A Requirement for Three Protein Components in Microsomal Stearyl Coenzyme A Desaturation*

P. W. Holloway

ABSTRACT: The enzymic conversion of stearyl coenzyme A into oleate was shown to require three protein components isolated from liver microsomes. A fraction, obtained by a combination of deoxycholate solubilization and *N*-ethylmaleimide treatment of microsomes, was resolved into two fractions by gel filtration in the presence of deoxycholate. Both of these column fractions were required, together with soluble DPNH–cytochrome b_5 reductase, for desaturation of stearyl coenzyme A. The large molecular weight fraction was heat labile, contained little phospholipid, and had no characteristic absorption spectrum. The small molecular weight fraction was relatively heat stable and contained large quantities of phospholipid and cytochrome b_5. The small molecular weight fraction was inactivated if the phospholipid was removed or the cytochrome b_5 was destroyed. These observations are further evidence for the involvement of the DPNH-specific electron-transport chain of microsomes in desaturation.

Stearyl-CoA desaturase activity in animals is found associated with the microsomal fraction of the cell (Bernhard *et al.*, 1959; Marsh and James, 1962; Holloway *et al.*, 1963). Attempts have been made to solubilize and purify the desaturase and recently Gurr and Robinson (1970) achieved a 5-fold increase in activity and found that the desaturase had a particle weight greater than 4×10^6. The requirement for reduced pyridine nucleotide and oxygen for the conversion of stearyl-CoA into oleate suggested that the microsomal electron-transport chain might be involved (Wakil, 1964; Oshino *et al.*, 1966). Microsomes are known to contain two electron-transport chains, one is TPNH specific and involves cytochrome P-450, the other is DPNH specific and involves cytochrome b_5. Of the two chains, the DPNH-specific chain has been suggested to be involved in the desaturase system of the microsomes (Oshino *et al.*, 1966; Jones *et al.*, 1969).

The recent finding that DPNH–cytochrome b_5 reductase was absolutely required for the desaturation of stearyl-CoA provided strong affirmation of this conclusion. It was reported (Holloway and Wakil, 1970) that *N*-ethylmaleimide-inhibited particles (N_2 fraction) derived from hen liver microsomes could not desaturate stearyl-CoA unless DPNH–cytochrome b_5 reductase was added. It appeared that the N_2 fraction contained the complete desaturase system except that the flavoprotein of the reductase system was inhibited. The present report describes the resolution of the N_2 fraction into two components both of which are required, together with DPNH–cytochrome b_5 reductase, for desaturation of stearyl-CoA.

Experimental Section

Hen liver microsomes were prepared as described previously (Jones *et al.*, 1969). The microsomes and fractions derived from them were assayed for stearyl-CoA desaturase activity by use of [1-^{14}C]stearyl-CoA, with the product, [1-^{14}C]oleate, being separated by thin-layer chromatography on $AgNO_3$-impregnated silica gel H (Jones *et al.*, 1969). The microsomes were "solubilized" and manipulated as described previously (Holloway and Wakil, 1970) to yield a purified particulate fraction P_2.

Protein was estimated by the biuret method of Gornall *et al.* (1949). Lipid content of microsomal subfractions was estimated from the phosphate content (Chen *et al.*, 1956) of a chloroform–methanol extract (Bligh and Dyer, 1959).

Preparation of the N-Ethylmaleimide-Treated Fraction (N_2). The P_2 fraction (Holloway and Wakil, 1970) (5 ml) was mixed with 7.0 ml of "solubilizing solution" (containing 60 ml of glycerol, 20 ml of 1 M KCl, 20 ml of 1 M potassium citrate (pH 7.7), and 10 ml of 10% sodium deoxycholate) (Holloway and Wakil, 1970; Lu and Coon, 1968), and 0.05 ml of 0.2 M dithiothreitol. The resulting mixture was homogenized in a Potter-Elvehjem Teflon homogenizer, and then allowed to stand for 20 min at 0°. To the mixture was added 1.2 ml of 0.2 M *N*-ethylmaleimide and, after rehomogenization, the mixture was incubated for 20 min at 37°. The excess *N*-ethylmaleimide was destroyed by dithiothreitol and the resultant mixture was centrifuged at 105,000*g* for 30 min. The clear yellow supernatant solution (12.5 ml) was removed and to it was added 0.33 volume (4.15 ml) of saturated ammonium sulfate solution (pH 7.2). The mixture was centrifuged at 105,000*g* for 30 min after which the floating yellow material was collected and dissolved in 0.02 M sodium bicarbonate (pH 7.7) containing 0.1 mM dithiothreitol and 0.2% sodium deoxycholate. This clear yellow solution was designated the "N_2 fraction."

