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# Purification and Properties of Rat Liver Microsomal Stearyl Coenzyme A Desaturase

**(fatty acid desaturation/membrane-bound enzymes)**

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**ABSTRACT** **The terminal enzyme of the NADH-dependent stearyl coenzyme A desaturase system has been isolated from rat liver microsomes. This desaturase is a single polypeptide of 53,000 daltons containing 62% nonpolar amino-acid residues and one atom of non-heme iron. The purified protein forms high molecular weight aggregates that can be dispersed by detergent procedures. Desaturase activity requires NADH, stearyl coenzyme A, oxygen, lipid, and the three enzymes, cytochrome $b_5$ reductase (EC 1.6.2.2), cytochrome $b_5$, and desaturase. Cytochrome $b_5$ is the direct electron donor to the desaturase, which appears to utilize the iron in the oxidation-reduction sequence during desaturation of stearyl coenzyme A.**

The characterization of the liver microsomal stearyl coenzyme A desaturase system by workers in several laboratories (1–11), using spectral methods and partially purified components from hen and rat liver microsomes has shown that the oxygen- and NADH-dependent formation of oleyl coenzyme A involves cytochrome $b_5$ reductase (EC 1.6.2.2; NADH:ferricytochrome $b_5$ oxidoreductase), cytochrome $b_5$, and a terminal, cyanide-sensitive oxidase, the desaturase. The present report describes the isolation of the terminal component, the desaturase, from liver of rats induced for this enzyme. A sequence of extractions of contaminating proteins from microsomes, followed by solubilization of the desaturase by detergents and subsequent purification, results in a protein preparation that appears to be homogeneous by several criteria. It contains one molecule of non-heme iron, which appears to participate in stearyl coenzyme A desaturation in the presence of oxygen and reduced cytochrome $b_5$.

## METHODS AND MATERIALS

Sodium deoxycholate (Matheson, Coleman and Bell) was purified as described (12). Sephadex G-75 was obtained from Pharmacia, and Whatman DEAE–cellulose (DE-52), from Reeve Angel. Triton X-100 was the purified product of Packard Instrument Co. The [$^{14}$C]deoxycholate was obtained from Mallinckrodt Chemical Works, the NADH, stearyl coenzyme A, and bathophenanthroline sulfonate were products of Sigma Chemical Co., and [$^{14}$C]stearyl coenzyme A was a product of New England Nuclear. The egg lecithin was obtained from Supelco in a chloroform solution. We prepared clear liposomes by removing solvent in a nitrogen stream, adding 0.02 M Tris-acetate buffer, pH 8.1 (Tris buffer), to yield a suspension of 12.5 mg/ml, sonicating under nitrogen at 0° for 15–20 min at 40 mW, and centrifuging the slightly turbid suspension for 15 min at 100,000 × *g* to yield the clear liposome suspension, which was stored under nitrogen at 0° and used for no longer than 10 days.

Abbreviation: Tris buffer, Tris-acetate buffer (pH 8.1).

Cytochrome $b_5$ was prepared from either calf or rabbit liver microsomes by the detergent extraction procedure that yields the complete form of this heme protein (13). The proteins from the two species were indistinguishable as electron donors for the desaturase. The soluble catalytic fragment of cytochrome $b_5$ was prepared by tryptic digestion of cytochrome $b_5$ (13). Either rabbit or calf liver cytochrome $b_5$ reductase was prepared by the procedure originally described for isolation from rabbit liver (12). The preparation of the catalytic fragment of the reductase has also been described (14).

The method of Weber and Osborn (15) was used for acrylamide gel electrophoresis in 9% crosslinked gels and in the presence of 0.2% sodium dodecyl sulfate after preincubation for 1 hr in 1% sodium dodecyl sulfate and mercaptoethanol. Protein bands were stained by the procedure of Fairbanks *et al.* (16). Lipid-extractable phosphorus was measured by the method of Chen (17), protein by the procedure of Lowry *et al.* (18), and reducing sugar by the phenol–sulfuric acid method (19). Flavin determinations involved measurement of the fluorescence of a trichloroacetic acid extract or a solution of desaturase in 1% sodium dodecyl sulfate at 530 nm with excitation at 470 nm. A slight modification of the bathophenanthroline sulfonate procedure (20) was used for iron analysis. Initial electron spin resonance measurements were made with a Jeol model JES-M spectrometer at 100°K. Acid-labile sulfide was determined as described by King and Morris (21).

