Biochimica et Biophysica Acta, 684 (1982) 263–271
Elsevier Biomedical Press

BBA 79476

CROSS-LINKING STUDIES OF THE PROTEIN TOPOGRAPHY OF RAT LIVER MICROSOMES

LEONARD S. BASKIN and CHUNG S. YANG

Department of Biochemistry, CMDNJ-New Jersey Medical School, Newark, NJ 07103 (U.S.A.)

(Received April 23rd, 1981)

Key words: Cross-linking; Protein topography; Dithiobis(succinimidyl propionate); Membrane protein; (Rat liver microsome)

A cleavable cross-linking reagent, dithiobis(succinimidyl propionate), DSP, was used to study the topography of the proteins in the endoplasmic reticulum membrane of rat liver. Reaction of untreated (control), phenobarbital- or 3-methylcholanthrene-induced microsomes with 0.5 mM DSP for 30 min at 0°C resulted in the cross-linking of a protein with a molecular weight of about 52 000 to form an apparent dimer. In phenobarbital microsomes, a smaller amount of a 52 000-dalton protein also appeared in a dimer in the absence of DSP if *N*-ethylmaleimide was not included during homogenization. In phenobarbital and 3-methylcholanthrene microsomes, a 48 000-dalton protein was cross-linked by DSP to a protein of about 57 000. In all three types of microsomes, a protein with an M_r of about 52 000 was also cross-linked to a protein of about 79 000. In phenobarbital and control microsomes, cross-linking resulted in an oligomeric protein of approximate molecular weight 180 000 which contained three proteins, two with M_r of about 52 000 and one about 79 000. Under the cross-linking conditions, little or no denaturation of cytochrome *P*-450 and NADPH-cytochrome *c* reductase was observed. The aryl hydrocarbon hydroxylase activity was significantly inhibited by the bifunctional cross-linking reagent, DSP, but not by the monofunctional reagent *N*-succinimidyl-3-(4-hydroxyphenyl) propionate. However, attempts to regenerate the aryl hydrocarbon hydroxylase activity by cleavage of the disulfide linkage with 2-mercaptoethanol or dithiothreitol were not successful.

Introduction

The endoplasmic reticulum membrane of mammalian cells contains about 70% protein by weight [1]. Among these proteins are the NADPH-dependent monooxygenase system consisting of cytochrome *P*-450 and NADPH-cytochrome *P*-450 reductase; the NADH-dependent desaturase system consisting of NADH-cytochrome b_5 reductase, cytochrome b_5, and desaturase; and some other functionally related enzymes such as epoxide hydrase and UDP-glucuronyltransferase. The lateral topography and molecular interaction of these enzymes in the endoplasmic reticulum membrane are not well understood. While some results point to a heterogeneous distribution of microsomal enzymes, [2,3] other studies suggest considerable lateral mobility of the cytochrome b_5, *P*-450 and the reductases in the membrane [4–10].

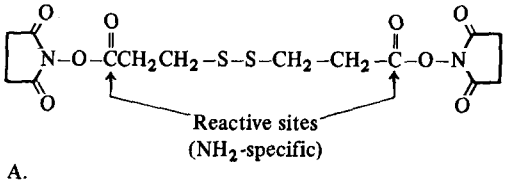
Experimental information on the detailed structural topography of the endoplasmic reticulum membrane is difficult to obtain. Electron microscopy of biological specimens are interpretable at best to a level of 20–30 Å [11,12]. X-ray diffraction spectroscopy or electron diffraction which is capable of resolution below 5 Å is of limited value in endoplasmic reticulum membrane model building. Chemical cross-linking is one generally applicable technique that can provide useful structural information in the range of 5 to 20 Å [11,13–15]. Towards this end Kreibich et al. [16] found that cross-linking with

Abbreviations: SDS, sodium dodecyl sulfate; DSP, dithiobis(succinimidyl propionate); SHP, *N*-succinimidyl-3(4-hydroxyphenyl) propionate.

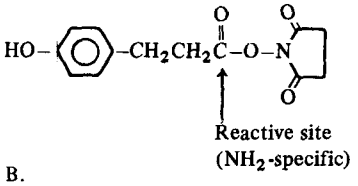
low concentrations of glutaraldehyde or the cleavable reagent methyl-4-mercaptobutyrimidate led to preferential cross-linking of the large subunits of bound ribosomes to two integral membrane proteins of the ribosome-binding site of rat liver rough microsomes. McIntosh and Freedman [17,18] have reported that cross-linking of β-naphthoflavone-treated rabbit liver microsomes with cupric phenanthroline led to the cross-linking of two proteins (53 000 and 57 000 daltons) with a linkage other than a disulfide bond. Omura et al. [19] cross-linked 3-methylcholanthrene-induced rat liver microsomes with glutaraldehyde and used an antibody (anti-*P*-450) column to assay for the cross-linking between cytochrome *P*-450 and the reductase. Their results suggested some extent of topographical association of the two enzymes. Previously, we have used the cross-linking approach to study the molecular organization in protein micelles consisting of purified cytochrome *P*-450 and NADPH-cytochrome *P*-450 reductase [20]. The present work intends to extend our study on the topography of microsomal proteins by chemical cross-linking with DSP in a nearest-neighbor analysis of proteins in the membrane.

