Involvement of Tryptophans at the Catalytic and Subunit-Binding Domains of Transcarboxylase†

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ABSTRACT: Transcarboxylase from *Propionibacterium shermanii* is a multisubunit enzyme. It consists of one central hexameric subunit to which six outer dimeric subunits are attached through twelve biotinyl subunits. Both the central and the outer subunits are multi-tryptophan (Trp) proteins, and each contains 5 Trps per monomer. The roles of the Trps during catalysis and assembly of the enzyme have been studied by using *N*-bromosuccinimide (NBS) oxidation as a probe. Modification of ~10 Trps of the total 90 Trps of the intact enzyme results in loss of activity. Both the substrates, viz., methylmalonyl-CoA and pyruvate, afford protection (~50%) against inactivation caused by NBS. Analyses of tryptic peptide maps and intrinsic fluorescence studies have indicated that modification of 10 Trps of the whole enzyme does not cause extensive conformational changes. Therefore, the Trps appear to be essential for catalytic activity. NBS modification of the individual subunits at pH 6.5 has demonstrated differential reactivity of their Trps. Modification of the exposed/reactive Trps of either one of the subunits significantly affects the subunit assembly with the complementary unmodified subunits to form active enzyme. It is proposed that Trps are involved at the subunit-binding domains of either the central or the outer subunit of transcarboxylase, in addition to those critical for catalysis.

Transcarboxylase (EC 2.1.3.1) from *Propionibacterium shermanii* is a multisubunit biotinyl enzyme with a complex quaternary structure (Green et al., 1972; Wood & Zwolinsky, 1976). It consists of one hexameric central (12S) subunit to which 6 dimeric outer (5S) subunits are linked through 12 biotinyl (1.3S) subunits (Figure 1). The enzyme catalyzes the transfer of a carboxyl group from methylmalonyl-CoA to pyruvate, forming propionyl-CoA and oxalacetate. A total of 12 CoA ester substrate sites, 2 per monomer, are located on the central subunit (Poto et al., 1978), whereas the outer subunit contains the keto acid substrate sites (Chuang et al., 1975). The biotinyl subunit contains 123 amino acid residues, and the biotin prosthetic group is in amide linkage with the ϵ-NH_2 of Lys-89 (Maloy et al., 1979). A large body of information is available on the interacting sequences and essential residues of the biotinyl subunit in relation to the overall assembly and catalysis of transcarboxylase (Ahmad et al., 1975; Kumar et al., 1982a,b; Wood & Kumar, 1985; Wood et al., 1987; Samols et al., 1988).

The intact enzyme can be dissociated into individual subunits (Figure 1), and the isolated subunits can be reassembled to yield active enzyme (Wood et al., 1975; Bahler et al., 1981). During assembly, there are specific interactions involving (i) the outer and the biotinyl subunits and (ii) the central subunit and the biotinyl subunits. The intrinsic fluorescence of both the outer and central subunits is enhanced as a result of interactions with the biotinyl subunit and its various functional domains (Kumar & Wood, 1982; Kumar et al., 1982a,b). The biotinyl subunit does not contain tryptophans and hence is nonfluorescent. Both pyruvate (substrate) and oxalate (inhibitor) quench the intrinsic fluorescence of the outer subunit, whereas both malonyl-CoA and methylmalonyl-CoA (substrates) quench the intrinsic fluorescence of the central subunit

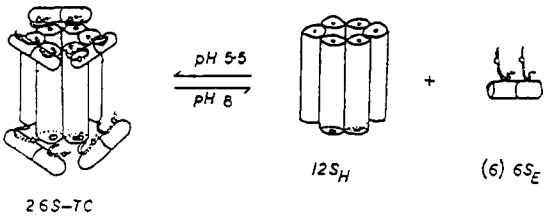

FIGURE 1: Schematic representation of the 26S form of transcarboxylase (M_r 1.2 × 10^6). At pH 8, the outside 5S subunits with the biotinyl 1.3S subunits still attached (designated 6S) dissociate from the central 12S subunit. The biotin is represented as a hexagon.

(Kumar et al., unpublished results). These studies indicate that one or more tryptophans may possibly be involved both at the subunit-binding domains and at active-site regions located on the outer and central subunits, respectively. In the present study, we have further characterized the tryptophanyl residues essential for assembly and catalysis of transcarboxylase by selective *N*-bromosuccinimide (NBS)[1] oxidation of the tryptophans of both the intact enzyme and the isolated outer and the central subunits.

