PROTEIN FACTOR INVOLVED IN FATTY ACID DESATURATION OF LINOLEIC

ACID

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## INTRODUCTION

It has been established that the enzyme system involved in
stearyl-CoA desaturation reaction has three integral components of
the microsomal membrane: the NADH-cytochrome $b_5$ reductase, cytochrome
$b_5$ and the desaturase (Gaylor et al, 1970; Holloway et al, 1970;
Holloway, 1971; Oshino et al, 1971, Shimakata et al, 1972). All
these components have been separated and purified (Strittmatter et
al, 1974; Enoch et al, 1976).

Linoleic acid and other fatty acids are considered to be
desaturated in liver microsomes by a similar system, requiring ATP,
CoA, $Mg^{++}$, NADH and oxygen (Brenner et al, 1966; Brenner et al,
1969; Castuma et al, 1972).

In our laboratory we were able to separate an additional
"soluble factor" loosely bound to the microsomes that was necessary
for the full linoleic acid desaturation activity of isolated
microsomes "in vitro" (Catalá et al, 1972; Catalá et al, 1975;
Nervi et al, 1975). Further information is reported here.

## EXPERIMENTAL PROCEDURE

$1-|^{14}C|$-linoleic acid, 58 mCi/mmole, was provided by the
Radiochemical Center, Amersham, England. Cofactors for desaturation
reaction were provided by Boehringer Argentina, Buenos Aires, Argen-

tina.  Sephadex and DEAE cellulose were provided by Sigma Chem. ( ).
St. Louis, Mo.

Microsomes were prepared as mentioned before (Catalá et al,
1975).  The microsomes were extracted with a solution of 0.25 M
sucrose, 0.04 M NaF, 0.15 M KCl, 1.5 mM glutathione, 0.33 mM
nicotinamide and 0.04 M phosphate buffer (pH 7.0).  The extraction
was performed in the cold using the following proportions: 5 mg
of microsomal protein suspended in the homogenizing solution (1:1
v/v) was dropped on 3 ml of the extraction solution and shaken
during 15 min.  They were centrifuged at 110,000 xg in the cold.
Two fractions were obtained: the pellet, containing the extracted
microsomes (Me), and the supernatant (Sp), which contains the
"soluble factor".

Protein was determined either by a microburet method (Munkres
et al, 1965) or by the method of Lowry et al (1951).  Catalase ac-
tivity was determined according to Beers et al (1952).

Fatty acid desaturation was assayed incubating 60 nmoles of
labeled linoleic acid and 2.5 mg of microsomal protein for 25 min
at 35°C in 1.5 ml of a solution containing 0.25 M sucrose, 0.15 M
KCl, 1.5 mM glutathione, 0.04 M NaF,  1.3 mM ATP, 0.06 mM CoA, 0.87
mM NADH, 5 mM MgCl$_2$, 0.33 mM nicotinamide and 0.04 M phosphate
buffer (pH 7.0).  Reaction was stopped with KOH solution and fatty
acids sterified, analyzed by gas-liquid radiochromatography and
$\gamma$-linolenate formed calculated according to Catalá et al (1975).

The potency of the different fractions obtained in the
purification steps to reactivate the capacity of extracted microsomes
(Me) to desaturate linoleic acid  was measured using the same
procedure, except that the amount of Me used was the remainder after
the extraction of 2.5 mg of complete microsomes (M).

The partial purification of the factor was controlled by meas-
uring the specific reactivation capacity of the different fractions
added to Me and was expressed by the increase of nmoles of $\gamma$-linolen-
ate formed by Me after the addition of the investigated fraction.
It was calculated per min per mg of protein added to Me.

Partial concentration of the material was performed with XM-100
Amicon membrane, Sephadex G-100 and DEAE-cellulose chromatography.
This partially purified protein was obtained from two sources: the
material loosely bound to the microsomes (Sp) and the cytosol (C).

400 ml of Sp, containing 120 mg of protein was concentrated
to 20 ml through the XM-100 Amicon membrane.  The final concentration
was 2.2 mg/ml. 5 ml of this fraction, called Sp FI, was applied to
a 1.5 x 75 cm Sephadex G-100 column equilibrated with potassium

phosphate buffer 0.02 M, 0.1 mM EDTA, (pH 7.4). The front fractions corresponding to volumes 35 to 45 ml were pooled and called Sp FII. 10 ml of this solution was directly poured on a 1 x 4 cm DEAE cellulose column, previously equilibrated with Tris-HCl buffer 0.02 M (pH 8.5). The unadsorbed material was collected washing the column with 15 ml of the same buffer. This fraction was called Sp FIII and was able to reactivate the Me in the linoleic acid desaturation reaction.

