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TWO-DIMENSIONAL ELECTROPHORESIS OF MEMBRANE PROTEINS

FACTORS AFFECTING RESOLUTION OF RAT-LIVER MICROSOMAL PROTEINS

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A comparison has been made of published techniques for the resolution of rat liver microsomal proteins by two-dimensional electrophoresis. The method of Kaderbhai and Freedman (Biochim. Biophys. Acta 601 (1980) 21–20) gives good resolution of acidic proteins but excludes hydrophobic integral membrane proteins of p*I* > 7, including cytochrome *P*-450 apoproteins. The method of Vlasuk and Walz (Anal. Biochem. 105 (1980) 112–120) gives good resolution of proetins of p*I* 5–8, including cytochromes *P*-450, but fails to resolve a major acidic protein of p*I* < 5. Isoelectric focusing of microsomal proteins is improved by the use of high concentrations of urea and low concentrations of sample proteins. Zwitterionic detergents of the general formula $R \cdot N^+(CH_3)_2 \cdot CH_2CH_2CH_2SO_3^-$ are effective in solubilizing microsomal proteins, either alone or in presence of non-ionic detergent; compounds with a long alkyl chain (C_{14} or C_{16}) are most effective. Isoelectric focusing of microsomal proteins solubilized by zwitterionic detergents did not give good resolution, probably because of incomplete dissociation and denaturation of the proteins. These detergents could not be used in the presence of high concentrations of urea. Although no single method of two-dimensional electrophoresis gives complete resolution of the whole range of microsomal proteins, conditions can be optimized for specific sets of proteins of interest. The technique can be used to monitor differences in microsomal composition between rat strains, or following induction, and for a variety of other studies.

Introduction

Many questions about the protein components of the endoplasmic reticulum membrane – questions about the transverse and lateral dispositions of these proteins, about their biosynthesis and post-translational modification, and about their specific induction – could be successfully tackled if the large number of proteins in this subcellular fraction could be completely resolved by a reproducible analytical technique. Conventional one-dimensional gel electrophoresis resolves microsomal proteins into 50–60 bands [1,2] but it is clear that resolution is incomplete and many bands, especially in the molecular weight range 45 000 to 60 000 are superposed.

A standardized technique for resolution of microsomal proteins by electrophoresis in two dimensions, based on the general procedure of O'Farrell [3], would be enormously valuable. Separation by isoelectric focusing, in addition to SDS-polyacrylamide gel electrophoresis, should allow resolution of the many superposed bands of proteins of similar molecular weight, and should also allow one to detect post-translational modifications – both of membrane and luminal content proteins – which are often not accompanied by significant changes in molecular weight. However, there are major difficulties in the isoelectric focusing of membrane proteins, which are associated with the hydrophobicity of integral membrane proteins. Such proteins are generally soluble only

in the presence of powerful ionic detergents, while isoelectric focusing requires that the proteins be in solution in a form in which their intrinsic polyelectrolyte characteristics are not obscured. In the endoplasmic reticulum, the numerous cytochrome *P*-450 species are among the most prominent hydrophobic integral proteins.

Two procedures for the resolution of microsomal proteins by two-dimensional gel electrophoresis have been published which attempt to circumvent these difficulties. One [4] employs an initial extraction to reduce the concentration of hydrophobic polypeptides, followed by solubilization in non-ionic detergent, urea and SDS for isoelectric focusing; the SDS is removed from the proteins early in the isoelectric focusing. The other [5] employs acidification of the sample to a pH close to that of the anodic buffer, in the presence of a high concentration of urea, to denature and solubilize basic, hydrophobic polypeptides. Neither technique is entirely successful in solubilizing and resolving all microsomal polypeptides.

In this work, we have set out to compare the two published methods for resolution of microsomal polypeptides and to examine factors affecting resolution in an attempt to design a standardized procedure. In particular, we have tested the suitability of using zwitterionic detergents for solubilization of the sample prior to the first-dimensional separation. These compounds contain a sulphonic acid group and a quaternary ammonium centre, and are therefore zwitterionic, with zero net charge over a wide pH range. They should have the solubilizing power of conventional ionic detergents without conferring any net charge on the solubilized proteins [6]. We conclude that no single method of two-dimensional electrophoresis gives optimal recovery and resolution of all the polypeptides in this fraction, but that for any given set of polypeptides of interest, conditions can be chosen to give excellent and reproducible resolution, making possible a wide range of applications.