Materials and Methods

Materials. The *N*-hydroxysuccinimidyl ester reagents shown in Scheme I, DSP and *N*-succinimidyl-3-(4-hydroxyphenyl) proprionate (SHP) were obtained from the Pierce Chemical Co. SDS, (DX2495), was obtained from Matheson Coleman and Bell. A gel electrophoresis molecular weight calibration kit was obtained from Pharmacia Inc. Cross-linked bovine serum albumin and other molecular weight standard proteins were obtained from Sigma Chemical Co. Phenobarbital was obtained from Merck & Co. and 3-methylcholanthrene from Mann Research Laboratories.



A.



B.

Scheme I. A. Bifunctional cross-linking reagent, DSP. B. Monofunctional reagent, SHP.

Microsome preparation. Liver microsomes were prepared from control (i.e., untreated), phenobarbital-treated or 3-methylcholanthrene-treated 100–120-g male Sprague-Dawley rats as previously described [10]. They will be referred to as control, phenobarbital, or 3-methylcholanthrene microsomes, respectively. The microsomes were suspended in 0.25 M sucrose and stored at −95°C. In experiments studying the effect of *N*-ethylmaleimide, it was added to the homogenization buffer (0.05 M Tris/1.15% KCl, pH 7.4) as an ethanol solution to give a final concentration of 8 mM *N*-ethylmaleimide and 1% ethanol.

Cross-linking reactions. Microsomes were suspended in a pH 7.4 buffer containing 0.1 M sodium phosphate, 3 mM $MgCl_2$, and 0.1 mM EDTA at a concentration of 4.8 mg protein/ml and sonicated 2 × 15 s. For cross-linking experiments, DSP was added to a final concentration of 0.5 mM in dimethylsulfoxide (final concentration 1%) and the reaction was allowed to proceed at 0°C for 30 min.

The reaction was stopped by mixing 500 μl sample with 50 μl 30% (w/w) SDS and 25 μl 0.8 M *N*-ethylmaleimide (in ethanol). After 15 min at 65°C, 5 μl 4 mM cytochrome *c* was added and the mixture was incubated for an additional 15 min at 65°C. This solution was mixed with 150 μl 60% (w/w) sucrose in 50 mM Tris-HCl, pH 6.8 and 25 μl 0.15% bromophenol blue. The samples were usually stored at −10°C and warmed at 37°C for 30 min prior to gel electrophoresis.

SDS-polyacrylamide gel electrophoresis. Unless otherwise stated, two-dimensional gel electrophoresis was carried out essentially as previously described [20]. Protein (160 μg) was applied to a 5 × 100 mm cylindrical 3.5% polyacrylamide gel [21]. The running buffer contained 50 mM sodium borate at pH 8.5. Electrophoresis was carried out at 24°C at 8 mA/gel for 5–6 h.

Gel electrophoresis in the second dimension was carried out at 24°C overnight at 30–40 V through buffered 1% agarose containing 10% 2-mercaptoethanol on a 3–15% polyacrylamide gradient gel slab (3 mm thickness) with a discontinuous 3% stacking gel similar to that of Laemmli [22]. An electrode buffer of 0.05 M Tris-HCl/0.38 M glycine/0.1% SDS (pH 8.3) was used. The liquified agarose was applied at 60°C. Molecular weight standards were run in slots made in the agarose. Gels were stained with Coomassie Blue R 250 and destained by the gradient procedure of Fairbanks et al. [23].

Other assays. Protein concentration was determined according to the method of Lowry et al. [24] with crystalline bovine serum albumin as a standard. Aryl hydrocarbon hydroxylase activity was determined according to the fluorometric method of Nebert and Gelboin [25] as described by Yang et al. [10]. Cytochrome *P*-450 concentration was measured with a Cary 17 recording spectrophotometer by the method of Omura and Sato [26].

Results

Optimal conditions for the cross-linking. Since DSP is easily cleavable and can be used at neutral pH, we chose this amino group directed reagent (which spans 11.9 Å between reaction centers) for our study. Cross-linking can be analyzed by diagonal gel electrophoresis following cleavage of the disulfide bond of the bifuctional reagent.

The microsomal protein concentration of 4.8 mg/ml was chosen to allow an appropriately small volume of sample to be applied to the first dimension gel as this gave better resolution in the second dimension run. The optimal concentration of 0.5 mM DSP was selected to minimize the appearance of very high molecular weight cross-linked material at the top of the first dimension gel. Cross-linking at 30°C usually led to the appearance of very high molecular weight material at the top of the first dimension gel. When cross-linking was carried out at 0°C, these high molecular weight aggregates were significantly decreased or not detected. Furthermore, when cross-linking with DSP was carried out at 30°C for 30 min, the microsomal aryl hydrocabon hydroxylase activity was inhibited by more than 70%; the NADPH-cytochrome *c* reductase activity was inhibited by 25–45% and cytochrome *P*-450 was not significantly affected. Similar experiments carried out at 0°C resulted in 35% inhibition of aryl hydrocarbon hydroxylase while cytochrome *P*-450 reductase activity and cytochrome *P*-450 were unaffected. For these reasons the cross-linking studies described here were those carried out at 0°C. The optimal time of reaction was found to be 30 min.