Amino-acid analysis was preformed by the technique of Spackman *et al.* (22), and cysteine content was determined as cysteic acid (23). Tryptophan and tyrosine were determined by the spectral procedures of Beaven and Holliday (24) in 1% sodium dodecyl sulfate. $NH_2$-terminal amino-acid analysis used the microdansyl technique after two sequences of subtractive Edman degradation (25).

*Assay Procedures.* The procedure of Jones *et al.* (4) with $^{14}$C-labeled substrate was used to standardize the rapid spectral method previously described for microsomal preparations (26). In the latter case, reoxidation of cytochrome $b_5$ at 424 nm, after NADH oxidation in the presence and absence of stearyl coenzyme A, was followed, as suggested by the studies of Oshino *et al.* (7). With purified desaturase and other components, the disappearance of NADH fluorescence with and without substrate also provided a continuous assay and initial rate data.

For all assays, the added complexity is the requirement for proper preincubation of the desaturase at various stages of purification with the other protein and lipid components of the system. At fractions 1–5 in the preparation, 5 μl of enzyme was brought to 2.3% sodium deoxycholate by the addition of an appropriate volume of 10% sodium deoxycholate in 0.02

M Tris buffer at 25°C. Quickly, 2 μl of 200 μM cytochrome $b_5$ and 10 μM reductase (or the catalytic fragment of reductase) in 0.02 M Tris buffer and 3 μl of sonicated egg lecithin suspension (12 mg/ml, in 0.02 M Tris buffer) were added in succession. Within 30–40 sec, 0.19 ml of 0.1 M Tris buffer was added to achieve the dilution of detergent required for activity in the subsequent assay. At fractions 6–9 the desaturase was in an appropriate detergent mixture. The order of addition of components at 25°C was then 3 μl of desaturase preparation, 2 μl of the cytochrome and reductase solution, 3 μl of egg lecithin suspension, and 0.19 ml of the 0.1 M Tris buffer. The final purification steps (fractions 10 and 11) were carried out either in the presence or absence of egg lecithin. When no phospholipid was added, 2–4 μl of the purified enzyme was mixed with 0.5 μl of 10% Triton X-100 and incubated for 10 min at 0°C, 2 μl of cytochrome and reductase solution were then added at 0°C and incubation was continued for another 10 min before addition of 3 μl of egg lecithin. After an additional 2 min at 0°C, 0.19 ml of Tris buffer at room temperature was added and the assay procedure was initiated. When egg lecithin was added during the final purification steps, 0.7 μl 10% sodium deoxycholate in 0.02 M Tris buffer was added to 2 μl of the cytochrome and reductase solution and incubated for 10 min at 0°C. The purified desaturase (4 μl, containing phospholipid) was then added and again incubated at 0°C for 10 min before addition of 0.19 ml of the Tris buffer.

Cytochrome $b_5$ reoxidation at 424 nm at 25°C was used routinely (26). The blank rate of NADH oxidation was first determined by addition of 1 μl of 1.5 mM NADH in 0.02 M Tris buffer to any one of the preincubated assay mixtures. The cytochrome $b_5$ was reduced rapidly and then reoxidized after exhaustion of the NADH. The initial linear rate of cytochrome reoxidation was extrapolated to obtain the time for complete reoxidation of the NADH and reduced cytochrome $b_5$. The NADH addition was then repeated after the addition of 3 μl of 2 mM stearyl coenzyme A to the assay cuvette, and the time for complete reoxidation was again measured. The difference between the time for reoxidation in the presence and absence of substrate was then used to calculate nmol of oleyl coenzyme A formation per min, assuming a stoichiometry of 1 mol of NADH per mol of oleyl coenzyme A formed (26). The blank NADH oxidation, usually between 20 and 30% of the rate with substrate, includes reductase and cytochrome $b_5$ autooxidation as well as an appreciable cyanide-sensitive autooxidation, presumably of the desaturase (see *Results*). The other two assay procedures were conducted in a similar manner except that [$^{14}$C]oleate was measured in one case, and NADH fluorescence disappearance in the other. The agreement among the three methods was ±10%.