Materials and Methods

Materials. Reagents were generally AR grade, except for urea, which was Aristar, and gel components, which were electrophoresis grade. Ampholines were from LKB (Sweden), zwitterionic detergents (zwittergents) were from Calbiochem-Behring (USA) and Sepharose 2B was from Pharmacia Fine Chemicals (Sweden). Molecular weight markers for SDS polyacrylamide gel electrophoresis were from Sigma (U.K.) and were β-galactosidase, phosphorylase *b*, bovine serum albumin, pyruvate kinase, ovalbumin, aldolase and cytochrome *c*. [^{14}C]Formaldehyde (10–20 mCi/mmol) was purchased from Amersham International (U.K.). Materials for autoradiography were EN^{3}HANCE (New England Nuclear, F.R.G.) and Kodak X-ray film (U.K.).

Microsomes. Male Sprague-Dawley CD strain rats (150–200 g) were starved overnight before being killed by cervical dislocation. The livers were removed into ice-cold 0.25 M sucrose/50 mM Tris-HCl (pH 7.5)/25 mM KCl/5 mM $MgCl_2$ (buffer 1). All subsequent procedures were carried out at 0–4°C. Livers were rinsed and minced with scissors in buffer 1 before being homogenized in 1.5 vol of buffer 1 using a glass-Teflon motor-driven homogenizer (six strokes). The homogenate was centrifuged at $600 \times g_{av}$, 10 min, and the supernatant was then centrifuged at $13\,000 \times g_{av}$, 10 min. The resultant post-mitochondrial supernatant was loaded on to a column of Sepharose 2B and the microsomes were eluted with buffer 1 (following Refs. 7, 8). The microsome suspension was centrifuged at $105\,000 \times g_{av}$, 90 min, and the pellet was resuspended to about 20 mg/ml in 0.1 M phosphate (pH 7.4)/20% (v/v) glycerol. Microsomes were stored in aliquots at −20°C. Protein was determined by the procedure of Lowry et al. [9] using crystalline bovine serum albumin as standard.

Radioactive labelling by reductive methylation. Nonidet P-40 was added to 30 μl of a microsomal suspension (15 mg/ml protein) to a final detergent concentration of 4% (w/v). To this was added 5 μl (approx. 50 μCi) of H^{14}CHO, and then after 30 s, 10 μl freshly made 2 M $NaCNBH_3$. The reaction was allowed to proceed on ice for 30 min and then quenched by addition of 200 μl 0.1 M sodium/potassium phosphate buffer (pH 7.4)/2% (w/v) Nonidet P-40. The reaction mixture was dialyzed overnight against 100 ml of the quenching solution and was then stored at −70°C. The method is based on that of Finger and Choo [10].

Solubilization by zwitterionic detergents. Microsomes were diluted to 2 mg/ml final protein concentration with addition either of zwitterionic detergent (to 2%, w/v) or of deionized Triton X-100 plus zwitterionic detergent (each 1%, w/v). The preparations were centrifuged at $105\,000 \times g_{av}$, 90 min; the supernatants containing solubilized protein were retained and protein was determined as above.

Isoelectric focusing – general methods. Isoelectric focusing was performed in cylindrical gels, 11 cm long, 0.27 cm internal diameter. Except in experiments with zwitterionic detergents (see below), the gel composition was polyacrylamide (monomer at 4% (w/v))/9 M urea/2% (w/v) Nonidet P-40. The composition of ampholines was varied. For optimal resolution of neutral and acidic proteins, wide-range ampholines alone (pH 3.5–10) were used; a mix of 0.5% (w/v) pH 8–9 plus 1.5% (w/v) pH 3.5–10 extended the basic region of the gel and gave optimal resolution of basic proteins including cytochrome *P*-450 apoproteins. The cathode buffer was 20 mM NaOH, the anode buffer 10 mM H_3PO_4. Gels were prefocused for 30 min at 200 V and focusing was for 12–18 h at 400 V, with maximum current of 6 mA (eight tubes).