Materials and Methods

Materials

N-Acetyltryptophanamide, methylmalonyl-CoA, sodium pyruvate (dimer free), and 8-anilino-1-naphthalenesulfonate ammonium salt were purchased from Sigma; *N*-bromosuccinimide was from Aldrich; sodium dodecyl sulfate and urea (sequanal grade) were from Pierce; cellulose phosphate was from Whatman; and sodium silico tungstate was from EM

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[1] Abbreviations: DTT, dithiothreitol; NBS, *N*-bromosuccinimide; ANS, 8-anilino-1-naphthalenesulfonate; PMSF, phenylmethanesulfonyl fluoride; Trp, tryptophan; SDS, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography; DNPS-Cl, 2,4-dinitrophenylsulfenyl chloride.

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Table I: Modification of Trps of Transcarboxylase (TC), Effect of Substrates on the Modification, and Activity of the Modified Enzyme

sample	max modification of Trps (no. of Trps modified)[a]		modification with 60 μM NBS		
	denatured	native	no. of Trps modified	activity[b] (units/mg)	inhibition (%)
TC	80–90	40	10	0	100
TC + methylmalonyl-CoA (740 μM)	80–90	33	3–5	10.7	53
TC + pyruvate (720 μM)	80–90	32	3–5	12.3	46

[a] The Trp content is expressed on the basis of M_r of TC of 1.2×10^6. [b] About 5% variation was observed between different transcarboxylase preparations with specific activity ranging from 20 to 35. TC was 0.47 μM.

Science. The other chemicals used were of reagent grade.

Methods

Preparation of Transcarboxylase and the Subunits. Transcarboxylase was isolated from *P. shermanii* (52W strain) and assayed by the method of Wood et al. (1969). The outer subunit complexed with two biotinyl subunits (designated 6S subunit) and the central subunit in hexameric form were isolated by stepwise dissociation of transcarboxylase bound to a cellulose phosphate column (Bahler et al., 1981). The isolated subunits were found to be homogeneous as evidenced by SDS–PAGE.

N-Bromosuccinimide (NBS) Oxidation of Transcarboxylase and Its Subunits. For NBS oxidation, appropriate protein solutions (1–2 mg/mL) were prepared in 250 mM potassium phosphate buffer, pH 6.5, containing 0.1 mM DTT and 0.1 mM PMSF. The progress of the oxidation reaction was monitored at 280 nm by using a Carl Zeiss spectrophotometer. A stock solution of NBS (4.5 mM) was used for modification studies. The number of tryptophans (Trp) oxidized was determined by the method of Spande and Witkop (1967a). For the estimation of Trp, the following molecular weights were used: 1.2×10^6, transcarboxylase; 1.4×10^5, the outer dimeric 6S subunit; and 3.6×10^5, the central hexameric subunit.

Fluorescence Measurements. Fluorescence was recorded by using an Aminco-Bowman spectrofluorometer equipped with a temperature-control system. All measurements were recorded at 25 °C. The emission spectra of transcarboxylase and its subunits were recorded at excitation wavelengths reported earlier (Kumar & Wood, 1982). The emission spectra were obtained by scanning from 300 to 400 nm with the spectrometer in the ratio mode and an emission band-pass of 8 nm and excitation band-pass of 2 nm. The emission intensity values were corrected for self-absorption of the protein and the reagent (McClure & Edelman, 1967) and for the inner filter effect (Hélène et al., 1969).

Reconstitution of Subunits To Form Active Transcarboxylase. The native and the modified subunits were reconstituted according to the method described previously (Wood et al., 1975; Bahler et al., 1981). The activity of the reconstituted transcarboxylase was determined and expressed as μmol of oxalacetate min^{-1} mg^{-1} of the central 12S subunit.

Electron Microscopy. Electron microscopy was performed with a JEM-100C (JEOL) instrument equipped with an anticontamination device. The reconstituted samples were processed for microscopy as described previously (Kumar et al., 1982a).

Trypsinization of Transcarboxylase. About 20 nmol of transcarboxylase in 250 mM sodium acetate, pH 5.5, containing 0.1 mM DTT and 0.1 mM PMSF was oxidized (20 min at 25 °C) with 300 μM final concentration of NBS. The reaction was stopped by the addition of a 10-fold molar excess of *N*-acetyltryptophanamide and dialyzed against 100 mM NH_4HCO_3, pH 8.0, at 4 °C for 48 h prior to trypsinization.

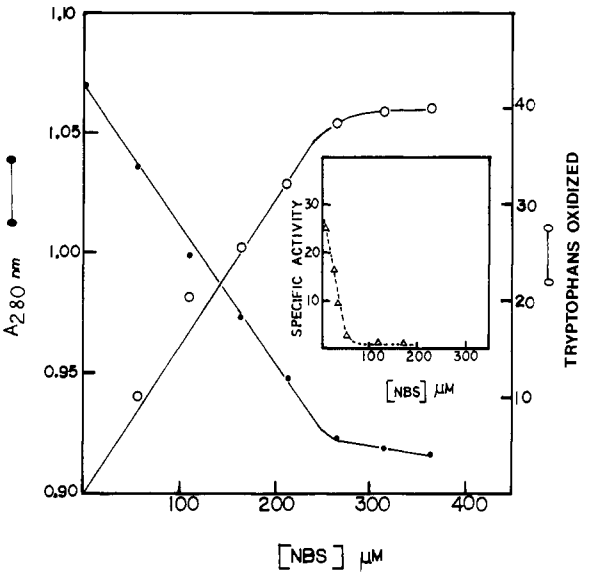

FIGURE 2: Correlation of the oxidation of the Trp (○), absorbance at 280 nm (●), and (inset) the activity of transcarboxylase (△) with the concentration of NBS. The modification was carried out at pH 6.5 with 0.92 μM transcarboxylase.