*Purification of Desaturase.* Fifty Long-Evans strain rats, weighing between 125 and 150 g, were starved for 48 hr, fed Purina laboratory chow for 24 hr, starved a second 48-hr period, and refed with Nutritional Biochemical Corp. "Fat Free" test diet for 20 hr on a schedule that permits the animals to be killed at the beginning of a day.

All procedures were carried out at 0–5°C with centrifugation at 120,000 × *g* unless otherwise specified. Livers were removed quickly from 50 rats (360–420 g) during a 20- to 30-min period and placed in cold 0.25 M sucrose, 10 mM Tris-acetate, 1 mM EDTA buffer, pH 8.1. The liver was washed twice with 10 volumes of the sucrose buffer, blotted, weighed, minced with scissors, and homogenized with 6 ml of sucrose buffer per g of liver in glass homogenizers with loose-fitting Teflon pestles. Mitochondria, nuclei, and cell debris were removed as a pellet by centrifugation at 18,000 × *g* for 15 min. The supernatant fluid was diluted with 3 volumes of distilled water, and 10 ml of 0.8 M $CaCl_2$ were added per liter of preparation. Centrifugation of this suspension for 5 min at 5000 × *g* served to pack the microsomal cell fraction. The clear supernatant fluid was discarded, and the microsomal pellets were suspended in sufficient 0.1 M Tris buffer to yield 200 ml of suspension after homogenization. After addition of 8 ml of 0.5 M EDTA, pH 8.1, the suspension was centrifuged for 30 min. The supernatant fluid was discarded and the pellets were homogenized with sufficient 0.02 M Tris buffer to yield a volume of microsomal suspension half the original weight of liver (*fraction 1*).

This suspension was brought to 0.45% sodium deoxycholate by the addition of 10% sodium deoxycholate in 0.02 M Tris buffer, sonicated for 40 sec at 60 mW, and centrifuged for 60 min. The supernatant fluid was carefully removed with a syringe and discarded. The relatively loosely packed material above the glycogen pellet was then removed with 60 ml of 0.02 M Tris buffer by agitating with a Vortex mixer. The suspension (about 100 ml) was brought to 0.75% sodium deoxycholate by further addition of the 10% deoxycholate solution (assuming that the wet pellets were at 0.45% deoxycholate), and sonicated for 40 sec (*fraction 2*). After fraction 2 was centrifuged for 60 min, the supernatant fluid was again removed carefully and the yellow-brown layer was resuspended with 36 ml of 0.02 M Tris buffer to yield about 55 ml of suspension, which was brought to 1% deoxycholate by addition of the 10% deoxycholate solution and sonicated for 40 sec (*fraction 3*). The loosely packed pellet obtained by centrifuging this suspension for 60 min was suspended with 36 ml of 0.02 M Tris buffer and sonicated for 40 sec to yield about 50 ml of *fraction 4*, which was again centrifuged for 60 min. The yellow-green pellet was suspended in 10 ml of 0.02 M Tris buffer, sonicated for 40 sec, and stored overnight (*fraction 5*, about 16 ml). Before fraction 5 the enzyme preparation cannot be stored overnight without loss of 20–50% of activity.

