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Two-dimensional Gel Analysis of Proteins from Mouse Fetuses with Trisomy 19 after DEAE-Sepharose Chromatography

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Summary

Isoelectrofocusing two-dimensional polyacrylamide gel electrophoresis (IEF-2D-PAGE) offers the opportunity to detect typical alterations in the protein pattern of trisomic mouse foetuses at a given time of development. The fractionation of the cell lysate by differential centrifugation into various subcellular components (nuclei, membranes, polyribosomes, cytoplasmic proteins) and fractionation of the proteins through DEAE-Sepharose chromatography allows detection of protein differences.

It is possible to detect eight differences in the protein patterns between trisomy 19 (Ts 19) mouse foetuses and euploid mouse fetuses at day 15. Five of these differences are quantitative in nature, three are qualitative. One of these proteins is synthesized in Ts 19 foetuses at a higher level than in euploid mouse fetuses (primary gene dosage effect). The other seven proteins are reduced or not present in trisomic foetuses (consequences of primary gene dosage effects).

The molecular mass of the individual proteins ranges from 13 to 41 kDa.

1. Introduction

Trisomy 21 is the most frequent autosomal chromosomal aberration in humans (Scoggin & Patterson, 1982). In human Ts 21-cells compared with normal cells superoxide dismutase (SOD-1) is increased by about 50%. But other enzymes, which are not coded on the extra chromosome, also show increased activities; for example alkaline phosphatase in fibroblasts of Ts 13, Ts 18 and Ts 21 (Hösli & Vogt, 1979) and galactokinase in Ts 21-erythrocytes (Krone *et al.* 1964), while glutathione peroxidase which is coded on chromosome 3 is increased in Ts 21-erythrocytes (Wijnen *et al.* 1978).

Trisomic mouse foetuses present a model to investigate such trisomies. It is possible to induce trisomies of all autosomal chromosomes in the mouse (Gropp, Tettenborn & Lehmann, 1969). Since the presence of an extra chromosome in the genome of an organism will change the function of the cell, the protein pattern should be changed. There are many possibilities for the alteration of cellular concentrations of single proteins in trisomics. (i) The products of genes located on Ts chromosome are increased one and a half times (gene dosage effect); (ii) primarily affected proteins influence the synthesis, processing or degradation of other proteins; (iii) regulatory proteins located on Ts chromosome will alter the transcription rate of some genes in the genome; (iv) a polypeptide, coded for by the aneuploid chromosome, forms complexes with other cell constituents, and thus the gene dosage effect may alter the concentration of normal complexes or cause the production of abnormal complexes (Weil & Epstein, 1979; Klose & Putz, 1983). Ts 19-foetuses have, as compared to other trisomies of the mouse, the advantage that they will sometimes be born alive and survive for some weeks (Gropp, 1982). Fundele *et al.* (1981) have shown that the activity of glutamate oxalacetate transaminase (GOT-1) in liver, heart, brain, skeletal muscle and erythrocytes and phosphoglycerate mutase in liver, brain and erythrocytes are increased by about 50% in Ts 19-cells.

To record biochemical alterations, proteins from trisomic and euploid cells have been analysed with IEF-2D-PAGE for different trisomies of the mouse by Klose & Putz (1983), who used the sensitive IEF-2D-PAGE, followed by the less sensitive Coomassie blue R-250 staining. Proteins from human Ts 21-cells from cell cultures were likewise analysed by IEF-2D-PAGE but following autoradiography or fluorography (Weil & Epstein 1979; Klose, Zeindl & Sperling, 1982; Patterson *et al.* 1982; Van Keuren, Goldman & Merril, 1982; Scoggin *et al.* 1983).

The basis of the present investigation was mouse foetuses (15 days) with Ts 19, and normal controls of the same litter. Before analysing the cytoplasmic pro-

teins with IEF-2D-PAGE, the proteins were fractionated through DEAE-Sepharose chromatography. After electrophoresis the protein pattern was documented using a sensitive silver-staining method.