TPCK-treated trypsin was added at a protein to trypsin ratio of 100:1 and incubated at 37 °C for 24 h. Unmodified transcarboxylase was processed similarly for trypsinization.

HPLC Analysis. A Du Pont HPLC system along with a C-8 (Synchropak) reverse-phase column was used for the tryptic peptide mapping of both the unmodified and the NBS-modified transcarboxylase. The following solvent system was used: solvent A was 0.1% trifluoroacetic acid in H_2O; solvent B was 0.1% trifluoroacetic acid in CH_3CN.

Results

Determination of Total and Exposed Trps of Transcarboxylase. The total number of Trps of the intact transcarboxylase was determined by spectrophotometric titration with NBS of the enzyme denatured in 8 M urea. From analysis of several enzyme preparations the Trp content was found to vary between 80 and 90 (Table I). This variation may arise from small differences in the number of outer subunits bound to the central subunit in various enzyme preparations. Among several enzyme preparations tested in the absence of urea, consistently about 40 Trps/mol of TC were modified at pH 6.5, as shown in Figure 2. A similar value was obtained at pH 5.5 (data not shown). The remaining residues are presumably less reactive and may be present either in the interior of the protein or at the subunit interface regions and thus are not accessible for modification. The number of Trps modified was proportional to the concentration of NBS until about 37 residues were modified (Figure 2).

Determination of the Effect of Modification of Trps of Transcarboxylase on Its Activity both in the Presence and in the Absence of Substrates. NBS inhibits transcarboxylase