Sample preparation techniques for isoelectric focusing. (i) Deoxycholate extraction (modified from Ref. 4). Microsomes were brought to 0.4% (w/v) deoxycholate, incubated for 30 min on ice and centrifuged at $105\,000 \times g_{av}$, 90 min. The supernatant (extract) deoxycholate was taken and stored at −20°C until required. It was then diluted to 10 mg/ml and dialysed against 9 M urea/1% (w/v) SDS/5% (v/v) β-mercaptoethanol for 1 h. The isoelectric focusing gel was initially overlaid (at the cathode end) with 8 M urea, and this was replaced for prefocusing by 9 M urea/8% (w/v) Nonidet P-40/0.5% (w/v) ampholines (pH 3.5–10)/5% (v/v) β-mercaptoethanol. After prefocusing, the overlay was removed and the sample containing 250 μg protein was applied at the cathode end and overlaid with a solution containing 5 M urea but otherwise identical to the prefocusing overlay.

(ii) Acidification (modified from Ref. 5). Microsomes were diluted to 10 mg/ml and then added to 1 vol. of lysis buffer comprising 10 M urea/3% (w/v) Nonidet P-40/2%(w/v) ampholines (pH 3.5–10)/5%(v/v) β-mercaptoethanol. Crystalline urea was added to saturation, and the sample was adjusted to pH 4.3 with 3 M HCl. A further volume of lysis buffer was then added, and the urea addition and acidification was repeated. This process was carried out at 25°C to increase the amount of urea which could be added.

The isoelectric focusing gel was set up anode uppermost, and the cathodic end was protected from electrode buffer by being placed in a well of lysis buffer contained in dialysis tubing. The anode was overlaid with lysis buffer for prefocusing. The overlay was then removed, sample (130 μg protein) was loaded at the anode and overlaid with 8 M urea/0.5% (w/v) ampholines (pH 3.5–10)/5% (v/v) β-mercaptoethanol, adjusted to pH 4.3.

(iii) Titration to alkaline extreme. This was carried out as for the method of Vlasuk and Walz [5], except that instead of being acidified, the sample was adjusted to pH 9.8 with 10 M NaOH. The sample overlay was likewise adjusted to pH 9.8, the electrode polarity was reversed and the sample was applied at the cathode.

(iv) Focusing in presence of zwitterionic detergent. Microsomes were diluted to 4 mg/ml and brought to 2% (w/v) Triton X-100/2% (w/v) zwittergent 3-16/1.5% (w/v) ampholines (pH 3.5–10)/1 mM EDTA/2 mM β-mercaptoethanol. Gels comprised 4% (w/v) acrylamide, 0.05% (w/v) Triton X-100, 0.05% (w/v) zwittergent 3-16, 2% (w/v) ampholines (pH 3.5–10) and either sucrose (30%, w/v), glycerol (20%, v/v) or 4 M urea. For gels containing sucrose or glycerol, the sample buffer contained glycerol (10%, v/v); for gels containing urea, sample buffer contained 4 M urea. Gels were overlaid with sample buffer and prefocused. Samples containing 200 μg protein were loaded at the cathode and overlaid with 2% (w/v) Triton X-100/2% (w/v) zwittergent 3-16/1.5% (w/v) ampholines (pH 3.5–10)/1 mM EDTA/2 mM β-mercaptoethanol/7% (v/v) glycerol. After focusing, the gels were frozen for 10 min before loading on to the second dimension, to avoid fragmentation.

Measurement of pH gradient.

Gels were focused after loading with overlay or lysis buffer and sliced into 0.5-cm segments. These were placed in 0.5 ml degassed water and left to

equilibrate overnight before the pH was measured.