N-Ethylmaleimide effect on phenobarbital microsomes. When phenobarbital microsomes were analyzed by the diagonal gel electrophoresis system, an off-diagonal spot appeared (spot 2, Figs. 1A and 1C) with an apparent M_r of about 52 000 even in the absence of the cross-linking reagent. The spot originated from a protein with an M_r of about 105 000. This and the fact that the cross-linking was cleaved by 2-mercaptoethanol suggest that the spot is derived from a disulfide-linked dimer. In 3-methylcholanthrene or control microsomes this dimeric protein was not seen. To establish whether this dimer was naturally cross-linked via a disulfide bond or formed during the homogenization and preparation of the phenobarbital microsomes, 8 mM *N*-ethylmaleimide was added to the homogenization buffer. This resulted in the disappearance of spot 2 (Fig. 1B). Several other fainter off-diagonal spots (Figs. 1B and 1C) still exist in the *N*-ethylmaleimide-treated sample, suggesting they are proteins with naturally occurring interchain polypeptide disulfide bonds. The location of off-diagonal spot 22 above the diagnonal may be indicative of a protein with an intrachain polypeptide disulfide bond.

Cross-linking of phenobarbital microsomes with DSP. When phenobarbital microsomes were cross-linked with DSP, off-diagonal spot 2 of M_r 52 000 (Fig. 2) which originated from a protein with M_r 107 000 (Table I) increased in amount relative to the uncross-linked control. Spots 1, 3, 5 and 7 also arose as a result of cleaving chemically cross-linked proteins. Spot 3 with M_r 52 000 originated from a protein of 176 000 (Table I). Spot 1 with M_r 48 000 originated from a protein of 107 000. Spot 5, a 79 000 protein, originated from one of 140 000, and spot 7, a 79 000 protein, originated from one of 176 000. Faint spots 12, 15–18, 20 and 22 also appeared in the control; except for spot 22 which is above the diagonal, all other spots appeared below the diagonal. The chemically cross-linked proteins appear to be

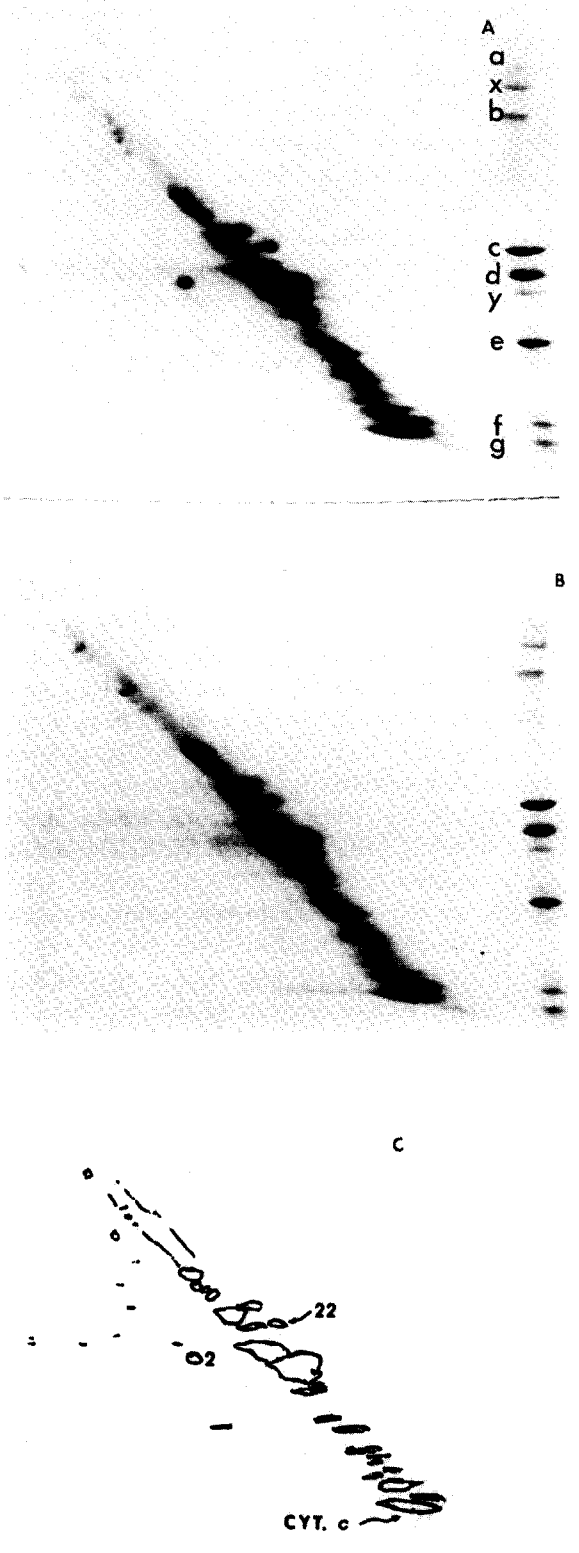


TABLE I

MOLECULAR WEIGHT OF OFF-DIAGONAL SPOTS OF CROSS-LINKED PHENOBARBITAL MICROSOMES

Data obtained from Fig. 2; M_r expressed in thousands.