Gel Filtration of the N_2 Fraction. The N_2 fraction (6 ml) was applied to a column (25 × 270 mm) of Sephadex G-200 (Pharmacia Fine Chemicals, Piscataway, N. J.) stabilized by a 0.5-cm layer of Sephadex G-25 on the top. The column was equilibrated with 0.02 M sodium bicarbonate (pH 7.7) containing 0.1 mM dithiothreitol and 0.2% sodium deoxycholate. The column was eluted with the same buffer and 3-ml fractions were collected. The applied sample separated into two yellow bands during the chromatography and these were routinely collected in tubes 20–27 and 28–42. Tube 20 coincided with the void volume of this column. The first fraction, eluted immediately after the void

* From the Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia 22901. *Received November 3, 1970.* This work was supported by Grant P-559 from the American Cancer Society. This work was done during the tenure of an Established Investigatorship of the American Heart Association.

volume (tubes 20–27), was applied to a column of Sephadex G-25 equilibrated with 0.01 M potassium phosphate buffer (pH 7.2) containing 0.1 mM dithiothreitol, to remove the deoxycholate. The cloudy eluate was poured into an equal volume of stirred saturated ammonium sulfate solution (pH 7.2) and the precipitate collected by centrifugation at 20,000*g* for 15 min. The pellet was suspended in a minimum volume of 0.01 M potassium phosphate (pH 7.2) and stored at −20°. This fraction is designated the "N_2G_1 fraction."

The second fraction eluted from the Sephadex G-200 column (tubes 28–42) was concentrated in an Amicon concentrator (Amicon Corp., Lexington, Mass.) using an XM-100 membrane and minimum pressure. This concentrated material was stored at −20° and is designated the "N_2G_2 fraction."

Characterization of N_2G_1 and N_2G_2. The stearyl-CoA desaturase activity of N_2, N_2G_1, and N_2G_2 was determined as for hen microsomes except that the standard incubation also included 0.06 mg of protein of partially purified soluble DPNH–cytochrome b_5 reductase (specific activity 6.0 μmoles dichlorophenol–indophenol reduced per min per mg of protein at 37°).

The cytochrome b_5 content of microsomes and microsomal subfractions was determined from the DPNH reduced minus oxidized spectrum. The difference in optical density between 424 and 410 mμ was taken as a measure of the cytochrome b_5 content. Purified DPNH–cytochrome b_5 reductase was added to both cuvets, at a final concentration of 0.01 mg/ml, before each difference spectrum was measured.

N_2G_2 was extracted with 10% aqueous acetone (Lester and Fleischer, 1961). After collection of the precipitated material by centrifugation at 12,000*g* for 2 min the acetone was poured off and the material was dried under vacuum at 0°. After 1 hr the material was taken up in 0.01 M potassium phosphate (pH 7.2) and designated "acetone-extracted N_2G_2".

Trypsin treatment of the N_2G_2 fraction (1 ml) was performed at 37° with 1 ml of trypsin solution (1 mg/ml of 0.01 M potassium phosphate, pH 7.2). After 1 hr 1 ml of trypsin inhibitor solution (2 mg/ml) was added. The mixture (trypsin–N_2G_2) was assayed for desaturase and cytochrome b_5 as described above. A control experiment consisted of the separate incubation of the trypsin solution and the N_2G_2. The trypsin was mixed with inhibitor and then with N_2G_2 before assay (inhibited trypsin–N_2G_2).

DPNH–Cytochrome b_5 Reductase and Cytochrome b_5. DPNH–cytochrome b_5 reductase was isolated from the postmitochondrial supernatant of rat liver homogenates by the method of Takesue and Omura (1968). The solubilized enzyme was purified by ammonium sulfate fractionation and gel filtration (Strittmatter, 1967). The gel elute was further purified on hydroxylapatite Bio-Gel HTP (Bio-Rad Laboratories, Richmond, Calif.) using a linear gradient of 0.01 M potassium phosphate (pH 7.2) to 0.5 M potassium phosphate (pH 7.2). The fractions with highest 2,6-dichlorophenolindophenol reductase activity were pooled and the protein was precipitated with solid ammonium sulfate and was collected by centrifugation. The specific activity of the reductase was 6.0 μmoles of dichlorophenolindophenol reduced per min per mg of protein, at 37°. The isolated reductase was approximately 5% pure when its specific activity was compared to that reported for the homogeneous protein (Takesue and Omura, 1970). Cytochrome b_5 was isolated from washed hen liver microsomes as described previously for rat liver microsomes (Omura *et al.*, 1967), and will be referred to as trypsin–cytochrome b_5 (Ito and Sato, 1968). Based on absorption spectra the trypsin–cytochrome b_5 was approximately 25% pure (Garfinkel, 1958).