SDS-polyacrylamide gel electrophoresis. The second-dimension resolution was carried out using the SDS-polyacrylamide system and Laemmli [11] buffers, with a 4% stacking gel and 7.5–16% (w/v) acrylamide gradient separating gel slab, 2 mm thick. Focused tube gels were held in position with 1% (w/v) agarose/2.3% (w/v) SDS/10% (v/v) glycerol/0.06 M Tris-HCl (pH 6.8)/5% (v/v) β-mercaptoethanol/0.02% (w/v) Bromophenol blue. Electrophoresis was carried out at 250 V, 30 mA/gel until the marker dye was within 3 cm of gel bottom. After electrophoresis, gels were fixed overnight at 25% (v/v) propan-2-ol/10% (v/v) acetic acid, before staining with 0.25% (w/v) Coomassie brilliant blue R250/50% (v/v) methanol/5% (v/v) acetic acid. For silver staining, gels were fixed overnight in 50% (v/v) methanol and stained according to Wray et al. [12]. Gels for autoradiography were fixed overnight in 25% (v/v) propan-2-ol/10% (v/v) acetic acid, treated with EN^{3}HANCE (according to the makers' instructions) and dried (Bio-Rad gel drier) on to Whatman 3MM paper. Autoradiography was carried out for 1 week at −80°C, using Kodak X-ray film.

Results and Discussion

Comparison and modification of published procedures

The procedure of Kaderbhai and Freedman [4] is based on a modification for membrane proteins [13] of the original O'Farrell method [3]. Ames and Nikaido [13] introduced solubilization of the sample proteins in SDS followed by the addition of a large excess of nonionic detergent to displace the ionic detergent from the solubilized proteins. Isoelectric focusing is then from the cathode (basic) and free SDS is rapidly removed by electrophoresis. When applied to microsomal membranes, this method was unsuccessful, since a large fraction of the sample protein came out of solution on removal of SDS and therefore formed an aggregate at the basic end of the isoelectric focusing gel. To circumvent this, Kaderbhai and Freedman [4] introduced an initial extraction of the membrane preparation to obtain a sample in which the concentration of highly hydrophobic polypeptides was reduced. Two-dimensional gel electrophoresis of such extracts gives excellent resolution, particularly of polypeptides in the acidic region (Fig. 1). In this region there is no streaking and repeat gels of identical samples are entirely reproducible. Almost 100 neutral and acidic polypeptides are clearly resolved. Three major acidic polypeptides of M_r 50–60 kDa are resolved which, on SDS-polyacrylamide gel electrophoresis alone, overlap with each other and with the numerous cytochrome *P*-450 isoenzymes. One of these major polypeptides has been identified as the enzyme protein disulphide-isomerase [14]. However, the deliberate exclusion of some of the most hydrophobic polypeptides gives the basic region of the gel a featureless appearance with some streaking and few, poorly resolved spots. A variable amount of aggregate forms and proteins of $pI > 7$ clearly come out of solution during isoelectric focusing.

The procedure of Vlasuk and Walz [5] is more radical in that the solubilized sample is titrated to pH 4.3, close to that of the anode buffer, and loaded at the anode (acidic) end of the isoelectric focusing gel. Solubilization is by non-ionic detergent and a high concentration of urea. Basic polypeptides are far better resolved by this technique than by the former, and the basic region of the gel gives a clear, detailed and complex pattern of

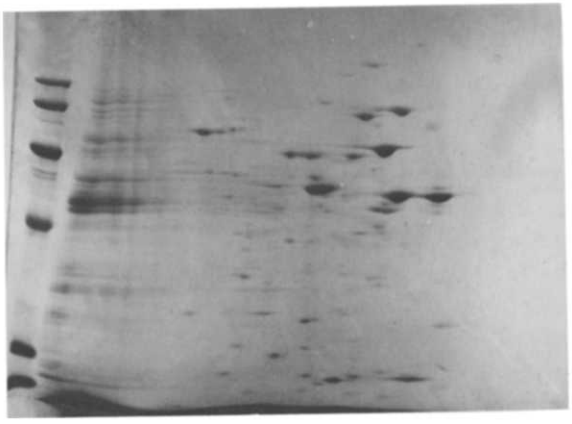

Fig. 1. Two-dimensional electrophoresis of microsomal proteins using the extraction method. Microsomal proteins were extracted into deoxycholate and subjected to two-dimensional electrophoresis with isoelectric focusing from the cathode, following the method of Kaderbhai and Freedman [4]. The sample loaded contained 250 μg protein and staining was with Coomassie blue. See Materials and Methods for full details.