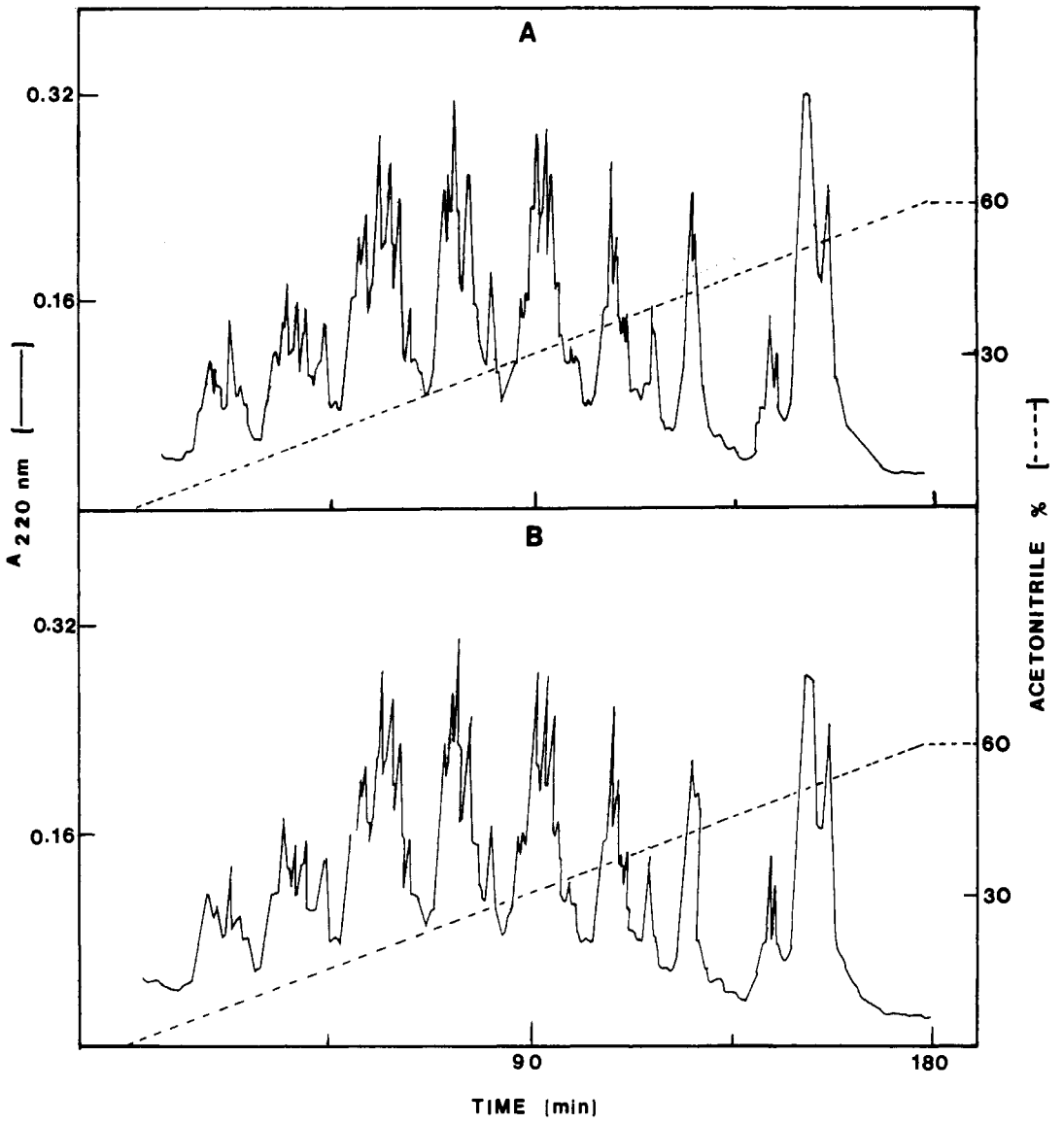

FIGURE 3: High-performance liquid chromatography analysis of the tryptic digests of (A) native and (B) NBS (60 μM) modified transcarboxylase. The peptides were eluted with a linear gradient of 0%–60% acetonitrile containing 0.1% trifluoroacetic acid, and the elution was monitored at 220 nm.

activity as shown in Figure 2 (inset). More than 90% of the activity is lost with the addition of 60 μM NBS (final concentration). At this concentration of NBS, about 10 Trps are modified (Table I, Figure 2). Inactivation of transcarboxylase by NBS may be due to modification of Trp essential for activity at the substrate-binding site. To test this possibility, NBS modification of transcarboxylase was carried out both in the presence and in the absence of methylmalonyl-CoA, and the number of modified Trps in the enzyme was determined. As seen in Table I, about 33 Trps were modified in the presence of either methylmalonyl-CoA (740 μM) or pyruvate (720 μM). At 60 μM NBS, only 3–5 Trps were modified in the presence of substrates. About 47% of the activity is protected by methylmalonyl-CoA (740 μM) against inhibition by NBS (60 μM) (Table I). Pyruvate (720 μM) also afforded protection to a similar extent. Therefore, both the pyruvate- and the methylmalonyl-CoA-binding domains of transcarboxylase may contain Trps that are essential for the catalytic activity.

Demonstration That Extensive Conformational Change Has Not Occurred during NBS Modification of Transcarboxylase. The observed loss of activity with NBS oxidation of the Trps of transcarboxylase may be either due to modification of essential Trps or a consequence of extensive conformational change in the enzyme. If gross conformational changes have occurred, then the susceptibility of NBS-treated transcarboxylase toward trypsin action should be different from that of the unmodified enzyme. In Figure 3, HPLC tryptic peptide profiles, derived from both native and NBS (60 μM) treated transcarboxylase, are presented. No significant differences in the two profiles in terms of number of peptides generated

Table II: Intrinsic Fluorescence Properties of Native and NBS[a]-Modified Transcarboxylase, the Central 12S Subunit, and the Outer 6S Subunit

sample	rel fluorescence intensity[b]	quenching (%)	emission max (nm)
transcarboxylase	12.4		339
+NBS	10.5	15.3	339
central 12S subunit	0.98		335
+NBS	0.82	16.3	335
outer 6S subunit	2.59		339
+NBS	2.16	16.6	339

[a] The final NBS concentration in the reaction mixture was 60 μM. The other details were as shown in Figure 2. Appropriate corrections for the inner filter effect, self-absorption, and dilution were made. [b] The relative fluorescence intensity corresponds to the emission maximum wavelength of the respective proteins.

Table III: Modification of Trps of the Central 12S Subunit and the Outer 6S Subunit and Effect on Activity of Reconstituted Enzyme

	max modification of Trps (no. of Trps modified)[a]		modification with 60 μM NBS		
				reconstitution[b]	
sample	denatured	native	no. of Trps modified	activity (units/mg)	inhibition (%)
12S subunit	5.0	2.8	0.8	0	100
6S subunit	4.8	2.0	1.0	3	96[c]

[a] The Trp content for the 12S subunit is expressed per monomer (M_r 0.6 × 10^5) and for the 6S subunit per monomer (M_r 0.72 × 10^5). [b] The reconstitution is performed by using modified subunit with the complementary unmodified subunit. [c] Activity of enzyme reconstituted from unmodified 12S and 6S subunit was 77.3 units/mg of 12S subunit. With modified 12S subunit and unmodified 6S subunit there was no activity, and with the modified 6S subunit and unmodified 12S subunit the activity was 3.0. There was no activity with both modified 6S and 12S subunits.

