original activity. The enzyme was completely protected from inactivation on preincubation with OSB, ATP, and Mg^{2+} together.

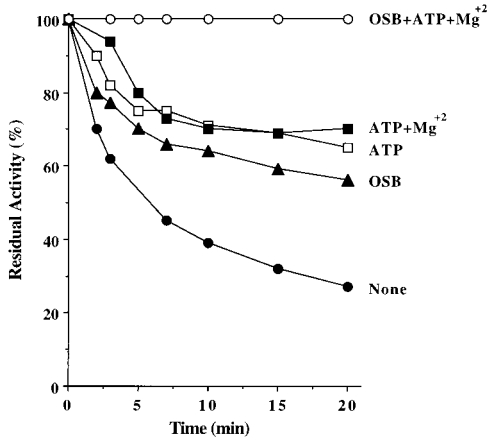

FIG. 7. Protection against DEP inactivation of OSB-CoA synthetase. OSB-CoA synthetase (1 μM) was preincubated with 3 mM either OSB, ATP, ATP plus Mg^{2+}, or OSB plus ATP plus Mg^{2+} in 20 mM HEPES buffer, pH 7.0, in a total volume of 300 μl at 30°C for 10 min, and 300 μM DEP was added. At the specified times, 25-μl aliquots were withdrawn and assayed for activity as described in the legend to Fig. 2.

Effect of His^{341}→Ala mutation on the kinetic properties of OSB-CoA synthetase. From the results presented above, it is evident that ATP can protect 70% of the enzyme from DEP inactivation. The results further suggest that modification of a single histidine residue is sufficient to inactivate the enzyme. Previously, by sequence analysis of the *menE* gene, which encodes OSB-CoA synthetase, an ATP binding motif has been identified (21). Since a histidine is present in the motif, it was thought that modification of this histidine (located at position 341) might be responsible for the loss of enzymatic activity. Hence, by site-directed mutagenesis, the His codon (CAT) was replaced with an Ala codon (GCC). The altered enzyme was overexpressed and purified, and its properties were compared to that of the native enzyme. The activity of the purified mutant enzyme was only 35% of that of the native enzyme. Further, the K_m for ATP of the mutant enzyme increased to 400 μM, compared to 73.5 μM for the native enzyme. This 5.4-fold increase in the K_m compared to that of the native protein suggests that the His→Ala mutant enzyme has a greatly decreased affinity for ATP.

DISCUSSION

Site-specific chemical modification and characterization of the modified product are versatile tools for delineating the requirements for the catalytic activity of enzymes. The use of DEP for chemical modification of the histidine residues in numerous proteins is well documented (6, 8, 24, 27). Histidine has been shown to be involved in phosphorylation and dephosphorylation in enzymes such as succinyl-CoA synthetase (1). Because of its importance, in this study, the histidine residues involved in the catalysis by the enzyme OSB-CoA synthetase have been explored by using a combination of chemical modification and kinetic analysis.

Kinetic analysis of the time-dependent inactivation of OSB-CoA synthetase by DEP shows that it is a bimolecular reaction which follows pseudo-first-order kinetics. The increase in A_{242} on treatment with DEP and its reversal by hydroxylamine provide strong evidence for the formation of carbethoxyhistidine. Possible disubstitution on the imidazole ring and carboxyethylation at both the N_1 and N_3 positions are excluded, since such a derivative would have spectral properties different from those of the monosubstituted derivative and will not regenerate histidine on treatment with hydroxylamine. Formation of a dicarboxyethylated imidazole derivative by ring cleavage is also excluded, since such a derivative would not be reversible by hydroxylamine (18). The CD spectrum of the DEP-modified enzyme was identical to that of the native enzyme, suggesting that the enzyme inactivation was not due to nonspecific secondary structural changes in the protein.

Although DEP can also react with other amino acid side chains, such as those of tyrosine, cysteine, and lysine, only ethoxyformyl tyrosine can be decarbethoxylated by hydroxylamine (14). However, *o*-carbethoxylation of tyrosine would have resulted in a dramatic decrease in the absorption spectrum at 278 nm. Examination of the absorption spectra (Fig. 3) reveals no such changes, thus ruling out the possibility of tyrosine modification (14, 18).

Modification of cysteine and lysine can be ruled out, since ethoxyformyl cysteine and ethoxyformyl lysine residues cannot be decarbethoxylated and are stable on treatment with hydroxylamine (14, 18). Further, the complete reversal of activity in the DEP-inactivated enzyme on treatment with hydroxylamine

rules out the possibility of carbethoxylation of any amino acid other than histidine.