2. Material and Methods

(i) *Source of mouse foetuses*

Mouse fetuses with normal and trisomic karyotypes were obtained from Professor Gropp and Dr Winking (University of Lübeck, Germany). Ts 19 was induced in mouse embryos by mating male mice (which were double heterozygous for two partially homologous Robertsonian metacentric chromosomes), with female laboratory NMRI mice (Gropp *et al.* 1969, 1974, 1975, 1976).

Normal foetuses from the same litter as the Ts-foetuses were used as controls. The karyotype of each foetus was determined by preparing chromosomes from the foetal membranes. Whole foetuses at day 15 of pregnancy were investigated (day of vaginal plug = day 0).

(ii) *Tissue homogenization*

All experiments were performed by analysing 1, 3 or 5 trisomic foetuses in parallel with the same number of normal foetuses. About 0·15–1·0 g foetal material was cut into small pieces and transferred to a glass homogenizer containing 4–16 ml 130 KTM3-SH buffer (130 mM-KCl, 30 mM-Tris-HCl, 3 mM-Mg-acetate, 1 mM dithioerythreitol, 0·25 M sucrose, pH 7·6) and ruptured by applying one stroke. Nuclei were removed by centrifugation at 500 *g* for 10 min, 4 °C.

Membranes and mitochondria were pelleted by centrifugation of the postnuclear supernatant for 10 min at 4000 *g*, 4 °C (Lubitz, Reichert & Birkmayer, 1980). The supernatant was layered on to a 10–50% (w/v) sucrose gradient in 130 KTM3-SH buffer and was centrifuged at 82500 *g* for 4·5 h, 4 °C (Reichert, 1985). Fractions containing free cytoplasmic proteins were collected and concentrated by ammonium sulphate precipitation (0–75%). Proteins were dissolved in BIV buffer (10 mM-Tris-HCl, 1 mM-MgCl, pH 7·4).

(iii) *DEAE-Sepharose chromatography*

About 5 mg cytoplasmic proteins in BIV buffer were diluted with 3 parts 10 NT 50 (10 mM-NaCl, 50 mM-Tris-HCl, pH 7·5) and applied to the column (2 × 10 cm). Proteins were eluted by a 10–750 mM-NaCl gradient. Fractions of 1·2 ml were collected and the absorption was determined at 280 nm. Individual protein fractions were mixed with 5 volumes acetone and incubated at −20 °C overnight.

Proteins were pelleted by centrifugation at 8000 *g* for 10 min at 4 °C, dissolved in double-distilled H_2O and lyophilized.

(iv) *IEF-2D-PAGE and silver staining*

Proteins (0·05 A_{280} were analysed by IEF-2D-PAGE as described earlier (O'Farrel, 1975; Unteregger, Zang & Issinger, 1983) and visualized by the silver-staining method according to Dr H. Beier and H. Blum (personal communication, University of Würzburg, Germany) with some modifications (Reichert, 1985) (Table 1). Comparisons of the protein patterns were carried out visually.

3. Results

(i) *Presentation of Data*

Figs 1(*a*,*b*); 3(*a*,*b*); 3(*c*,*d*); 3(*e*,*f*) and 3(*g*,*h*) always represent gel pairs of three independent analyses. It is possible that in the following figures some differences in the protein patterns are not caused by trisomy 19 but can be explained by polymorphism. Therefore only distinct repeatable differences are indicated in the figures.

There was no difference in the protein pattern between Ts 19 and normal controls in the conserved polysomal proteins and nuclear proteins (not shown).

Table 1. *Staining procedure for the silver-based gel electrophoresis*

Steps	Solutions	Duration of agitation
Fix	50% CH_3OH, 12% HAC	3 h or more
Wash	10% ETOH, 5% HAC	1 h, change once
Pre-incubation	$Na_2S_2O_3$ (0·22 g/l)	1 h
Wash	ddH_2O	5 min, change once
Incubation	$AgNO_3$ (2 g/l)	9 min
Rinse	ddH_2O	15 s, change once
Reduce silver	HCHO (0·5 ml/l), $Na_2S_2O_3$ (15 mg/l), 3% Na_2CO_3	6 min
Stop	47% CH_3OH, 10% HAC	Store

ETOH, ethanol; HAC, acetic acid; ddH_2O, double-distilled water.