TABLE I: Stearyl-CoA Desaturase Activity in Fractions Derived from Hen Liver Microsomes.[a]

Fraction	Stearyl-CoA Desaturase, mμmoles of Oleate Formed
Microsomes	0.42
P_2	1.71
N_2 without added reductase	0
N_2	0.83
N_2G_1	0.01
N_2G_2	0.57
N_2G_1 + N_2G_2[b]	1.94
N_2G_1 + N_2G_2[b] without added reductase	0

[a] The stearyl-CoA desaturase activity of hen liver microsomes and protein fractions derived from the microsomes was determined as described previously (Jones *et al.*, 1969). The reaction mixture contained 60 mM potassium phosphate (pH 7.2). 100 μM DPNH, 20 μM [1-^{14}C]stearyl-CoA (30,000 cpm), 0.2 mg of protein, and water to a final volume of 0.5 ml. Incubations were at 37° for 15 min in air. The fractions N_2, N_2G_1, N_2G_2, and N_2G_1 + N_2G_2 were assayed in the presence of soluble DPNH–cytochrome b_5 reductase (0.06 mg of protein, specific activity 6.0 μmoles of 2,6-dichlorophenolindophenol reduced per min per mg of protein), unless otherwise indicated. [b] 0.2 mg of protein of both fractions was used.

Results

The P_2 fraction, isolated by deoxycholate solubilization of hen liver microsomes followed by dilution with water, was enriched in both DPNH–cytochrome *c* reductase and DPNH–stearyl-CoA desaturase activities (Table I), as previously reported (Holloway and Wakil, 1970). Treatment of the P_2 fraction with *N*-ethylmaleimide, followed by centrifugation and ammonium sulfate precipitation, yielded a particulate fraction (N_2) lacking both desaturase and reductase activities. The desaturase activity was restored to the N_2 fraction by addition of a partially purified soluble DPNH–cytochrome b_5 reductase (Table I). The same restoration of desaturase activity was obtained previously when a highly purified preparation of reductase was used (Holloway and Wakil, 1970). The N_2 fraction, although particulate in the absence of detergent, formed clear "solutions" in the presence of deoxycholate and was chromatographed on a column of Sephadex G-200 equilibrated with a buffer containing 0.2% deoxycholate. The majority of protein applied to the Sephadex G-200 column as N_2 fraction (28 mg) was recovered in two fractions: N_2G_1, 13.2 mg; and N_2G_2, 7.8 mg. The recovery of desaturase activity, mainly in N_2G_2, was very low but a combination of these two fractions produced a stimulation of desaturase activity (Table I).

Although both N_2G_1 and N_2G_2 were required for maximal desaturase activity, these two fractions had different compositions and properties. Both fractions contain protein but in

TABLE II: Analysis of Hen Liver Microsomes and Microsomal Subfractions for Lipid and Cytochrome b_5.[a]

Fraction	Lipid (μg P/mg of Protein)	Cytochrome b_5 ΔOD 424–410 red.–ox.
Experiment 1		
Microsomes	9.0	0.031
P_2	13.8	0.045
N_2	24.2	0.072
N_2G_1	5.6	0
N_2G_2	47.0	0.30
Acetone-extracted N_2G_2	4.9	0.39
Experiment 2		
N_2G_2		0.19
Heated N_2G_2		0.14
Trypsin–N_2G_2		0
Sephadex G-25 N_2G_2		0.24

[a] The hen liver microsomes and microsomal subfractions were analyzed for lipid phosphorus and cytochrome b_5 as described in Experimental Section. The P_2,N_2, N_2G_1, N_2G_2, acetone-extracted N_2G_2, and trypsin–N_2G_2 were prepared as described in Experimental Section. The "heated N_2G_2" was prepared by heating N_2G_2 at 60° for 15 min. The "Sephadex G-25 N_2G_2" was prepared by passing N_2G_2 through a Sephadex G-25 column equilibrated in 0.01 M potassium phosphate (pH 7.2). The material which was completely excluded was designated "Sephadex G-25 N_2G_2." The cytochrome b_5 data were obtained with either 0.1 or 0.2 mg of protein per ml, but have been recalculated to give the ΔOD for a solution of 1 mg of protein/ml.