Two volumes of 2.5% Triton X-100 were added to fraction 5 and after sonication for 40 sec the suspension was allowed to stand for 30 min before centrifugation for 60 min. The pellet was suspended with 9 ml of 2.5% Triton X-100 and sonicated for 40 sec. Slowly, first 0.2 ml of 10% sodium deoxycholate in 0.02 M Tris buffer and then 0.19 ml of 0.8 M $CaCl_2$ was added with stirring (*fraction 6*). After 30 min, this suspension was centrifuged for 30 min. The supernatant fluid (about 9 ml) was brought to 25% propylene glycol concentration by the addition of the concentrated solvent and again centrifuged for 30 min to yield about 13 ml of supernatant fluid (*fraction 7*). Fraction 7 was layered on a 5 × 2-cm DEAE–cellulose column equilibrated with 0.01 M Tris buffer and eluted with the same buffer. The desaturase emerged in the void volume, and 13 ml were collected and centrifuged for 30 min to yield 13 ml of supernatant fluid (*fraction 8*). This enzyme solution was either stored at −70°C or immediately placed on a 105 × 2-cm Sephadex G-75 column that was equilibrated with 0.2% sodium deoxycholate, 0.01 M Tris buffer. The column was developed with the same buffer, at a flow rate of about 9 ml/hr. The protein emerged in the void volume (168–192 ml,

TABLE 1. *Purification of desaturase*

| Fraction* | Protein (mg) | Activity† (nmol/min) | Specific activity (nmol/mg per min) |
|---|---|---|---|
| 1 | 4500 | 13,000 | 2.9 |
| 2 | 1270 | 12,500 | 9.8 |
| 3 | 520 | 11,900 | 22.9 |
| 4 | 350 | 9,200 | 26.3 |
| 5 | 290 | 8,500 | 29.2 |
| 6 | 157 | 8,200 | 52 |
| 7 | 19.8 | 7,000 | 354 |
| 8 | 12.6 | 5,000 | 396 |
| 9 | 10.6 | Not done | Not done |
| 10 | 6.6 | 2,200 | 333 |
| 11 | 5.9 | 2,100 | 355 |

* Fractions 1–10 were from one preparation; fraction 11 was from another preparation.

† By cytochrome $b_5$ reoxidation (see *Methods and Materials*).

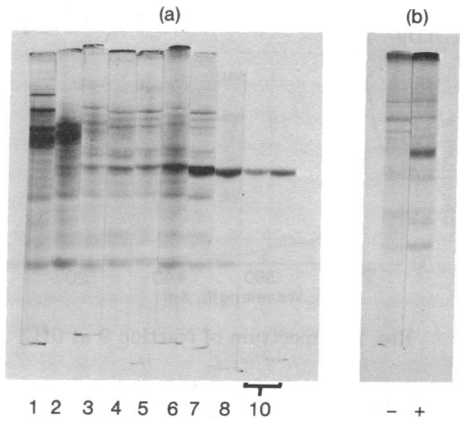


FIG. 1. Acrylamide disc-gel electrophoresis (a) of fractions 1–8 and 10 of a typical preparation with two protein concentrations of fraction 10; and (b) of fraction 7 obtained from starved (−) and starved-refed (+) rats. See *Methods and Materials* for details and *text* for protocol for (b).

*fraction 9*), and the Triton, propylene glycol, and most of the residual lipid were included and did not begin to elute until 210–220 ml.

Low phospholipid enzyme was isolated by diluting fraction 9 with 2 volumes of 0.1 M Tris buffer and collecting the precipitate after 15 min by centrifugation for 60 min. The precipitate was washed free of much of the deoxycholate by resuspending with 15–20 ml of 0.1 M Tris buffer, homogenizing in an all-glass homogenizer, and centrifuging for 30 min. This precipitate was homogenized with 2–3 ml of 0.1 M Tris buffer and stored at 0°C or −70°C (*fraction 10*).

Alternatively, the enzyme was isolated with phospholipid by adding 1.3 ml of the egg lecithin suspension (16 mg of phospholipid) to fraction 9 before dilution with the 0.1 M Tris buffer. The same centrifugation and washing procedure as described for the egg lecithin-free preparation was then used to obtain *fraction 11*, which was also stored at 0°C or −70°C.