spots (Fig. 2). Streaking of the highly insoluble basic polypeptides is not entirely overcome, but the technique does permit resolution of these very intractable polypeptides with good reproducibility (compare Fig. 2 and Fig. 3, below). The neutral and acidic region is similar to that observed with the former technique, except that a very acidic major polypeptide (M_r 54000, pI < 4.3) is either not detected or forms a faint streak at the acidic end of the gel. This polypeptide is clearly too acidic to enter the isoelectric focusing gel using the standard procedure of Vlasuk and Walz [5] and is not observed in their published electrophoretograms. Minor modifications of the technique, such as acidification to pH 4.0, have not been successful in producing reproducible retention and focusing of this polypeptide (not shown).

The important step in the technique of Vlasuk and Walz [5] is the acidification of the sample, not simply its adjustment to the electrode pH. When a sample was prepared in a similar manner but adjusted to pH 9.8 and loaded at the cathode (basic) end of the gel, considerable aggregation occurred at the point of loading, and the basic region of the gel was poorly resolved, but the acidic proteins (including that of M_r 54000, pI < 4.3) were well resolved (not shown). It is therefore clear that the most insoluble proteins in microsomal membranes are (slightly) basic proteins, in particular various species of cytochrome *P*-450, and that these are most successfully resolved by isoelectric focusing when solubilized at a pH far from their isoelectric point.

Whatever method of sample preparation is employed, resolution is improved by maintaining a high concentration of urea during isoelectric focusing (see Materials and Methods), by appropriate choice of ampholytes (see Materials and Methods) and by working at the minimum protein concentration. Problems of insolubility, aggregation and instability of the pH gradient are all exacerbated by use of high proton loads. The requirement for low protein concentrations makes it imperative to use sensitive methods of visualization to detect minor components. Microsomal membranes contain more than 100 resolvable polypeptides. With a total sample of 100 μg the minor spots cannot be reliably detected by conventional staining by Coomassie brilliant blue. Greater sensitivity can be achieved by use either of a modified method of silver staining (not shown) or by radioactive labelling of sample proteins, followed by autoradiography (Fig. 3). The reductive methylation technique of Finger and Choo [10] allows either $^{14}C(H^{14}CHO + NaBCNH_3)$ or $^{3}H(HCHO + NaB^{3}H_4)$ to be introduced as a

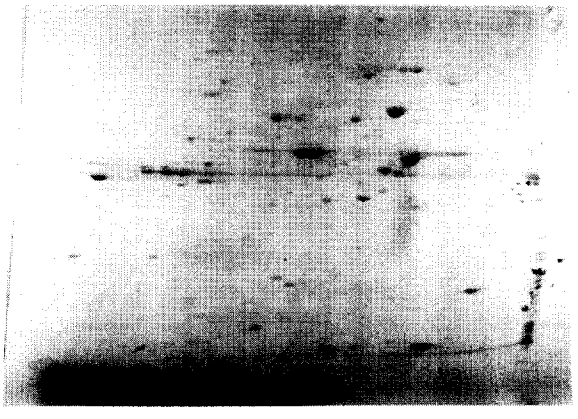

Fig. 2. Two-dimensional electrophoresis of microsomal proteins using the acidification method. Microsomal proteins were solubilized in urea and non-ionic detergent and subjected to two-dimensional electrophoresis with isoelectric focusing from the anode following the method of Vlasuk and Walz [5]. The sample loaded contained 130 μg protein and staining was with Coomassie blue.