TABLE III: Properties of Microsomal Subfractions N_2G_1 and N_2G_2.[a]

Components[b]	Stearyl-CoA Desaturase, mμmoles of Oleate Formed
Experiment 1	
N_2G_1 (0.2)	0.07
N_2G_2 (0.07)	0.20
N_2G_1 (0.2) + N_2G_2 (0.07)	1.24
N_2G_1 (0.2) + heated N_2G_2 (0.07)	1.19
Heated N_2G_1 (0.2) + N_2G_2 (0.07)	0.14
Experiment 2	
N_2G_1 (0.2) + N_2G_2 (0.05)	1.30
N_2G_1 (0.2) + trypsin–N_2G_2 (0.05)[c]	0.13
N_2G_1 (0.2) + inhibited trypsin–N_2G_2 (0.05)[c]	1.05
N_2G_1 (0.2) + acetone-extracted N_2G_2 (0.05)	0.24
N_2G_1 (0.2) + acetone-extracted N_2G_2 (0.05) + DOC[d]	0.22
N_2G_1 (0.2) + Sephadex G-25 N_2G_2 (0.05)	0.83
N_2G_1 (0.2) + Sephadex G-25 N_2G_2 (0.05) + DOC[d]	0.97
Experiment 3	
N_2G_1 (0.2) + N_2G_2 (0.10)	1.88
N_2G_1 (0.2) + N_2G_2 (0.05)	1.30
N_2G_1 (0.2) + N_2G_2 (0.05) + cyt b_5 (0.02)[e]	1.10
N_2G_1 (0.2) + cyt b_5 (0.02)[e]	0.07

[a] The stearyl-CoA desaturase activity of N_2G_1, N_2G_2, and N_2G_1 plus N_2G_2 was determined as described in Table I. The components were obtained as described in the Experimental Section and Table II. [b] The numbers in parentheses after each component refer to milligrams of protein used in the assay. [c] The volume of these components used would have contained 0.05 mg of N_2G_2 protein if no digestion had occurred. [d] A volume of 0.2% sodium deoxycholate equal in volume to the volume of N_2G_2 used above. Final concentration 0.02%. [e] The cyt b_5 refers to trypsin–cytochrome b_5 prepared as described in the Experimental Section.

addition, the N_2G_2 fraction contains large amounts of lipid (Table II, expt 1). Spectral examination of the two fractions suggested that N_2G_2 contained cytochrome b_5 and a typical cytochrome b_5 reduced minus oxidized difference spectrum was obtained when the reduction was performed either by dithionite or by DPNH plus DPNH–cytochrome b_5 reductase (Table II, expt 1). The large molecular weight fraction N_2G_1 contained no cytochrome b_5 and less lipid per milligram of protein than the N_2 fraction or the original microsomes (Table II, expt 1). A further difference between the two fractions was in their heat stabilities. Although the ability of N_2G_1 to exhibit desaturase activity, when combined with N_2G_2 and DPNH–cytochrome b_5 reductase, was destroyed by heating at 60° for 15 min, N_2G_2 was not affected by this treatment (Table III, expt 1).

The high cytochrome b_5 content of the N_2G_2 fraction suggested that this pigment was involved in the desaturase reaction and attempts were made to correlate the known properties of cytochrome b_5 with those of the N_2G_2 fraction. The stabilities of the enzymic (Table III, expt 1) and spectral (Table II, expt 2) properties of N_2G_2 to heating at 60° for 15 min agree with the known stability of cytochrome b_5 (Nobrega *et al.*, 1969). Cytochrome b_5 can be isolated by trypsin digestion and is stable to further attack by trypsin; hence, it was surprising that the N_2G_2 fraction was destroyed by trypsin (Table III, expt 2). However, this loss of activity was paralleled by a destruction of the cytochrome b_5 in the N_2G_2 as determined spectrally (Table II, expt 2). The N_2G_2 fraction could not be replaced by purified trypsin–cytochrome b_5 and the trypsin–cytochrome b_5 did not cause a stimulation when added to an incubation where a nonsaturating level of N_2G_2 was used (Table III, expt 3).