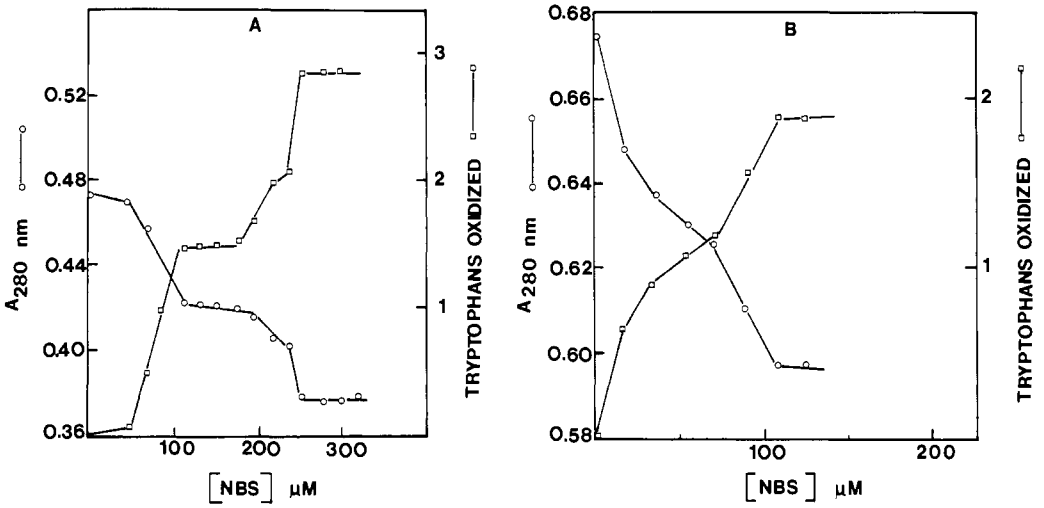

FIGURE 4: Correlation of the number of Trps oxidized by NBS (□) and the absorbance at 280 nm (○): (A) central 12S subunit (1.31 μM); (B) outer 6S subunit (4.8 μM). The values of Trps are per monomer of respective subunits.

and their respective elution times are seen. On the basis of this criterion, it appears that NBS modification of the most exposed Trp has not caused extensive conformational changes in transcarboxylase. Further support for this view has come from a comparative analysis of the intrinsic fluorescence of the native and NBS (60 μM) modified transcarboxylase as shown in Table II. If extensive conformational change has occurred due to modification, then both large changes in the fluorescence and/or a shift in the emission maximum will occur. But on modification of the most reactive 10 Trps, the fluorescence intensity of transcarboxylase was quenched slightly (~15%) without any observable change in the emission maximum. Likewise, there was only small quenching (~16%) of the intrinsic fluorescence of both the outer and central subunits as a result of NBS modification, and there was no change in the emission maximum. Thus, it appears that NBS (60 μM) modification of Trps has not caused an extensive alteration in the structure of transcarboxylase.

Determination of the Total and Exposed Trps of the Central and Outer Subunits. The total number of Trps of the central and outer subunits of transcarboxylase was determined by using NBS oxidation of the corresponding urea-denatured proteins. As shown in Table III there are 5 Trps each per monomer of the central and outer subunits, respectively. This is in close agreement with the value reported by Zwolinski et al. (1977). In the native state, at pH 6.5 only 3 Trps per monomer of the central subunit were modified of 5, whereas 2 of the 5 Trps per monomer of the outer 6S subunit were oxidized (Figure 4 and Table III). There is differential reactivity among the 5 Trps per monomer of the central subunit (Figure 4A); initially 1.5 Trps per monomer were modified between 100 and 200 μM NBS. At a higher concentration of NBS (250 μM) 1.5 more residues were modified (Figure 4A). Two Trps remained unreactive at pH 6.5. With the outer 6S subunit, the modification of Trps occurs in two stages (Figure 4B); the most exposed residue was modified first with 50 μM NBS, and there was a subsequent modification of a second Trp at higher NBS concentrations (100–120 μM). The three remaining Trps were not reactive at pH 6.5.