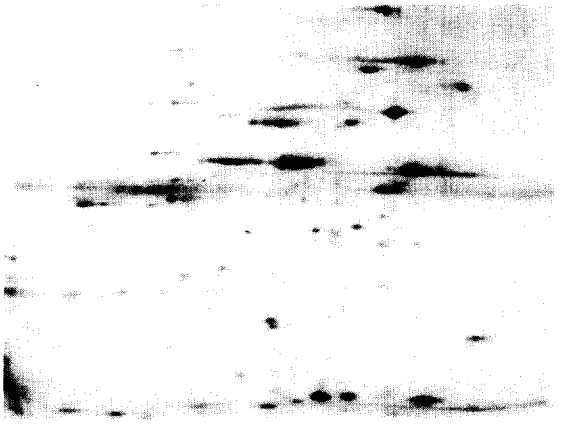

Fig. 3. Two-dimensional resolution at low protein loading using [^{14}C]methylation and autoradiography. Microsomes were solubilized and reductively methylated by the method of Finger and Choo [10] using $H^{14}CHO$, and were then resolved as in Fig. 2, but with 50 μg of protein loaded (200000 dpm) and detection by autoradiography.

methyl group on a lysyl side-chain. The positive charge is retained so that the modification does not perturb the isoelectric focusing properties of the sample proteins (cf. Fig. 2; note that Figs. 2 and 3 show good reproducibility despite being different samples, processed and detected by different methods). These methods allowed excellent visualization of minor spots with as little as 50 μg protein loaded.

Solubilization and isoelectric focusing in presence of zwitterionic detergents

Zwitterionic detergents of the general formula $R \cdot N^+(CH_3)_2 \cdot CH_2CH_2CH_2SO_3^-$ are commercially available with an alkyl group (R) ranging in size from C_8 to C_{16}. Such compounds should be very effective in solubilizing proteins, without affecting their net charge. As such, they should in theory be excellent for the isoelectric focusing of hydrophobic integral membrane proteins. It has previously been shown that purified cytochrome *P*-450 species, which are notoriously difficult to solubilize for isoelectric focusing [15], can be focused in presence of such zwitterionic detergents [16].

The ability of these detergents to solubilize total rat liver microsomal membrane proteins was tested both in the absence and presence of the non-ionic detergent Triton X-100 (Table I). Optimum solubilization was obtained with the longer chain-length zwitterionic detergents in the presence of Triton X-100. SDS-polyacrylamide gel electrophoretic analysis of the solubilized material showed that effectively all microsomal proteins were solubilized (not shown). Attempts were made to resolve by isoelectric focusing microsomal proteins solubilized by either the C_{14} or C_{16} zwitterionic detergent plus Triton X-100. Initial results showed that only poor resolution was obtained, with loading either at cathode or anode, and further problems were caused by the mechanical weakness of the isoelectric focusing gels. In principle, both these problems might be overcome by the inclusion of a high concentration of urea in the isoelectric focusing gel, which would both impart strength to the gel and contribute further to the denaturation of the polypeptides, hence improving resolution. However, the zwitterionic detergents have a very low critical micelle concentration and hence form a flocculant precipitate in the presence of high concentrations of urea. Isoelectric focusing in the presence of 4 M urea, the maximum permissible concentration, gave no improvement over that in its absence. Isoelectric focusing was also carried out in presence of glycerol (20%, v/v) or sucrose (30%, v/v), but neither markedly improved the mechanical strength of the gels. Fig. 4 shows a two-dimensional gel electrophoretogram in which the first dimension was isoelectric focusing of a zwittergent-solubilized sample in the presence of

TABLE I

SOLUBILIZATION OF MICROSOMAL PROTEINS BY ZWITTERIONIC DETERGENTS OF VARIOUS CHAIN LENGTH IN PRESENCE AND ABSENCE OF TRITON X-100

Solubilization was either in the presence of 2% (w/v) zwittergent (A) or in the presence of 1% (w/v) zwittergent + 1% (w/v) Triton X-100 (B). The total concentration of microsomal protein was 2.0 mg/ml. See Materials and Methods for full details.