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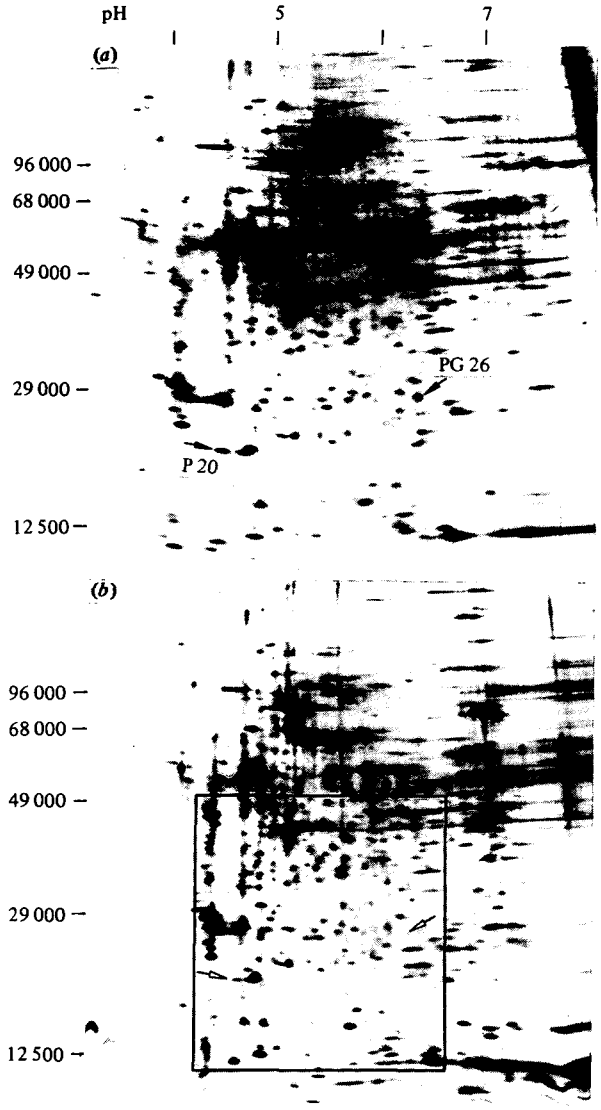

Fig. 1. IEF-2D-PAGE from cytoplasmic proteins: protein pattern from (*a*) normal mouse foetuses; (*b*) trisomic foetuses. The differences between Ts 19 mouse foetuses and normal controls are marked thus. Solid arrows indicate the presence of a newly appearing protein in either the trisomic mice or the normal controls. Open arrows indicate reduction or complete absence of a particular protein. The box in Fig. 1(*b*) indicates the area of interest which is shown in Fig. 3.

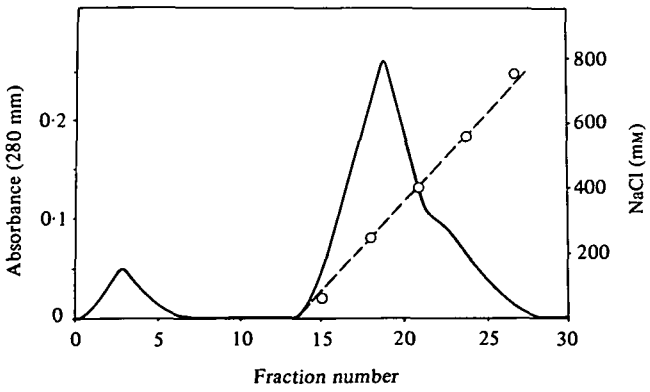

Fig. 2. DEAE-Sepharose chromatography of cytoplasmic proteins. The solid line indicates the elution profile of the proteins at 280 nm. The dashed line shows the applied salt gradient.

Fig. 1 shows proteins of the cytoplasmic fraction of diploid mouse foetuses (1*a*) and Ts 19-mouse foetuses (1*b*). In Ts 19 fetuses protein PG26 (26 kDa/pI 6·2) is absent and protein P20 (20 kDa/pI 4·6) is reduced in intensity. For a better documentation of these differences it was necessary to fractionate the proteins through DEAE-Sepharose chromatography (Fig. 2).