Spot number	M_r	M_r of origin	ΔM_r
1	48	107	59
2	52	107	55
3	52	176	124
5	79	140	61
7	79	176	97
12	59	289	230
15	63	216	153
16	108	248	140
17	118	185	67
18	128	283	155
20	140	205	65
22	69	–	–

integral membrane proteins based on three types of evidence: (1) their molecular weights are relatively high compared to rat liver ribosomal proteins which are of molecular weight less than 42 000 [27], (2)

Fig. 1. Two-dimensional gel electrophoresis of phenobarbital microsomes. The liver was homogenized in 0.05 M Tris-HCl, pH 7.4, containing 0.15 M KCl: A, in the absence of *N*-ethylmaleimide and B, in the presence of 8 mM *N*-ethylmaleimide. The microsome sample (160 μg protein) was subected to electrophoresis in the first dimension on a cylindrical gel and subsequently subjected to electrophoresis in the second dimension in the presence of 2-mercaptoethanol on a slab gel. Panel C is a tracing of panel A. The spot numbers were selected after reviewing the data from phenobarbital, 3-methylcholanthrene and control microsomes. They were numbered sequentially according to increasing M_r. The column on the right side shows the mobilities of the protein standards: a (top band), thyroglobulin (330 kdalton); x, unidentified band in the kit; b, ferritin, half unit (220 kdalton); c, albumin (67 kdalton); d, catalase (60 kdalton); y, unidentified band; e, lactate dehydrogenase (36 kdalton); f, ferritin (18.5 kdalton); and g, cytochrome c (12.4 kdalton). Because the standards were applied in a well on the top of the slab gel, these proteins entered the separation gel faster than the sample proteins. For example, the cytochrome *c* band in the standard column was slightly below the cytochrome *c* spot in the sample (at the bottom of the diagonal line). For molecular weight determinations, a standard curve plotting log M_r versus R_f (relative to the mobility of cytochrome *c*) was used. The M_r of the off-diagonal spots and their extrapolated origins were determined by their R_f values. This approach was also used for the data in Fig. 2–4.

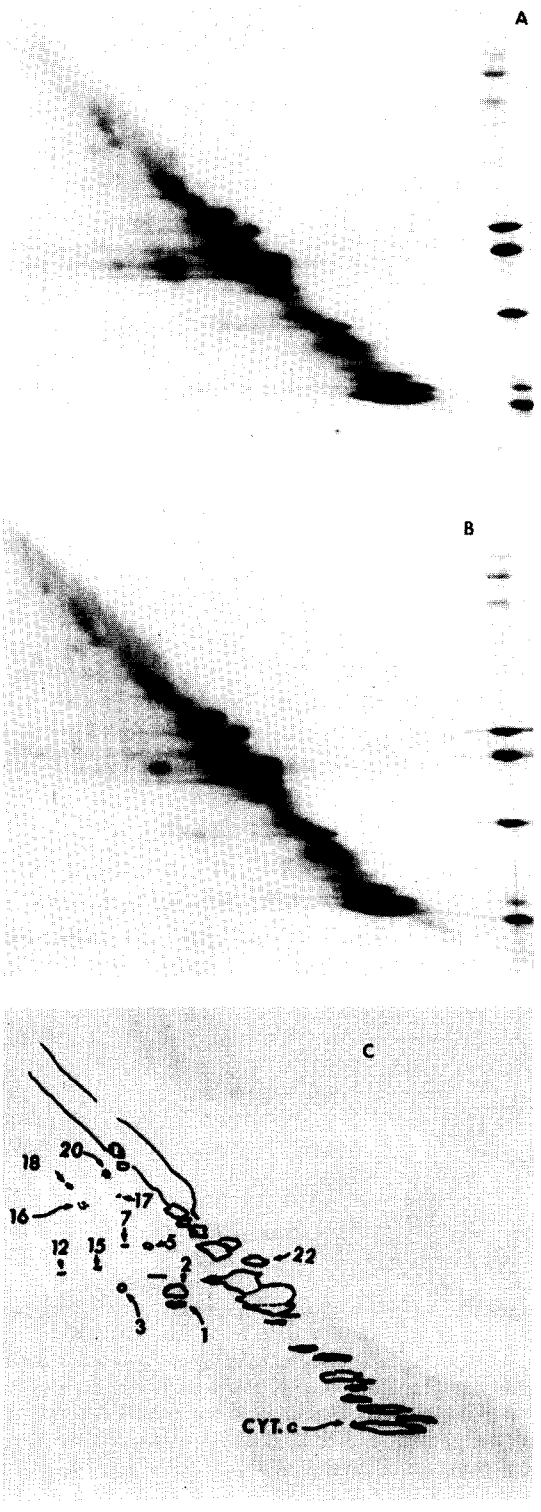


Fig. 2. Diagonal gel electrophoresis of cross-linked phenobarbital microsomes. A, microsomal protein (4.8 mg/ml) was cross-linked at 0°C for 30 min with 0.5 mM DSP, and 160 μg protein were subjected to two-dimensional gel electrophoresis. B, control, without DSP. C, tracing of panel A.

the EDTA-KCl wash step during the microsome preparation effectively removed most of the RNA from the microsomes (unpublished results) and indicates that most of the ribosomes attached to the rough endoplasmic reticulum were stripped during the washing procedure [28], and (3) washing the microsomes with 0.6 M KCl at pH 7.5 prior to cross-linking does not affect the basic pattern of these off-diagonal spots (unpublished results).