Zwittergent	Protein concentration in supernatant (mg/ml)	
	A	B
3-08 (C_8)	0.5	1.7
3-10 (C_{10})	1.6	1.5
3-12 (C_{12})	1.3	1.8
3-14 (C_{14})	1.4	1.9
3-16 (C_{16})	1.6	2.2

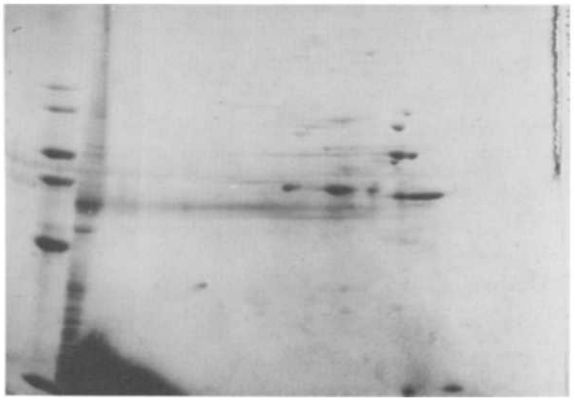

Fig. 4. Two-dimensional resolution of microsomal proteins solubilized by zwitterionic detergent. Microsomes were solubilized in zwittergent 3-16 and Triton X-100 and subjected to isoelectric focusing and SDS-polyacrylamide gel electrophoresis as described in Materials and Methods. The sample loaded contained 200 μg protein and staining was by Coomassie blue.

sucrose (30%). Some major soluble polypeptides were reasonably resolved, but most polypeptides appeared as extended streaks and the resolution was considerably worse than those obtained with the published procedures (cf. Figs. 1,2). In Fig. 4, isoelectric focusing was from the cathode; loading from the anode gave even poorer results.

As a result of the problems of incomplete dissociation of sample proteins, poor solubility of the detergents and fragility of the isoelectric focusing gels, zwitterionic detergents were not successful in bringing about the resolution of all microsomal proteins. Rational modification of conditions might surmount these problems, but at present little is known about these compounds or about their mechanism of solubilization. In a previous application [6] where they were used for isoelectric focusing of purified cytochrome *P*-450, only a basic pH range was used and focusing was carried out in large gels, which may have overcome the problem of mehanical fragility. Such gels would not be suitable as the first dimension of a two-dimensional resolution.

Assessment of the techniques and applications

The results of studies with a number of techniques and minor modifications suggest that neither published technique for two-dimensional electrophoresis of microsomal proteins, nor any single modification, gives ideal resolution of all microsomal polypeptides covering the whole range of molecular weight, solubility and isoeletric point. Nevertheless, for any given set of polypeptides, conditions can be found which give complete resolution with little streaking and good reproducibility. Hence conditions can be chosen depending on the proteins of major interest and the aim of the work. Gels of the same sample, prepared and run under the same conditions, give excellent reproducibility in terms of the numbers of spots resolved, their relative positions and their approximate relative intensities. Complete quantitative reproduciblity requires facilities for parallel preparation, electrophoresis and processing of multiple samples, and computer normalization of gel dimensions to correct for mechanical changes during fixation and staining. Nevertheless, useful conclusions can be drawn from qualitative comparisons.

The acidification method [5] gives good resolution of peptides of p*I* 5–8, including the highly hydrophobic, slightly basic polypeptides which cause greatest difficulty in working with this organelle. Since these proteins include cytochrome *P*-450 apoproteins, and other enzymes of foreign compound metabolism, such as epoxide hydratase, this method, or slight variants of it (see above), is preferred for analyses of variation of content of drug-metabolizing enzymes as a function of strain, sex, induction treatment, etc. For example, we have found, in untreated male animals, differences in the content of putative cytochrome *P*-450 apoproteins between the colony of Sprague-Dawley rats used for most of this work (see Fig. 2) and a colony of Wistar rats (Fig. 5). Vlasuk et al. [16] have recently reported differences of a similar kind between control Holtzman and Long-Evans rats. Comparison of their data and ours suggests that each strain or colony has a characteristic uninduced protein profile in the molecular weight range 45 000–60 000 and p*I* range 7–8. Another application is in studies of induction. Treatment of rats with 2-acetylaminofluorene is known to produce several-fold induction of epoxide hydratase activity [17,18]; resolution by two-dimensional electrophoresis of microsomal proteins from 2-acetylaminofluorene-treated rats showed pronounced induction of a specific protein (Fig. 6), corresponding in molecular weight to purified rat microsomal epoxide hydratase (cf. Ref. 18).