The other source of soluble factor was the cytosol. It was called C in Table 2. 3 ml of cytosol containing 90 mg of protein was directly applied to a 1.5 x 75 cm Sephadex G-100 column, equilibrated as mentioned before. The front peak, corresponding to volumes of 29-42 ml, was called C FI. 13 ml of this fraction, containing 17 mg protein, was applied to a 2.5 x 2 cm DEAE-cellulose column and the unadsorbed material was collected in the same way as mentioned before. This fraction was called C FII. Each fraction was assayed as mentioned before.

RESULTS AND DISCUSSION

Complete microsomes (M), extracted with a low ionic strength solution lost most of their linoleic acid desaturation activity. The extracted microsomes (Me) recovered the desaturation activity after the readdition of supernatant (Sp). The activity of Me was also recovered when the cytosol (C) was added to Me. Sp had no activity "per se" (Table 1).

Partial concentration of the material was obtained by XM-100 Amicon membrane, Sephadex G-100, and DEAE-cellulose, as is described in the experimental part. This partially purified protein fraction was obtained from two sources, the material loosely bound to the microsomes and the cytosol. Table 2 shows the reactivation capacity of different purified fractions when added to Me. The reactivation capacity is expressed as the increase in nmoles of γ-linolenate synthesized per min and per mg of protein fraction added to Me.

After the G-100 step, the activity of Sp was increased three times and the cytosol activity four times. The DEAE-cellulose step concentrated similarly the activity of both fractions. The active fractions were separated in both cases at the lowest concentration of Tris-HCl buffer, showing a low superficial charge. The concentration achieved was 38 times for the Sp material and 74.6 times for the cytosolic material. Quantities as low as 10 μg of protein factor of both fractions were enough to recover almost 100% of the desaturation capacity of the extracted microsomes. Therefore, it could be possible that the cytosolic supernatant factor is the same as the one found stuck to the unwashed microsomes and separated from the Sp.

Table 1.  Extraction of a microsomal factor involved in linoleic acid desaturation.

Assay conditions and extraction of microsomes (see methods).

| FRACTIONS | RELATIVE DESATURATION ACTIVITY |
|---|---|
| M (complete microsomes) | 100.0 |
| Me (extracted microsomes) | 40.0 |
| Sp (supernatant) | not detectable |
| Me + Sp | 96.2 |
| Me + C (cytosol/110.000 xg) | 98.1 |

The capacity of Sp to reactivate Me is inhibited by trypsin digestion showing that a protein structure is necessary in Sp to reactivate Me.  Besides, heated or boiled Sp are also inactives. Different diets modify Me activity but have no effect on Sp. (Nervi et al, 1975).

Table 2.  Partial purification of a protein factor involved in linoleic acid desaturation.

Assay conditions and extraction of microsomes (see methods)

| FRACTIONS | μg OF PROTEIN FRACTION ADDED TO Me | SPECIFIC REACTIVATIONS CAPACITY OF DIFFERENT FRACTIONS ON Me <br> $\Delta$nmoles  $\gamma$-18:3 <br> min x mg protein added to Me |
|---|---|---|
| Me | -- | -- |
| Me + Sp FI | 500 | 0.30 |
| Me + Sp FII | 100 | 0.88 |
| Me + Sp FIII | 10 | 11.4 |
| Me + C | 1000 | 0.15 |
| Me + C FI | 200 | 0.60 |
| Me + C FII | 10 | 11.2 |

The presence of lipids has been detected in Sp.  The resolution
of lipid class was achieved in silica gel G thin layer chromatography
showing the presence of phosphatidyl choline, triacylglycerols,
cholesterol and cholesterol esters.  However the extraction of
lipids with ether did not deactivate Sp. (Catala et al, 1975).

The capacity of albumin to reactivate stearate $\Delta 9$ desaturase
was investigated by Jeffcoat et al (1976).  Although the ability of
albumin to reactivate the Me $\Delta 6$ desaturation capacity had already been
studied by Catalá et al (1975), it was reinvestigated in the present
work.  Fig. 1 shows that Me is reactived by Sp but very little
effect is shown by albumin.  This means that Sp would be more active
and specific than albumin to reactivate Me in this reaction.  The
effect exerted by albumin is only shown at low concentrations.

Fig. 2 shows the effect of linoleic acid concentration on $\Delta 6$
desaturase activity of complete and extracted microsomes.  It is
apparent that the specific activity of $\Delta 6$ desaturase for different
substrate concentrations is lower for Me than for complete
microsomes (M).  Therefore Sp enhances the desaturation of linoleic
acid.  Maximal velocity is higher for M than for Me in our reaction
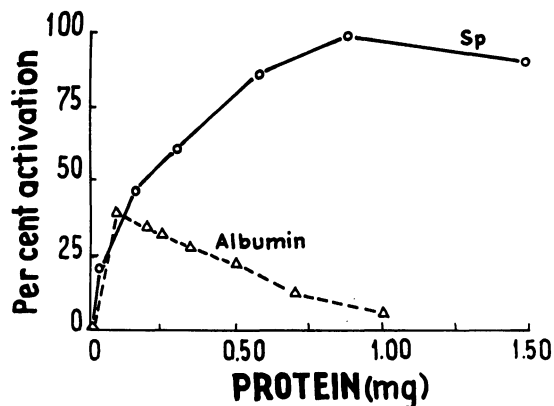conditions.