## RESULTS

The purification procedure (Table 1) results in a 120- to 130-fold purification with a 30–40% yield at fraction 8. Subsequent manipulation of the desaturase, to remove Triton and some tightly bound phospholipid, does not result in further apparent purification and involves appreciable loss of protein. The observed activities must be considered minimal values, since the major problem in assaying the enzyme as purification proceeds is proper dispersion of the highly aggregated desaturase.

Disc-gel electrophoresis (Fig. 1a) shows the course of purification and provides some indirect data concerning the disaggregation-activity relationship. It is clear that one major polypeptide is concentrated during purification. By use of appropriate protein standards, this polypeptide, under denaturing and reducing conditions, has an apparent molecular weight of 53,000. Therefore, in the absence of high concentrations of detergents, e.g., fraction 10, the native polypeptide must form high-molecular-weight aggregates. Fig. 1b shows one type of evidence that identifies this polypeptide with the desaturase. In this experiment rats were divided during the feeding period into those that were starved but not refed the "fat-free" diet (−) and those that were refed (+). Liver from both groups was subjected to purification to fraction 7, and only in the induced animals (+) does a concentrated polypeptide appear at the position of the major component of fraction 10. There is some indication in Fig. 1a that fraction 10 may, in fact, be slightly less contaminated with minor, particularly smaller, peptide bands than fraction 8. Thus, some purification may occur on the Sephadex column which is not seen as an increase in specific activity because the preincubation conditions may fail to properly disperse all of the aggregated fraction 10 to yield desaturase which can interact rapidly with reduced cytochrome $b_5$. This assumption is consistent with the observation that fractions 10 and 11 from several preparations varied in specific activity from 240 to 360.

Preparations of fraction 10 contained 18.4 ± 1.8 nmol of iron/mg of protein. This corresponds to a minimum molecular weight of 55,000 ±10%. Consistently, the preparations with slightly lower iron content also had lower specific activities. Iron loss appears to result from desaturase instability during the 20-hr Sephadex gel filtration procedure. Fraction 10 also still contains 2–10 mol of phospholipid/mol of iron. However, there was no detectable acid-labile sulfur, flavin, heme, or reducing sugar ($\leq$0.2 mol per mol of protein) in these preparations. Preparations of fraction 11, to which phospholipid was added, contained about 1.3–1.7 mg of phospholipid/mg of protein. By including [$^{14}$C]deoxycholate in the latter steps in the purification during the isolation of desaturase when phospholipid is re-added, we found that 30 mol of the detergent per mol of enzyme appeared in fraction 11 despite the washing procedure used.

The spectrum of the purified desaturase at fraction 9 is shown in Fig. 2. Only a major protein band with a high tryptophan content, indicated by the 290-nm shoulder, is seen. The low absorbance in the 300- to 430-nm region is similar to that of ribonucleotide reductase (27), which also contains non-heme iron. The low extinction at 380–450 nm is consistent with the absence of heme. Since radioactive Triton X-100 was not available to us, the exact content of this detergent is difficult to ascertain. Nevertheless, the fact that Triton X-100 is included in the Sephadex column (see *Methods and Materials*) and only begins to appear more than 20 ml after the protein

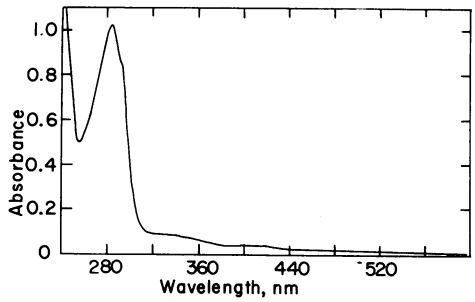


FIG. 2. Spectrum of fraction 9 at 0°C.