Effect of NBS Modification of Tryptophans of the Central 12S and 6S Subunits on Assembly To Form Active Transcarboxylase. The isolated central 12S subunit and the 6S subunits can be combined to form active enzyme (Figure 1). To test if Trps are critical for assembly, the central and the outer 6S subunits were treated individually with 60 μM NBS; approximately 1 Trp per monomer of the subunits was modified (Table III). The reaction was stopped with addition of a large excess of *N*-acetyltryptophanamide and dialyzed to remove the reagents and byproducts. These modified subunits were then investigated by combination with the complementary unmodified and modified subunit under optimal conditions for assembly (Wood et al., 1975). The reaction mixtures were then (i) tested for activity (Table III) and (ii) examined by electron microscopy for the formation of complexes (Figure 5). Active complexes assembled from the native subunits are shown in Figure 5A. In the reconstituted transcarboxylase, a majority of the complexes were assembled with the outer subunits on only one face of the large central 12S subunit. Occasionally, outer subunits can be seen on both faces of the central subunit (Figure 5A). There is little or no complexing with the modified 12S subunit plus the native 6S subunit (Figure 5B), and the mixture has no activity (Table III). With the modified 6S subunit plus the native 12S subunit it was difficult to obtain a uniform distribution of the molecules; however, careful inspection does reveal the presence of some complexes of transcarboxylase (Figure 5C). The activity was greatly reduced (96% inhibition) compared to that of the preparation from unmodified subunits (Table III). Complexes do form when both the 12S and 6S subunits are modified, but the molecules appear mostly as aggregates (Figure 5D) and