Cross-linking of 3-methylcholanthrene microsomes with DSP. When 3-methylcholanthrene microsomes were cross-linked with DSP, a 51 500 off-diagonal protein (spot 2, Fig. 3) appeared. This protein originated from a protein with an M_r of 102 000 (Table II). Spots 1, 4–6 and 8 also arose as a result of cleaving chemically cross-linked proteins. Spot 4, which is also of M_r 51 000, originated from a protein of 157 000. Spot 1 with M_r 47 000 originated from a protein of 102 000. Spot 6 with M_r 77 000 originated from a protein of 157 000, and spot 8 with M_r of 102 000 originated from a protein of 207 000. Spot 22 with a M_r of 71 000 appeared above the diagonal in a similar location to spot 22 in Fig. 2. Spots 10, 13 and 22 also occurred in the control.

Cross-linking of control microsomes with DSP. When control microsomes were cross-linked with DSP, a 52-kdalton protein (spot 2, Fig. 4) was found to form an oligomer with M_r 101 500 (Table III). Spots 3, 5, 7 and 9 also arose as a result of cleaving chemically cross-linked proteins. Spot 3, which also had M_r 52 000, originated from a protein of 180 000. Spot 5 with M_r 81 000 originated from a protein of 139 000. Spot 7 which was also 81 000 in M_r originated from a protein of 180 000. Spot 9 with M_r 110 000 originated from a protein of 255 000. Spots 10, 14, 19, 21 and 22 also appeared in the cross-linked control. Spot 22 with M_r 74 000 appeared above the diagonal in a similar location to the above-diagonal spot appearing in phenobarbital and 3-metylcholanthrene microsomes (spot 22, Fig. 2 and 3).

Effect of N-hydroxysuccinimidyl esters on monooxygenase activities of phenobarbital microsomes. When the microsomal aryl hydrocarbon hydroxy-

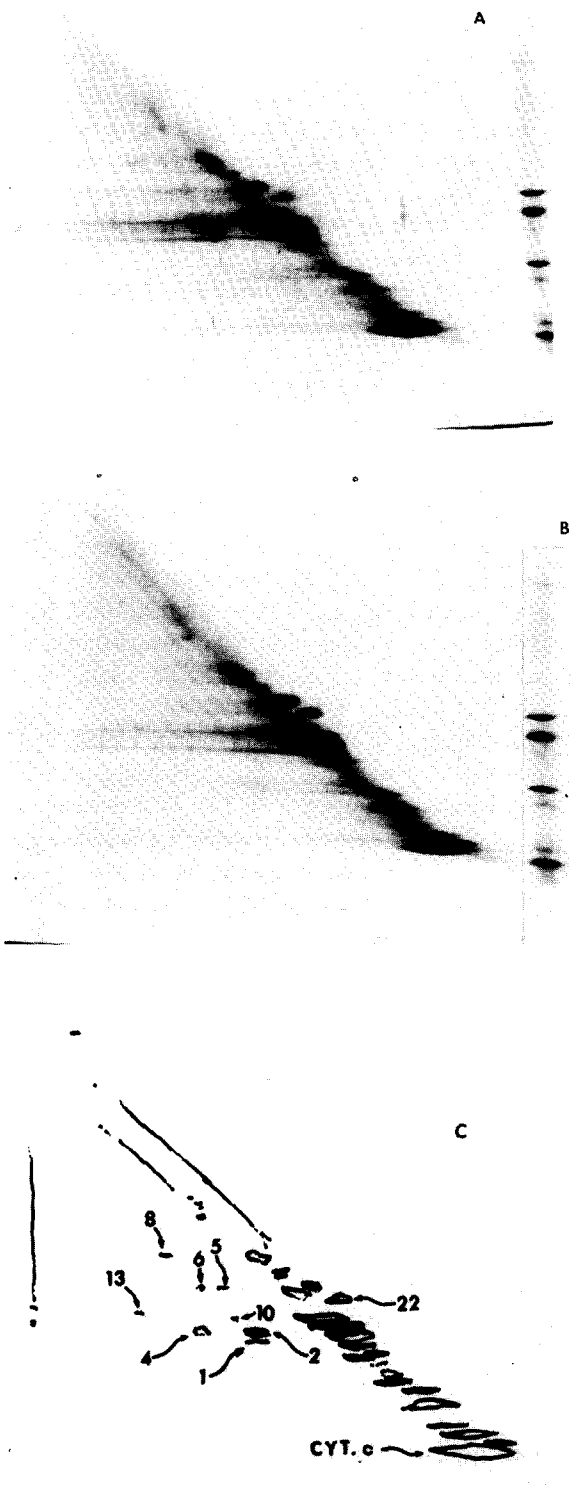


TABLE II

MOLECULAR WEIGHT OF OFF-DIAGONAL SPOTS OF CROSS-LINKED 3-METHYLCHOLANTHRENE MICROSOMES

Data obtained from Fig. 3; M_r expressed in thousands.

Spot number	M_r	M_r of origin	ΔM_r
1	47	102	55
2	51.5	102	50.5
4	51.5	157	105.5
5	77	135	58
6	77	157	80
8	102	207	105
10	58.5	117	58.5
13	58.5	235	176.5
22	71	–	–

lase activity of phenobarbital microsomes was measured after cross-linking with DSP at 0°C for 10 or 30 min, the aryl hydrocarbon hydroxylase was inhibited while the cytochrome *P*-450 content and the reductase activity were not significantly inhibited (Table IV). Since treating the microsomes with the corresponding monofunctional reagent, SHP, under identical conditions resulted in much less inhibition

TABLE III

MOLECULAR WEIGHT OF OFF-DIAGONAL SPOTS OF CROSS-LINKED CONTROL MICROSOMES

Data obtained from Fig. 4; M_r expressed in thousands.