(ii) IEF-2D-PAGE of cytoplasmic proteins after DEAE-Sepharose chromatography

Fig. 3 shows IEF-2D-PAGE patterns from selected column fractions. The upper panel shows 2D profiles from cytoplasmic proteins of normal 15-day-old foetuses (3*a,c,e,g*), the lower panel exhibits the same protein fraction from trisomic foetuses (3*b,d,f,h*).

After the fractionation of the cytoplasmic proteins through DEAE-Sepharose chromatography, eight distinct differences in the protein pattern between Ts 19 foetuses and normal controls were detected.

The protein patterns of Fig. 3(*a*) (normal) and 3(*b*) (trisomic) differ in protein P13 (13 kDa/pI 6·0), P20 (20 kDa/pI 4·6) and P22 (22 kDa/pI 5·3). P13 and P20 are reduced in trisomic foetuses, whereas P22 is not present. Protein P13 was eluted from the DEAE column with a salt gradient between 140 and 300 mM, P20 between 140 and 470 mM and P22 between 200 and 360 mM-NaCl. Protein P41 (41 kDa/pI 5·8) is enhanced in Ts 19 cells and was eluted from the column in a salt gradient between 250 and 470 mM-NaCl (Fig. 3*c,d*).

Figs 3(*e,f*) show that protein PG26 (26 kDa/pI 6·2) in the protein patterns of Ts 19 foetuses is observed in a reduced amount, whereas PM 26 (26 kDa/pI 6·4) found in normal controls is not present at all. PG26 was eluted from the column between 300 and 470 mM and PM26 between 360 and 410 mM-NaCl.

Figs 3(*g,h*) show differences for P23 (23 kDa/pI 4·8) and PR 26 (26 kDa/pI 5·9). P23 and PR26 are reduced in Ts 19 foetuses. Table 2 summarizes the differences in protein profiles indicated in Fig. 3.

4. Discussion

The results show eight distinct reproducible differences in the 2D protein pattern between cells from whole mouse foetuses (15 days) with Ts 19 and normal controls. This made possible by the separation of proteins which are present in small concentrations from proteins which are present in large concentrations in the cell. Such separations then allow the detection of rare proteins without disturbance through some dominant proteins (e.g. actin). Differences which are not present in IEF-2D-PAGE pattern of total cytoplasmic proteins, or which are perhaps only seen in traces, were clearly visible after DEAE-Sepharose fractionation (Reichert & Issinger, 1985). This is not surprising, as there are 5000–20000 different proteins in

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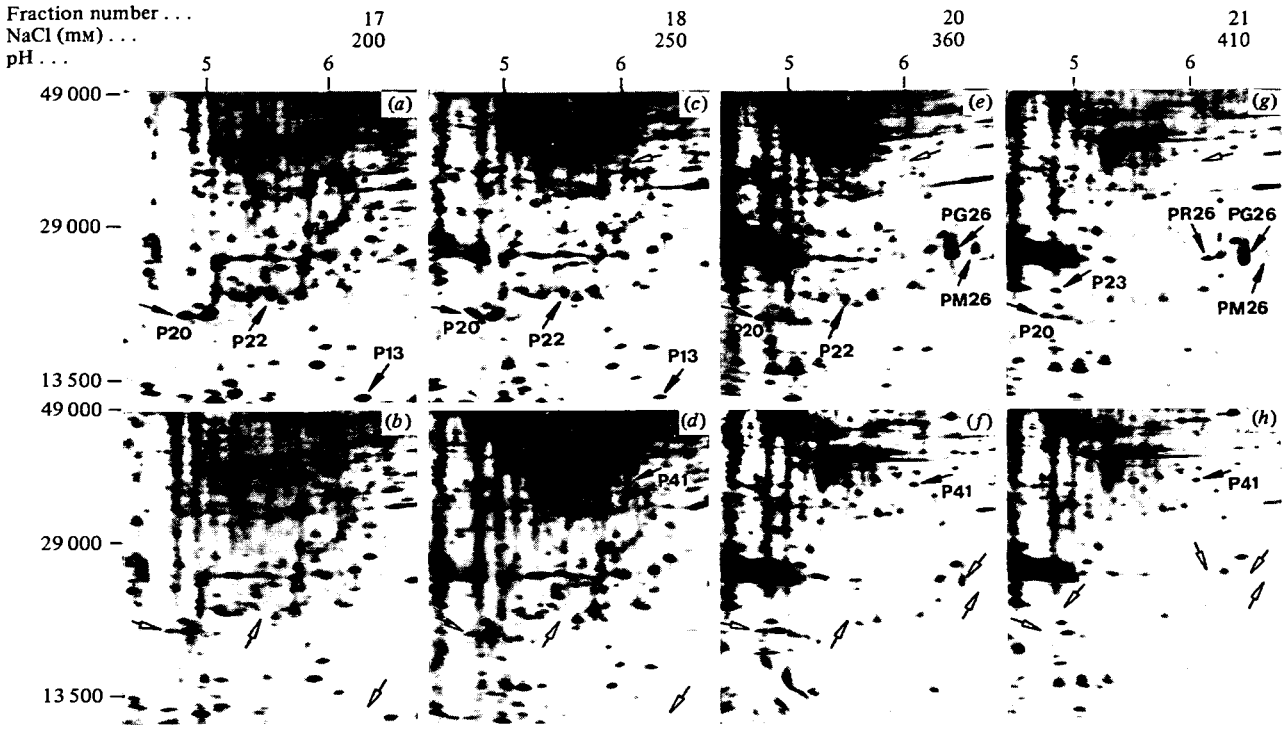