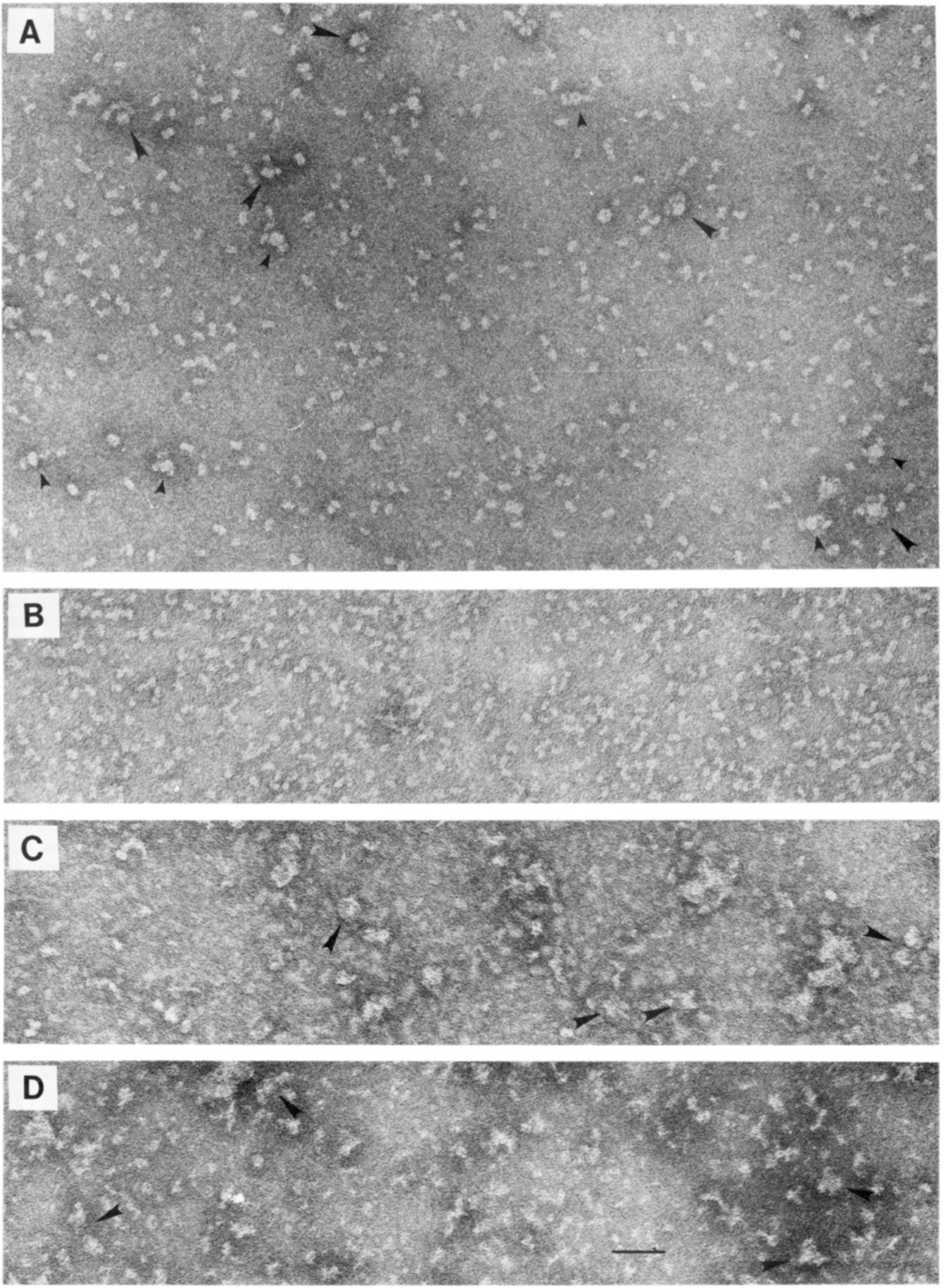

FIGURE 5: Representative electron micrographs derived from the reconstitution mixtures: (A) native 12S + native 6S; (B) NBS-treated 12S + native 6S; (C) native 12S + NBS-treated 6S; (D) NBS-treated 12S + NBS-treated 6S. For modification the following final concentrations of the proteins and NBS were used: 12S, 1.31 μM; 6S, 4.8 μM; NBS, 60 μM. Appropriate samples of unmodified and modified subunits were reconstituted in a molar ratio of 1:12 between the central 12S subunit and the outer 6S subunits, respectively. Examples of the native-type complexes are indicated by large arrows. The small arrows indicate a few unusual examples of complexes in which a single outer subunit is seen on opposite faces of the central subunit. The magnification is 250000×.

there was no transcarboxylase activity (Table III).

Discussion

We have studied the role of Trps in the catalytic and subunit assembly processes of transcarboxylase. The concentration-dependent modification of Trps of both the intact enzyme and the isolated central and the outer subunits by NBS at pH 6.5 have been determined and the enzymic properties of the native and modified proteins compared. From these studies we have concluded (i) that certain Trps are essential for activity and (ii) that the subunit-binding domains of the outer and the central subunits may contain one or more Trps that are critical for proper assembly of the subunits to form active transcarboxylase.

Several lines of evidence support the idea that at least one Trp may be essential for the catalytic activity. They are as follows: (a) Transcarboxylase activity is abolished by NBS oxidation of its Trps. More than 90% of the activity is lost with the modification of about 10 residues of the 40 reactive Trps of native transcarboxylase at pH 6.5 (Table I, Figure 2). (b) Substrates like methylmalonyl-CoA and pyruvate have afforded partial protection ($\sim$50%) against the inactivation of transcarboxylase by NBS (Table I). Additionally, 3–5 of the 80–90 Trps of the intact enzyme are modified by 60 μM NBS in the presence of 740 μM methylmalonyl-CoA or 720 μM pyruvate, whereas under identical conditions about 10 Trps were modified in the absence of substrates (Table I). (c) Modification of transcarboxylase with NBS appears to be specific for Trps. NBS is known to modify other amino acids in addition to Trp but with greatly reduced reactivity (Schmir & Cohen, 1961). However, there is a nearly linear relationship between the decrease in absorbance of transcarboxylase with the consumption of NBS (Figure 2), suggesting that only Trps are modified under these conditions (Spande & Witkop, 1967b). (d) Inactivation of transcarboxylase is not due to extensive structural changes as a result of NBS modification. This conclusion is supported by the fact that the susceptibility of both the unmodified and NBS-modified transcarboxylase to trypsin action was found to be similar (Figure 3). Also, the oxidation of about 10 Trps of the transcarboxylase did not greatly alter the fluorescence emission properties (Table II); only 15% of the fluorescence was quenched without any apparent change in the emission maximum.

The inactivation of transcarboxylase by NBS modification of its reactive Trps may arise from changes in the hydrophobicity of either the active site or the substrate-binding pocket. It has been shown that NBS oxidation of Trp-177 of papain altered the hydrophobic character of the active site, and as a consequence the ionization behavior of a histidine residue at the active site was somewhat changed (Imoto et al., 1974). The oxidation of Trp-62 in hen egg white lysozyme caused a decrease in its binding affinity toward the substrate (Lowe & Whitworth, 1974). It is possible that NBS modification of Trps of transcarboxylase might have caused a significant change in the hydrophobicity of the specific subsites involving the substrates (pyruvate and methylmalonyl-CoA) and the biotin prosthetic group of the 1.3S subunit, which serves as the carboxyl carrier during catalysis. In the following paper, it has been shown that a single Trp of the outer subunit (Trp-73) is involved at the pyruvate-binding site (Kumar et al., 1988). Also, fluorescence studies with the central 12S subunit have suggested that Trp may also be present at or near the CoA-binding site (Kumar, unpublished results). The biotin-binding site on avidin has been shown to contain Trp, the modification of which is shown to drastically decrease the biotin-binding affinity (Green, 1963). On the basis of the above considerations, it is proposed that the reactive Trps of transcarboxylase are critical for the catalysis by serving to maintain the proper hydrophobicity of either the active site or the subsites involved in the binding of the substrates and the biotin prosthetic group. Chemical modification of residues affecting the hydrophobicity of one or more of the above functionally critical sites of transcarboxylase might eventually lead to its inactivation.