Spot number	M_r	M_r of origin	ΔM_r
2	52	101.5	49.5
3	52	180	128
5	81	139	58
7	81	180	99
9	110	255	145
10	61	117	56
14	61	310	249
19	135	310	175
21	147	190	43
22	74	–	–

Fig. 3. Diagonal gel electrophoresis of cross-linked 3-methylcholanthrene microsomes. A, microsomal protein (4.8 mg/ml) was cross-linked at 0°C for 30 min with 0.5 mM DSP and 160 μg protein were subjected to two-dimensional gel electrophoresis. B, control, without DSP. C, tracing of panel A.

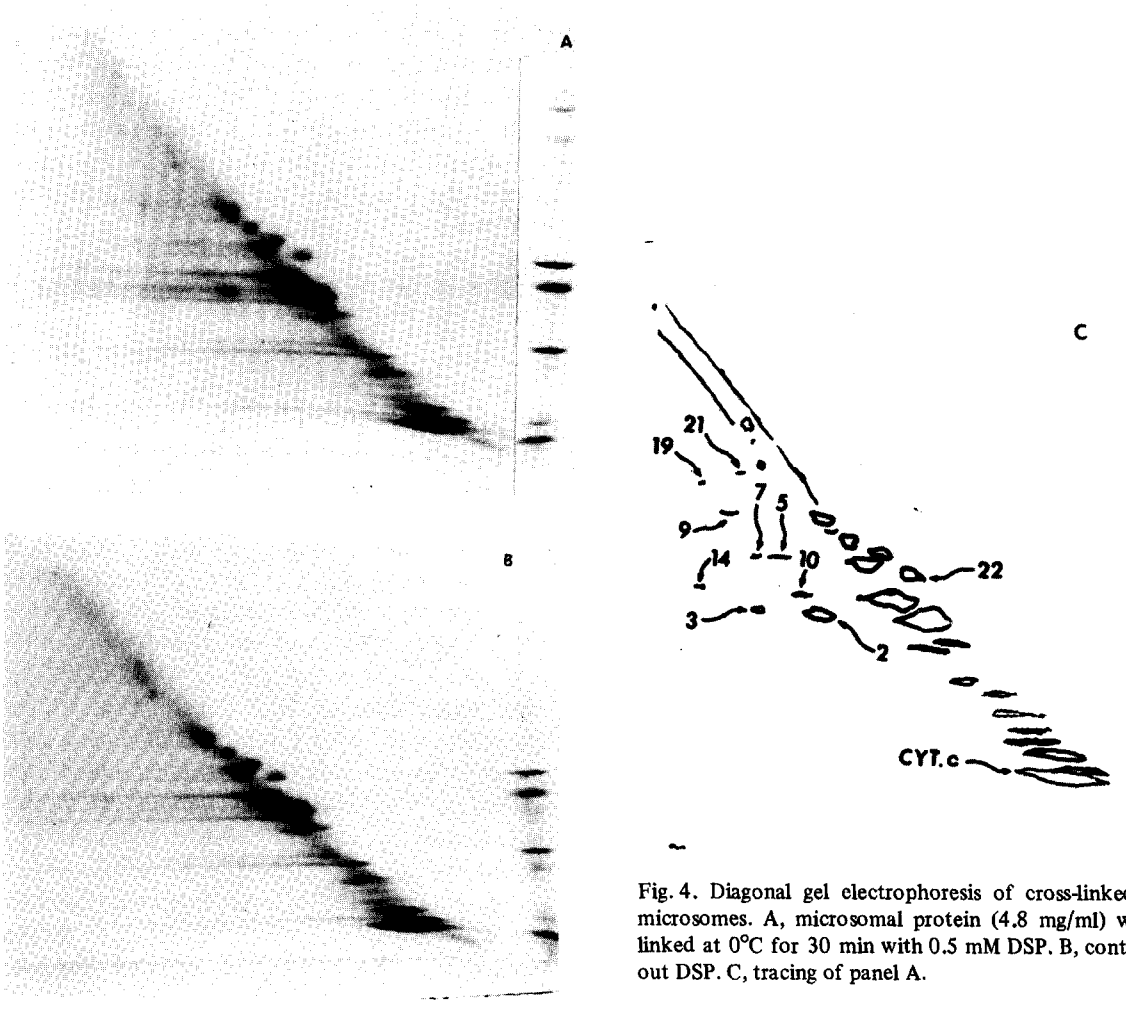


Fig. 4. Diagonal gel electrophoresis of cross-linked control microsomes. A, microsomal protein (4.8 mg/ml) was cross-linked at 0°C for 30 min with 0.5 mM DSP. B, control, without DSP. C, tracing of panel A.

TABLE IV

EFFECT OF *N*-HYDROXYSUCCINIMIDYL ESTERS ON MONOOXYGENASE ACTIVITIES

Phenobarbital microsomes (4.8 mg/ml protein) in 0.1 M phosphate/3 mM $MgCl_2$/0.1 mM EDTA, pH 7.4, were reacted with DSP or SHP at 0°C and stopped with 0.125 M glycine. The aryl hydrocarbon hydroxylase assay was carried out at 37°C. The activity is expressed as a percentage of the dimethyl sulfoxide (DMSO) control.