The necessity for a non-protein component in N_2G_2 for desaturase activity was demonstrated by the decreased desaturation when N_2G_2 was extracted with acetone (Table III, expt 2). The addition of deoxycholate to the acetone extracted N_2G_2 did not increase the desaturation. The removal of deoxycholate from N_2G_2 by Sephadex G-25 caused a slight decrease in desaturase activity (Table III, expt 2). Neither acetone extraction nor Sephadex G-25 chromatography affected the ability of DPNH plus DPNH–cytochrome b_5 reductase to reduce the cytochrome b_5 component of N_2G_2 (Table II).

The reconstituted system retained the cyanide sensitivity shown by the original microsomes (Wakil, 1964; Oshino *et al.*, 1966). The desaturase activities of the original micro-

somes, P_2, cytochrome b_5 reductase plus N_2 or plus N_2G_1 and N_2G_2, were all inhibited approximately 50% by 1 mM KCN. This would suggest that the cyanide-sensitive factor isolated by Gaylor *et al.* (1970) is retained in N_2G_1 or N_2G_2.

Reduced cytochrome b_5, both trypsin–cytochrome b_5 and that present in N_2G_2, was found to be autoxidizable. The rates of autoxidation of both types of reduced cytochrome b_5 were measured at a concentration of 0.5 mμmole/ml. Both sample and reference cuvets contained the cytochrome b_5 and purified DPNH–cytochrome b_5 reductase (0.01 mg/ml). The cytochrome in the sample cuvet was reduced by addition of 1 mμmole of DPNH. The effect of N_2G_1 (0.4 mg/ml) in both sample and reference cuvets was also measured. The initial rates of autoxidation, in millimicromoles per minute, as measured by decrease in absorbance at 424 mμ were: for trypsin–cytochrome b_5 alone 0.12, for trypsin–cytochrome b_5 plus N_2G_1 0.05, for N_2G_2 alone 0.06, and for N_2G_2 plus N_2G_1 0.11.

Discussion

In a previous report evidence was presented which demonstrated a requirement for DPNH–cytochrome b_5 reductase in stearyl-CoA desaturation (Holloway and Wakil, 1970). It was shown that an *N*-ethylmaleimide-inhibited particle (N_2) derived from hen liver microsomes had neither DPNH–cytochrome *c* reductase nor DPNH–stearyl-CoA desaturase activities. Desaturase activity was restored to N_2 by addition of soluble DPNH–cytochrome b_5 reductase. The present publication presents further evidence for the involvement of the DPNH-specific electron-transport chain of microsomes in stearyl-CoA desaturation.

The N_2 fraction although particulate in the absence of detergent is "soluble" in the presence of deoxycholate. The nature of membrane "solubilization" in detergents is incompletely understood. It has been suggested that the membrane is dissociated into small lipoprotein subunits (Green and Perdue, 1966), whereas other evidence suggests a complete separation into lipids and proteins does occur (Engelman and Morowitz, 1968; Bont *et al.*, 1969). If a complete dissociation into individual proteins does occur it should be possible to separate them by standard biochemical procedures, provided reaggregation is prevented by detergent. The N_2 fraction when dissolved in a deoxycholate-containing buffer and chromatographed on Sephadex G-200 in the same buffer readily separated into two yellow bands. The desaturase activity in either the large molecular weight fraction, N_2G_1, or the small molecular weight fraction, N_2G_2 was very low. Addition of N_2G_1 to N_2G_2 gave a marked stimulation (Table I).

The large molecular weight fraction (N_2G_1) contained little lipid and had no characteristic spectral properties. The proteinaceous nature of the required component in N_2G_1 was demonstrated by its rapid inactivation by heat. After heating at 60° for 15 min N_2G_1 no longer exhibited desaturase activity when combined with N_2G_2 and DPNH–cytochrome b_5 reductase. The smaller molecular weight fraction (N_2G_2) contained large amounts of lipid and had spectral properties identical to cytochrome b_5. When N_2G_2 was heated to 60° for 15 min neither the ability to catalyze desaturation in the presence of N_2G_1 and the reductase, nor the spectral properties were affected.

It appears that both the protein and lipid components of N_2G_2 are required for stearyl-CoA desaturation, as the ability of N_2G_2 to exhibit desaturase activity, when combined with N_2G_1 and DPNH–cytochrome b_5 reductase, was lost when N_2G_2 was extracted with aqueous acetone or treated with trypsin. The destruction of desaturase activity when N_2G_2 was extracted with aqueous acetone is significant in view of the reported requirement for lipid in stearyl-CoA desaturation in microsomes (Jones *et al.*, 1969). Aqueous acetone extraction would lead to loss of lipid and possibly deoxycholate. Although removal of deoxycholate from N_2G_2 by Sephadex G-25 chromatography did decrease the desaturase activity somewhat, the addition of deoxycholate to "acetone-extracted N_2G_2" did not restore the desaturase activity (Table III, expt 2). Deoxycholate did slightly stimulate when added to an incubation containing Sephadex G-25 N_2G_2. This suggests that the required component removed from N_2G_2 by aqueous acetone is lipid not deoxycholate.