The primary amino acid sequence of OSB-CoA synthetase deduced from the complete nucleotide sequence of the *menE* gene revealed the presence of 12 histidine residues per monomeric subunit of the enzyme (21). However, in this study, it has been calculated that only eight histidine residues are modified (Fig. 5). Hence, the remaining four residues must be inaccessible for DEP modification. Among the eight histidine residues modified, only one was found to be essential for the activity of the enzyme.

Studies of protection against DEP inactivation were performed by preincubating the enzyme with either OSB, ATP, ATP plus Mg^{2+}, or OSB, ATP and Mg^{2+} together before treatment with DEP. The enzyme was partially protected from DEP inactivation by ATP, ATP plus Mg^{2+}, or OSB, while it was completely protected by the combination of OSB, ATP, and Mg^{2+}. Protection studies were not performed with CoA, since the sulfhydryl group would react with DEP. However, complete protection from inactivation by the combination of OSB, ATP, and Mg^{2+} in the absence of CoA suggests the possibility that the histidine residue is involved in the formation of the presumptive adenylated intermediate in the reaction. Our kinetic experiments suggest that one histidine residue is essential for enzyme activity. Since, among the substrates tested for protection from DEP inactivation, ATP provided 70% protection, it was thought that the critical histidine might be located at the ATP binding motif.

There are two motifs that have been identified in ATP-utilizing enzymes, referred to as Walker A and Walker B (25, 26). The Walker A motif (G-X-X-X-X-G-K) has been identified in OSB-CoA synthetase as G^{338}-E-M-H^{341}-N-G-K^{344} (21). The presence of histidine in the variable region of this motif is reminiscent of succinyl-CoA synthetase, where His^{246}, the third amino acid upstream of the Walker A motif, has been found to be phosphorylated (1). It was thought that this histidine might be the one involved in the DEP inactivation of OSB-CoA synthetase. Hence, by site-directed mutagenesis, this histidine (His^{341}) was replaced with alanine. The resulting protein, when overexpressed, purified, and assayed, showed greatly diminished activity. Further, the K_m for ATP of the mutant enzyme increased fivefold. This increase in K_m is consistent with the notion that the critical histidine residue is involved in the binding of ATP. The partial protection against DEP inactivation provided by OSB alone and the complete protection achieved when it was present in combination with ATP might be due to the location of the OSB binding site in close proximity to the ATP binding site.

However, definite confirmation that His^{341} is the histidine residue involved in DEP inactivation must await site-directed mutational studies on the remaining 11 histidine residues in OSB-CoA synthetase.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant GM 50262 from the National Institute of Health.

We thank Gordon Kresheck for the CD spectra and James Erman for invaluable assistance with analysis of the data and critical reading of the manuscript.