The deoxycholate extraction method with loading at the cathode [4] gives excellent resolution of

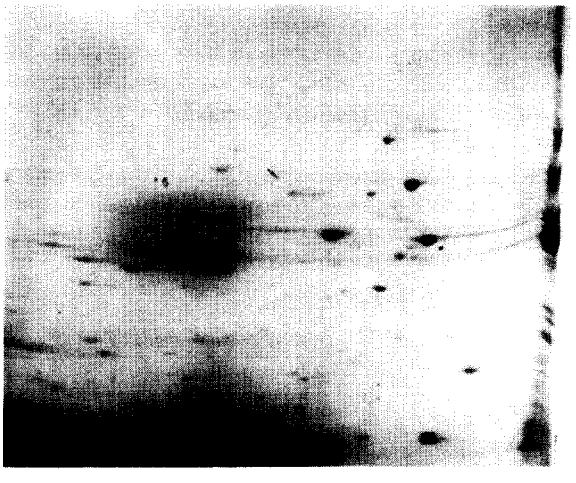

Fig. 5. Two-dimensional electrophoresis of microsomal proteins from Wistar rat livers. As for Fig. 2, except that the rats were Wistar rats from a colony at Shell, Sittingbourne Research Centre. We are grateful to Dr. T.K. Bradshaw for his help in obtaining this material.

neutral and acidic microsomal proteins, and is superior to the acidification method for studies on polypeptides of $pI < 5$. We have used this technique in studies on a major acidic microsomal polypeptide (labelled A in Ref. 4) which we have shown to be the enzyme protein disulphide-isomerase [14]. Loading at the cathode is also essential to resolve another major acidic polypeptide (B in Ref. 4) whose function has not yet been established and which is not found on gels of microsomal proteins resolved by the acidification method [5]. Both the published techniques are satisfactory for resolution of slightly acidic proteins such as albumin and proalbumin [18,19].

Thus, while no single technique has been devised which gives ideal resolution of all the 100 and more rat-liver microsomal proteins, careful choice of sample preparation, ampholyte composition and other conditions for isoelectric focusing can give excellent two-dimensional electrophoretic separation of a particular set of polypeptides of interest. In addition to the applications mentioned above, the approach will be useful in analysis of in vitro translation products (Ref. 20; Mills, E.N.C., unpublished data), in studies on microsomal protein targets modified in vitro or in vivo by reactive metabolites of foreign compounds [21], in studies on the transverse disposition of microsomal proteins based on susceptibility to modification by proteases, impermeant reagents etc. (Freedman, R.B., Kaderbhai, M.A. and Lambert, N., unpublished data) and on their lateral disposition using cross-linking reagents. The resolution of the technique is such that, in time, it should be possible to make a complete catalogue of the protein components of liver microsomal membranes.

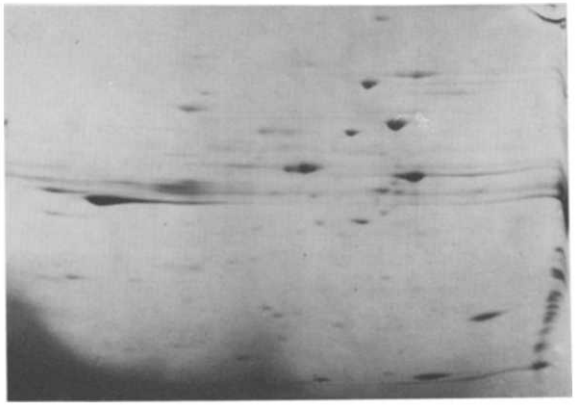

Fig. 6. Application of two-dimensional electrophoresis to monitor the induction of a specific microsomal protein. As for Fig. 5, except that the rats had been pretreated with 2-acetylaminofluorene (160 mg/kg as intraperitoneal injections for 3 successive days). These microsomes were the kind gift of Dr. M.A. Kaderbhai; see Ref. 18 for further discussion.

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