The known components of the DPNH-specific electron-transport chain of microsomes are the DPNH–cytochrome b_5 reductase and cytochrome b_5. Oshino *et al.* (1967) have demonstrated, in intact microsomes, that the desaturation of stearyl-CoA is dependent on oxidation of reduced cytochrome b_5. The present demonstration that the N_2G_2 fraction, which was required for stearyl-CoA desaturation, also contained a pigment with the spectral properties of cytochrome b_5 lends further support to the involvement of cytochrome b_5 in stearyl-CoA desaturation.

Attempts were made to confirm that the pigment in the N_2G_2 fraction was cytochrome b_5, and that this pigment was required for desaturase activity. Cytochrome b_5 as isolated from various sources is stable up to 60° (Nobréga *et al.*, 1969), and both the enzymic and spectral properties of N_2G_2 were likewise unchanged by heating at 60° for 15 min. The destruction of the activity of N_2G_2 (Table III, expt 2) by trypsin is at variance with the known stability of cytochrome b_5 to tryspin. However, it was found that under the conditions used for the tryptic digestion the cytochrome b_5 spectrum also had been destroyed (Table II, expt 2). Hence, the loss of desaturase activity upon tryptic digestion of N_2G_2 could be due to destruction of the cytochrome b_5, but might also be due to destruction of other heat stable protein components. Trypsin–cytochrome b_5 was unable to replace N_2G_2 in the desaturase system (Table III, expt 3) and this again supports the notion that N_2G_2 contains components, certainly lipid and perhaps proteins, other than cytochrome b_5, that are required for desaturation of stearyl-CoA. If two or more required components are present in N_2G_2 and one of these is replaceable by trypsin–cytochrome b_5 then the addition of trypsin–cytochrome b_5 to a desaturase incubation containing limiting amounts of N_2G_2 may cause a stimulation of desaturase activity. As shown in Table III (expt 3) no stimulation of desaturase activity was observed. This lack of stimulation indicates either that the component limiting, when lower levels of N_2G_2 are used, is something other than the cytochrome b_5 component in N_2G_2 or that the trypsin–cytochrome b_5 is not equivalent to the cytochrome b_5 in N_2G_2. Certainly, cytochrome b_5 isolated from microsomes by detergent solubilization procedures (detergent–cytochrome b_5) is different from that isolated by trypsin solubilization (trypsin–cytochrome b_5) (Ito and Sato, 1968).

Neither extraction with aqueous acetone nor Sephadex G-25 chromatography of N_2G_2 affected the ability of the cytochrome b_5 to be reduced by DPNH plus DPNH–cytochrome b_5 reductase (Table II, expt 1) although, as rates of reduction were not measured, it is not possible to say whether either of these procedures influenced the interaction of the cytochrome b_5 with the reductase. The reduced form of the

cytochrome b_5 in the N_2G_2 fraction like reduced trypsin cytochrome b_5 is autoxidizable by air. The rate of autoxidation of the reduced cytochrome b_5 in the N_2G_2 fraction is stimulated twofold by addition of N_2G_1, whereas the rate of autoxidation of trypsin–cytochrome b_5 is inhibited 50% in the presence of the N_2G_1 fraction. The accuracy of these observations, however, can be questioned in view of the turbidity of the samples, especially those containing the N_2G_1 fraction. With the reservation just expressed, these observations suggest that the large molecular weight fraction, N_2G_1, does contain a cytochrome b_5 oxidase specific for the cytochrome b_5 in N_2G_2. Although N_2G_1 was collected as a single fraction, it was not completely excluded from the gel and should be amenable to further purification by gel filtration.

The information presented suggests that N_2G_2 supplies both cytochrome b_5 and lipid to the desaturase system with the N_2G_1 supplying the remainder of the complex and perhaps playing a structural role. The composition of the animal desaturase system may then be analogous to the soluble desaturase system of *Euglena gracilis* (Nagai and Bloch, 1968). In this organism stearyl acyl-carrier protein is desaturated to oleic acid in the presence of three soluble proteins: the TPNH oxidase, ferredoxin, and the desaturase.

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