Fig. 1.  Effect of different concentrations of protein factor (Sp)
and bovine serum albumin on the restoration of linoleate desaturation
activity.

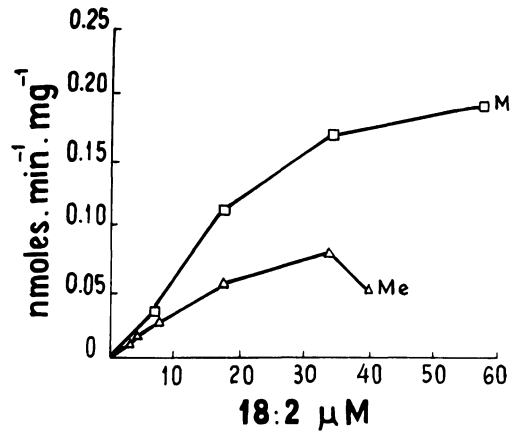Extraction of microsomes and assay conditions (see methods).

Fig. 2.  Effect of linoleic acid concentration on Δ6 desaturase
activity of complete microsomes (M) and extracted microsomes (Me).

Extraction of microsomes and assay conditions (see methods).

We also checked the effect of catalase on Me since a recent
report of Baker et al, (1976) has shown that this enzyme enhances
the stearyl-CoA desaturation.  For this reason the catalase activity
of Sp, Cytosol and other fractions tested in the reactivation of
Me were measured.  Sp and the cytosol have catalase activity but
the last one was relatively more active than the first one.  Fig. 3
shows the comparative reactivation capacity of Sp, cytosol, DEAE
Cellulose fraction and pure catalase on Δ6 desaturation and the
content of units of catalase of each fraction.  Results demonstrate
that there is no correlation between the Sp reactivation capacity
of Δ6 desaturation reaction and their catalase activity content.
Pure catalase has less reactivation capacity than Sp.

## CONCLUSIONS

A protein (or proteins) loosely bound to the microsomes is
necessary to obtain full activity in the linoleic acid desaturation
reaction in an "in vitro" system.  This protein (or proteins) is
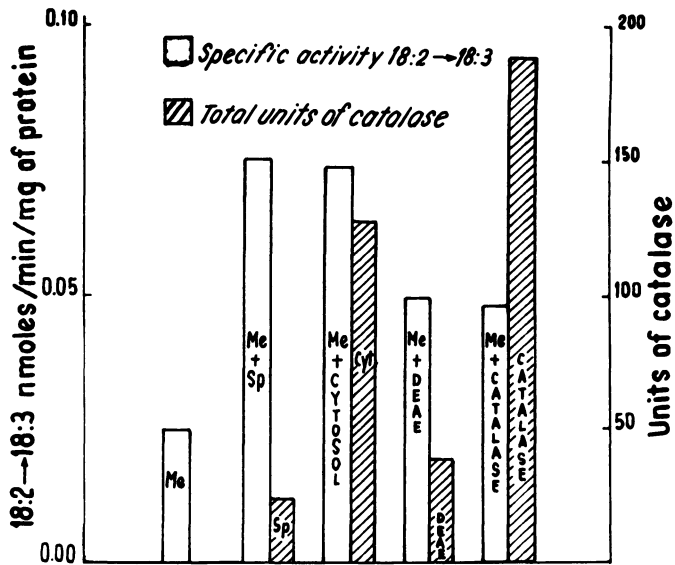extractable from unwashed microsomes with low ionic strength

Fig. 3.  Reactivation of Me by different protein fractions in the linoleic acid desaturation reaction.

Each fraction is indicated in the respective bar.

solution.  Cytosol has also capacity to restore Δ6 desaturation of Me.  Therefore it is considered that the protein factor present in the cytosol loosely binds to the microsomes and reactivates Δ6 desaturation.  It is sensitive to temperature and trypsin treatment. It contains lipids, and sonification does not affect it.  Albumin cannot substitute for the protein factor.

The protein factor contains catalase activity and pure catalase reactivates the desaturation.  However there is no correlation between the protein factor reactivation capacity on Δ6 desaturation and catalase activity content.  Pure catalase has less reactivation capacity than Sp although it contains many more units of catalase activity.

So the cytosol contains a protein factor able to increase the microsomal velocity of linoleic acid desaturation.  This effect would be due to an unidentified protein component and to catalase.

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