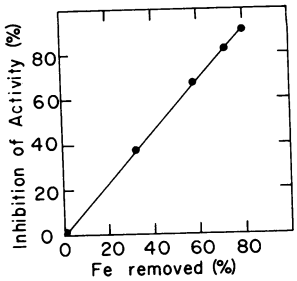


FIG. 3. Correlation between iron removal and loss of desaturase activity. A suspension of fraction 11 (12.7 nmol of iron), 16 nmol of cytochrome $b_5$, and 2 nmol of reductase in 2.65 ml of 0.1 M Tris buffer was incubated for 30 min at 32°C. After the mixture was cooled to 25°C, 20 $\mu$l of 10 mM bathophenanthroline sulfonate was added to 50-$\mu$l aliquots of the enzyme solution. To five such samples, 0, 15, 30, 45, and 60 nmol of NADH were added, respectively, and incubation at 25°C was continued until the NADH was oxidized, as indicated by cytochrome reoxidation. Aliquots of each sample were then assayed for activity and the amount of bathophenanthroline iron couplex formed.

has been eluted, and the absence of detectable absorbance characteristic of the Triton X-100 absorption spectrum in deoxycholate (maximal at 280 and 275 nm), suggest that little Triton remains in fractions 10 and 11. The single major absorption band is accounted for by the spectral contributions of 24 tyrosyl and 17 tryptophanyl residues, as determined by the spectral methods of Beaven and Holliday (24) and by tyrosine analysis (see below).

The amino-acid analysis of fraction 10 (Table 2) emphasizes the predominance of nonpolar residues in the desaturase. By the procedure of Capaldi and Vanderkooi (27), 62% of the amino acid residues are nonpolar. This provides an explanation for the marked tendency of the protein to polymerize in aqueous solvents during isolation. $NH_2$-terminal end group determinations by two cycles of Edman degradation on preparations of fractions 10 and 11 show a dominant $NH_2$-terminal serine followed by tryptophan. Low concentrations of several other residues (<10%) were detected, but these appear to arise from small peptide contaminants, since prior gel filtration on Bio-gel P-10 equilibrated with 1% sodium dodecyl sulfate removes them completely. Then only serine residues followed by tryptophan are seen by the dansyl technique as the $NH_2$-terminal sequence. The 53,000-dalton polypeptide seen on disc-gel electrophoresis, therefore, appears to be a single polypeptide species.

The essential components for NADH-dependent stearyl coenzyme A desaturase are the three enzymes (reductase, cytochrome $b_5$, and desaturase), the substrates (NADH, oxygen, and stearyl coenzyme A), and phospholipid. Detergents appear to serve the function of dispersing the proteins, in particular, the desaturase. If phospholipid was omitted in the assay of fraction 10, in which Triton X-100 is the detergent (see *Methods and Materials*), 30–35% of maximal activity was observed. Whether this resulted from the low residual lipid in fraction 10 or represents activity of the enzyme system in this detergent alone, is not yet clear. We were able to confirm the previous report of Shimakata *et al.* (11) that there is no detectable enzyme activity when the soluble catalytic fragment of cytochrome $b_5$ is substituted for the complete amphipathic heme protein. In contrast to Shimakata *et al.* (11), however, we find that in the absence of cytochrome $b_5$ neither reductase nor its catalytic fragment can serve as direct electron donor, although either form of the reductase can be used to reduce the cytochrome $b_5$ rapidly in the normal assay system.

TABLE 2. *Amino-acid composition* of fraction 10*

| | | |
|---|---|---|
| Lys 23 | Glu 29 | Met 12 |
| His 14 | Pro 21 | Ile 21 |
| Arg 22 | Gly 30 | Leu 58 |
| Asp 33 | Ala 35 | Tyr 24 |
| Thr 20 | Cys 8 | Phe 28 |
| Ser 33 | Val 25 | Try 17 |

Total residues = 458 Molecular weight = 53,162

* See *Methods and Materials* for procedures. The Lys residue value was chosen to agree with the minimum molecular weight determined by iron analysis and from disc-gel electrophoresis. Several preparations of fractions 10 and 11 yielded essentially identical results. Composition is given in residues per 23 lysine residues.