Previous studies (Kumar & Wood, 1982; Wood & Kumar, 1985) have suggested that possibly one or more Trps may be located at or near the subunit-binding domains of the central or the outer subunit. In this study, we have further established the following: (a) The modification of the exposed Trp of the central subunit drastically affects its reconstitution with the unmodified outer 6S subunit to form subunit complex (Figure 5B), and the mixture is enzymatically inactive (Table III). (b) The modification of the exposed Trp of the 6S subunit partly abolishes its ability to form subunit complexes with the central subunit (Figure 5C), and the reconstituted complex has a very low catalytic activity (Table III). These results favor the idea that Trps are involved at the subunit-binding domains of both the central and outer subunits of transcarboxylase.

The primary sequences of both the outer and the central subunits have been recently derived from their nucleotide sequences (Thornton et al., 1987; Samols et al., 1988). Among the five Trps of the outer subunit three Trps, viz., Trp-51, Trp-56, and Trp-73, are clustered together near the amino-terminal region, whereas the remaining Trps, viz., Trp-394 and Trp-440, are located near the carboxy terminal. Thus, the Trp content of the outer subunit reported in this study (5 per monomer) is in complete agreement with these sequencing studies. However, there is a discrepancy in the Trp content of the central subunit estimated in this study (5 per monomer) and those derived from nucleotide-sequencing studies (7 per monomer). It is possible that the central subunit is not completely denatured in 8 M urea solution, thus preventing certain Trps from being accessible to NBS modification and yielding lower estimates of the total Trps. It will be of great interest to identify the Trps of the outer and central subunits essential for the assembly and catalytic activity of transcarboxylase. Such information will be possible through systematic studies involving site-directed modification of the Trps of the subunits. Alternately, essential Trps could be identified by labeling Trps with 2,4-dinitrophenylsulfenyl (DNPS) chloride of isolated 5S, 12S, and 6S subunits and the intact transcarboxylase and comparing the incorporation of DNPS into specific peptides of the subunits and with the intact transcarboxylase. In the following paper (Kumar et al., 1988), using a similar labeling strategy, we have demonstrated and identified a single Trp involved at the pyruvate-binding site of transcarboxylase.

Registry No. TC, 9029-86-1; Trp, 73-22-3.

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Involvement and Identification of a Tryptophanyl Residue at the Pyruvate Binding Site of Transcarboxylase†

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ABSTRACT: Transcarboxylase (TC) from *Propionibacterium shermanii* consists of a central hexameric 12S subunit to which 6 outer dimeric 5S subunits are attached through 12 biotinyl 1.3S subunits. The enzyme catalyzes the transfer of a carboxyl group from methylmalonyl-CoA to pyruvate, forming oxalacetate and propionyl-CoA. The pyruvate binding site, located on the 5S subunit, was examined by monitoring the intrinsic fluorescence quenching accompanying the incremental addition of pyruvate to either TC or the 5S subunit. The quenching studies indicate that there are two binding sites for pyruvate with apparent dissociation constants of 0.23 and 1.25 mM for intact TC and of 0.18 and 1.20 mM for the outer 5S subunit. The microenvironment of the Trp(s) sensitive to pyruvate binding was analyzed by using the neutral quencher acrylamide. With TC, the fractional accessible fluorescence (f_a) was 0.64, whereas a f_a value of 0.56 was obtained in the presence of pyruvate. A 27% decrease in f_a was observed with the outer 5S subunit in the presence of pyruvate as compared to the free 5S subunit. By labeling the outer subunit in the absence of pyruvate with 2,4-dinitrophenylsulfenyl chloride (DNPS-Cl), a tryptic peptide containing DNPS-labeled Trp was isolated; the sequence was determined and identified with the amino-terminal residues 67-75 of the outer subunit that has been derived from DNA-sequencing studies. Trp-73 contained the DNPS label; its labeling was inhibited by pyruvate. A sequence comparison with other biotinyl enzymes shows that the sequence 67-75 is highly conserved. On the basis of these results, it is proposed that sequence 67-75 of the outer subunit may constitute a part of the pyruvate binding region of transcarboxylase.

Transcarboxylase from *Propionibacterium shermanii* consists of a central 12S subunit to which 6 outer 5S subunits are attached through 12 biotinyl 1.3S subunits [for review of the structure see references Wood and Zwolinsky (1976) and Wood and Kumar (1985)]. The biotinyl subunit has a biotin prosthetic group linked to the ϵ-NH_2 group of Lys-89 through an amide bond (Maloy et al., 1979). The enzyme catalyzes the transfer of a carboxyl group from methylmalonyl-CoA to pyruvate, forming oxalacetate and propionyl-CoA. This reaction consists of two partial reactions; the central 12S subunit catalyzes the first partial reaction in which a carboxyl group from methylmalonyl-CoA is transferred to the biotinyl subunit, forming propionyl-CoA and the carboxybiotinyl subunit. The second partial reaction is catalyzed by the outer 5S subunit, where the carboxyl group is transferred to pyruvate, forming oxalacetate, and the biotinyl subunit is regenerated (Chuang

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