	Reaction time (min)	*P*-450	Reductase	Aryl hydrocarbon hydroxylase
1% DMSO	30	100	100	100
DSP (0.5 mM)	10	108	121	84
DSP (0.5 mM)	30	88	113	71
DSP (1.5 mM)	10	98	111	83
DSP (1.5 mM)	30	98	108	65
SHP (1.0 mM)	10	92	134	99
SHP (1.0 mM)	30	102	123	84
SHP (3.0 mM)	10	102	106	97
SHP (3.0 mM)	30	105	126	93

of the aryl hydrocarbon hydroxylase activity (Table IV), it is tempting to assume that cross-linking of cytochrome *P*-450 and the reductase with other proteins would reduce their mobility and thus decrease the aryl hydrocarbon hydroxylase activity. However, attempts to regain the aryl hydrocarbon hydroxylase activity by cleaving the cross-linking with 2-mercaptoethanol or dithiothreitol have not been successful (results not shown).

Discussion

Chemical cross-linking offers an experimental approach for studying protein-protein association in solution and in biological membranes. The cross-linking, however, can take place either between proteins within a complex or when two proteins come together by collision, as shown by Reactions 1 or 2 and 3, respectively.

$$\mathrm{PP} + \text{c-c} \xrightarrow[k'_1]{} \overset{\text{c-c}}{\mathrm{PP}} \xrightarrow{k_1} \overset{\text{c-c}}{\mathrm{PP}} \qquad (1)$$

$$\mathrm{P} + \text{c-c} \xrightarrow[k'_2]{} \overset{\text{c-c}}{\mathrm{P}} + \mathrm{P} \xrightarrow{k_2} \overset{\text{c-c}}{\mathrm{PP}} \qquad (2)$$

$$\mathrm{P} + \mathrm{P} + \text{c-c} \xrightarrow{k_3} \overset{\text{c-c}}{\mathrm{PP}} \qquad (3)$$

where PP or P represents proteins existing as part of a complex or a monomer and c-c is the bifunctional cross-linking reagent.

$$\overset{\text{c-c}}{\mathrm{PP}} \text{ and } \overset{\text{c-c}}{\mathrm{PP}} \quad \text{or} \quad \overset{\text{c-c}}{\mathrm{P}} \text{ and } \overset{\text{c-c}}{\mathrm{PP}}$$

are the intermediate and products, respectively, of the cross-linking reaction. The rate constants are k'_1, k_1, k'_2, k_2 and k_3. Since k'_1 and k'_2 have about the same value and the rate constant of a unimolecular reaction (k_1) is much faster than that of a bimolecular reaction (k_2), the rate of Reaction 2 is much lower than that of Reaction 1. The rate of the trimolecular reaction (3) is even lower than Reaction 2. Thus, the cross-linking between proteins in a molecular complex is much faster than that between proteins that come together by collision. This point is also illustrated by the recent results of Ji and Middaugh [29].

Since experimental conditions were selected to avoid the production of cross-linked complexes with very large molecular weights (more than 700 000) the cross-linking observed is believed to be between proteins existing in molecular complexes or between those closely located in the membrane. The horizontal streaks in the second-dimension gels appeared both in the chemically cross-linked and non-cross-linked samples, but was more apparent in the former. This is clearly shown in the 52 000 molecular weight region in Fig. 2, suggesting that the 52-kdalton protein is also cross-linked to many other proteins in addition to those identified in Table 1. The presently observed extent of cross-linking, as judged by the intensity of the off-diagonal spots, is low. This may mean that only a small fraction of proteins exists in complexes or that the efficiency of the cross-linking is low. In addition to the chemically cross-linked proteins, off-diagonal spots were also observed in the absence of cross-linking reagent. These are presumably derived from proteins linked by disulfide bonds. Spot 22, which appeared above the diagonal in control, phenobarbital and 3-methylcholanthrene microsomes, is probably a polypeptide which is compact when bridged by intrachain disfulfide bonds, but opens up and migrates more slowly in an electrophoretic field when reductively cleaved with 2-mercaptoethanol.

In all three types of microsome an integral membrane protein with M_r about 52 000 (spot 2) was cross-linked by DSP to form an apparent dimer. Its molecular weight and relatively large abundance suggest the protein may be cytochrome *P*-450. This would be similar to the report of McIntosh and Freedman [17,18] that the rabbit liver cytochrome *P*-450 can be cross-linked to form dimers. Of further interest is the finding that in both phenobarbital and 3-methylcholanthrene liver microsomes, but not in control microsomes, a 47–48-kdalton protein (spot 1) was cross-linked with a 55–59-kdalton protein. This suggests that the spot 1 protein or the protein to which it was cross-linked is inducible. Epoxide hydrase which is induced by phenobarbital and 3-methylcholanthrene [30] may be the spot 1 protein. Further studies are under way to identify the off-diagonal endoplasmic reticulum membrane proteins. Upon cross-linking the microsomes at 0°C, there were no cross-linked oligomers with M_r above 300 000

involving the 52-kdalton protein, as might be expected if such large oligomeric clusters exist in the membrane. However, after cross-linking the microsomes at 30°C, some of the cross-linked proteins did not enter the 3% stacking gel of a one-dimensional Laemmli gel under non-reducing conditons (data not shown), suggesting the existence of cross-linked protein with M_r greater than 700 000.