A catalytic role for the non-heme iron of the desaturase is implied by the observed loss of activity upon iron removal by a ferrous chelator (Fig. 3). It was found that incubation of a preparation of fraction 11, which contained about 1.5 mg of lipid per mg of protein and 30 mol of deoxycholate per mol of desaturase, with reductase, cytochrome, and desaturase at 32°C for 30 min, was sufficient to obtain 70–80% of the desaturase activity. When such activated complexes of the three proteins and phospholipid were preincubated with a relatively high concentration of ferrous chelator, iron was removed only if the incubation mixture contained NADH. Iron in fraction 11 appears to be reduced by the NADH, reductase, and cytochrome $b_5$. The high concentrations of bathophenanthrolene sulfonate and desaturase during this incubation, before a 20-fold dilution for activity assay, results in iron removal proportional to the total amount of NADH added and, thus, the duration of desaturase reduction. Both the dependence of iron removal upon reduction by substrate and the direct correlation with loss of catalytic activity suggests a catalytic function for the non-heme iron in the desaturase. Moreover, as in the case of the enzyme in microsomes (3, 7), the activities of the purified enzyme fractions are completely and reversibly inhibited by 0.5 mM KCN. Preliminary electron paramagnetic resonance studies at 100°K with 70 nM desaturase as well as 20 nM enzyme reduced enzymatically with NADH as in Fig. 3, showed no iron signals. However, these data must be considered inconclusive, since both higher protein concentrations with oxidized and reduced

enzyme and lower temperatures may be necessary to detect a catalytically active high spin iron intermediate.

## DISCUSSION

The present studies with purified stearyl coenzyme A desaturase confirm earlier reports on microsomes and the partially purified enzyme (1–11) that reduced cytochrome $b_5$ is the normal electron donor for the desaturase in endoplasmic reticulum. The requirement for phospholipid reported by Jones *et al.* (4) is also observed with the purified enzyme, even in the presence of Triton X-100. Both the tendency of this protein to form high-molecular-weight, inactive aggregates during purification and the instability of the enzyme, particularly in detergents, account for the difficulties encountered in the development of the isolation procedure. These properties of the enzyme required rapid isolation and storage at −70°C in low detergent concentrations. Dispersion of aggregates of the desaturase for assay was a balance between detergent conditions that would disperse aggregates completely and still avoid denaturation.

As in the case of the other two components of the desaturase system, the desaturase appears to be a single polypeptide. However, it is larger (456 residues), and the high content of nonpolar residues in the complete protein (62%) contrasts with the flavo- and heme proteins. The latter are amphipathic proteins each containing a hydrophilic, catalytic segment and a smaller hydrophobic sequence (12, 13) involved in attachment to microsomal vesicles.

Since the dominant polypeptide in the final desaturase preparations (fractions 10 and 11) appears to be nearly homogeneous by several independent physical and chemical criteria and to contain functional iron, it is unlikely that a minor protein contaminant is responsible for the desaturase activity. The maximum activities observed with such preparations yield a turnover at 25°C of 21 mol of substrate per mol of desaturase per min. It is not yet clear, however, whether or not this represents a true maximum velocity in this complex system. The observed turnover number and the total activity in induced rat liver microsomes indicates that the desaturase may represent 0.7–0.8% of the total microsomal protein. The dietary induction of desaturase described by Oshino and Sato (8) and used here, thus results in at least a 20-fold increase in the concentration of this protein to form a significant quantitative component of the endoplasmic reticulum.

The apparent involvement of iron in the catalytic activity of the desaturase is remarkably similar to the situation reported recently by Pistorius and Axelrod (28) for soybean lipoxygenase as well as other non-heme iron proteins (29, 30). Here, too, the enzyme contains 1 mol of iron, which is removed under reducing conditions by orthophenanthroline. These authors also detected an electron paramagnetic resonance signal at g = 6 upon addition of linoleic acid to the lipoxygenase in the presence of oxygen. The utilization of the purified desaturase to probe mechanistic questions regarding interactions with cytochrome $b_5$, oxygen, and substrates must include similar electron paramagnetic resonance experiments under optimal conditions. Isolation of the desaturase also provides the third component required to reconstitute a complete liposomal desaturase system for a detailed examination of the essential protein and lipid interactions in the mobile nonpolar phase.

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