REFERENCES

1. **Beck, D., M. E. Spencer, and J. R. Guest.** 1985. The primary structure of the succinyl-CoA synthetase of *Escherichia coli*. Biochemistry **24:**6245–6252.
2. **Bentley, R.** 1990. The shikimate pathway—a metabolic tree with many branches. Crit. Rev. Biochem. Mol. Biol. **25:**307–384.
3. **Bentley, R., and R. Meganathan.** 1982. Biosynthesis of vitamin K (menaquinone) in bacteria. Microbiol. Rev. **46:**241–280.
4. **Bentley, R., and R. Meganathan.** 1983. Vitamin K biosynthesis in bacteria—precursors, intermediates enzymes, and genes. J. Nat. Prod. (Lloydia) **46:** 44–49.
5. **Bhattacharyya, D. K., U. Bandyopadhyay, and R. K. Banerjee.** 1993. Chemical and kinetic evidence for an essential histidine residue in the electron transfer from aromatic donor to horseradish peroxidase compound I. J. Biol. Chem. **268:**22292–22298.
6. **Blanke, S. R., and L. P. Hager.** 1990. Chemical modification of chloroperoxidase with diethylpyrocarbonate: evidence for the presence of an essential histidine residue. J. Biol. Chem. **265:**12454–12461.
7. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
8. **Cheng, K. C., and T. Nowak.** 1989. A histidine residue at the active site of avian liver phosphoenolpyruvate carboxykinase. J. Biol. Chem. **264:**19666–19676.
9. **Church, F. C., R. L. Lundblad, and C. M. Noyes.** 1985. Modification of histidines in human prothrombin: effect on the interaction of fibrinogen with thrombin from diethyl pyrocarbonate-modified prothrombin. J. Biol. Chem. **260:**4936–4940.
10. **Dansette, P., and R. Azerad.** 1970. A new intermediate in naphthoquinone biosynthesis. Biochem. Biophys. Res. Commun. **40:**1090–1095.
11. **Gomi, T., and M. Fujioka.** 1983. Evidence for an essential histidine residue in *s*-adenosylhomocysteinase from rat liver. Biochemistry **22:**137–143.
12. **Kwon, O., D. K. Bhattacharyya, and R. Meganathan.** 1996. Menaquinone (vitamin K_2) biosynthesis: overexpression, purification and properties of *o*-succinylbenzoyl-coenzyme A synthetase from *Escherichia coli*. J. Bacteriol. **178:**6778–6781.
13. **Levy, H. M., P. D. Leber, and E. M. Ryan.** 1963. Inactivation of myosin by 2,4-dinitrophenol and protection by adenosine triphosphate and other phosphate compounds. J. Biol. Chem. **238:**3654–3659.
14. **Lundblad, R. L.** 1995. Techniques in protein modification. CRC Press, Inc., Boca Raton, Fla.
15. **Meganathan, R.** 1996. Biosynthesis of the isoprenoid quinones menaquinone (vitamin K_2) and ubiquinone (coenzyme Q), p. 642–656. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
16. **Meganathan, R., and R. Bentley.** 1979. Menaquinone (vitamin K_2) biosynthesis: conversion of *o*-succinylbenzoic acid to 1,4-dihydroxy-2-naphthoic acid by *Mycobacterium phlei* enzymes. J. Bacteriol. **140:**92–98.
17. **Melchior, W. B., Jr., and D. Fahrney.** 1970. Ethoxyformylation of proteins. Reaction of ethoxyformic anhydride with α-chymotrypsin, pepsin, and pancreatic ribonuclease at pH 4. Biochemistry **9:**251–258.
18. **Miles, E. W.** 1977. Modification of histidyl residues in proteins by diethylpyrocarbonate. Methods Enzymol. **47:**431–442.
19. **Morjana, N. A., and G. A. Scarborough.** 1989. Evidence for an essential histidine residue in the *Neurospora crassa* plasma membrane H^+-ATPase. Biochim. Biophys. Acta **985:**19–25.
20. **Ovadi, J., S. Libor, and P. Elodi.** 1967. Spectrophotometric determination of histidine in proteins with diethylpyrocarbonate. Acta Biochem. Biophys. Acad. Sci. Hung. **2:**455–458.
21. **Sharma, V., M. E. S. Hudspeth, and R. Meganathan.** 1996. Menaquinone (vitamin K_2) biosynthesis: localization and characterization of the *menE* gene from *Escherichia coli*. Gene **168:**43–48.
22. **Shaw, D. J., E. C. Robinson, R. Meganathan, R. Bentley, and J. R. Guest.** 1983. Recombinant plasmids containing menaquinone biosynthetic genes of *Escherichia coli*. FEMS Microbiol. Lett. **17:**63–67.
23. **Takeuchi, M., N. Asano, Y. Kameda, and K. Matsui.** 1986. Chemical modification by diethylpyrocarbonate of an essential histidine residue in 3-ketovalidoxylamine A C-N lyase. J. Biochem. **99:**1571–1577.
24. **Topham, C. M., and K. Dalziel.** 1986. Chemical modification of sheep-liver 6-phosphogluconate dehydrogenase by diethylpyrocarbonate: evidence of an essential histidine residue. Eur. J. Biochem. **155:**87–94.
25. **Traut, J. W.** 1994. The functions of consensus motifs of main types of peptide segments that form different types of nucleotide-binding sites. Eur. J. Biochem. **222:**9–19.
26. **Walker, W., M. Saraste, M. J. Runswick, and N. J. Gay.** 1982. Distally related sequences in the α and β subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a nucleotide binding fold. EMBO J. **8:**945–951.
27. **Youn, H. D., Y. I. Yim, K. Kim, Y. C. Hah, and S. O. Kang.** 1995. Spectral characterization and chemical modification of catalase-peroxidase from *Streptomyces* sp. J. Biol. Chem. **270:**13740–13747.