In all three types of microsome there is evidence that a protein with M_r about 52 000 was cross-linked to a protein of about 79 000. In phenobarbital and control microsomes the 52- and 79-kdalton proteins originated from an oligomer of about 180 kdaltons. The alignment of the two protein spots on the gel after cleavage of the cross-link suggested that they were components of the same 180-kdalton protein. Furthermore, the intensity of the 52-kdalton spot was greater than that of the 79-kdalton spot. This was consistent with a stoichiometry of two 52-kdalton proteins and one 79-kdalton protein in the complex. In 3-methylcholanthrene microsomes a protein of 51.5-kdalton and another of 77-kdalton both originated from a protein of 157-kdaltons; the intensities of the spots were about the same, suggesting a 1 : 1 stoichiometry in the complex. It cannot be ruled out at the present time, however, that the cross-linked proteins in the 157-kdalton region are trimers of the 51.5-kdalton protein plus dimers of the 77-kdalton protein.

As indicated, the evidence regarding the stoichiometry and composition of the complexes appears variable among the different types of microsomes. Phenobarbital-induced cytochrome *P*-450b [31] and NADPH-cytochrome *P*-450 reductase from rat liver have molecular weights of 52 000 and 80 000, respectively, as measured by SDS-polyacrylamide gel elecphoresis [20]. It is intriguing to speculate that these integral membrane proteins are closely located or may have some of their population existing in molecular complexes.

Acknowledgements

This work was supported by Grants CA-16788 from the U.S. National Cancer Institute and IN-92 from the American Cancer Society. The authors acknowledge the excellent technical assistance of Mrs. Shu-Min Ning.

References

1 DePierre, J.W. and Dallner, G. (1975) Biochim. Biophys. Acta 415, 411–472
2 DePierre, J.W. and Ernster, L. (1977) Annu. Rev. Biochem. 46, 201–262
3 Matusuura, S., Fujii-Kuriyama, Y. and Tashiro, Y. (1978) J. Cell Biol. 78, 503–519
4 Rogers, M. and Strittmatter, P. (1974) J. Biol. Chem. 249, 895–950
5 Rogers, M. and Strittmatter, P. (1974) J. Biol. Chem. 249, 5565–5569
6 Yang, C.S. and Strickhart, F.S. (1975) J. Biol. Chem. 250, 7968–7972
7 Yang, C.S. (1977) J. Biol. Chem. 252, 293–298
8 Yang, C.S. (1977) Life Sci. 21, 1047–1058
9 Yang, C.S., Strickhart, F.S. and Kicha, L.P. (1978) Biochem. Pharmacol. 27, 2321–2336
10 Yang, C.S., Strickhart, F.S. and Kicha, L. (1978) Biochim. Biophys. Acta 509, 326–337
11 Peters, K. and Richards, F.M. (1977) Annu. Rev. Biochem. 46, 523–551
12 Ottenmeyer, F.P. (1979) Annu. Rev. Biophys. Bioeng. 8, 129–144
13 Ji, T.H. (1979) Biochim. Biophys. Acta 559, 39–69
14 Freedman, R.B. (1979) Trends Biochem. Sci. 4, 193–197
15 Das, M. and Fox, C.F. (1979) Annu. Rev. Biophys. Bioeng. 8, 165–193
16 Kreibich, G., Freienstein, C.M., Pereyra, B.N., Ulrich, B.L. and Sabatini, D.D. (1978) J. Cell Biol. 77, 488–506
17 McIntosh, P.R. and Freedman, R.B. (1979) FEBS Lett. 105, 217–223
18 McIntosh, P.R. and Freedman, R.B. (1980) Biochem. J. 187, 227–237
19 Omura, T., Noshiro, M. and Harada. N. (1980) in Microsomes, Drug Oxidations and Chemical Carcinogenesis. (Coon, M.J., Conney, A.H., Estabrook, R.W., Gelboin, H.V., Gillette, J.R. and O'Brien, P.J. eds.) pp. 445–453, Academic Press, New York
20 Baskin, L.S. and Yang, C.S. (1980) Biochemistry 19, 2260–2264
21 Davies, G.E. and Stark, G.R. (1970) Proc. Natl. Acad. Sci. U.S.A. 66, 651–656
22 Laemmli, U.K. (1970) Nature 227, 680–685
23 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1977) Biochemistry 10, 2606–2617
24 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
25 Nebert, D.W. and Gelboin, H.V. (1968) J. Biol. Chem. 243, 6242–6249
26 Omura, T. and Sato, R. (1967) Methods Enzymol. 10, 556–561
27 Wool, I.G. (1979) Annu. Rev. Biochem. 48, 719–754
28 Roobol, A. and Rabin, B.R. (1977) FEBS Lett. 14, 165–169
29 Ji, T.H. and Middaugh, C.R. (1980) Biochim. Biophys. Acta 603, 371–374
30 Lu, A.Y.H. and Miwa, G.T. (1980) Annu. Rev. Pharmacol. Toxicol. 20, 513–531
31 Ryan, D.E., Thomas, P.E., Korzeniowski, D. and Levin, W. (1979) J. Biol. Chem. 254, 1365–1374