Fig. 3. Close-up of IEF-2D-PAGE from selected DEAE-Sepharose column fractions; protein pattern from (*a,c,e,g*) normal mouse foetuses and (*b,d,f,h*) trisomic foetuses. (For arrows see Fig. 1.)

each cell (Duncan & McConkey, 1982). Since the experiments were repeated several times, differences in the protein pattern which were not due to trisomy 19 (e.g. polymorphisms, differential binding or elution on DEAE-Sepharose) could be virtually excluded.

The 50% increase in activity in Ts 19 cells of GOT-1 (liver, heart, brain, skeletal muscle and erythrocytes) and phosphoglycerate mutase (liver, brain, erythrocytes) is mainly explained by primary gene dosage effects (Fundele *et al.* 1981). The increased level of protein P41 is possibly caused by a primary gene dosage effect, as the amount of this protein in Ts 19 foetuses is enchanced compared to normal controls. The proteins P13, P20, PR26 are reduced in concentration in Ts 19 foetuses compared to normal controls (quantitative differences). P22 and PM26 are not present in Ts 19 foetuses, whereas PG26 is present in Ts19 foetuses in a very small amount (qualitative differences).

Therefore most of these differences are not due to primary gene dosage effects but by other influences of the gene expression rate. This can occur in many ways. First, it is possible that some proteins, coded on chromosome 19, regulate the expression of some genes. The 1·5-fold increase of such regulatory proteins could then lead to abnormal ratios between these and the receptor sites, and thus lead to altered rates of gene transcription. Secondly, some proteins coded on chromosome 19 could form regulatory complexes with other proteins. The 1·5-fold amount of one component of the complexes could imbalance the physiological concentration of the complexes and cause the

Table 2. *Quantitative and qualitative variant polypeptides in 2D protein pattern between Ts* 19 *mouse foetuses and normal controls*

Protein	Mouse foetus euploid	Mouse foetus Ts 19	Mr	pI	Fraction number	mM-NaCl
P13	++	+	13000	6·0	16–19	140–300
P20	++	+	20000	4·6	16–22	140–470
P22	++	−	22000	5·3	17–20	200–360
P23	++	+	23000	4·8	21–22	410–470
PG26	++	−	26000	6·2	19–22	300–470
PM26	++	−	26000	6·4	20–21	360–410
PR26	++	+	26000	5·9	21–22	410–470
P41	+	++	41000	5·8	18–22	250–470

++, Large amount of proteins; +, small amount of proteins; −, protein not present.

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production of abnormal complexes. This event could yield changes in the transcription rate of some genes in the genome (Weil & Epstein, 1979; Klose & Putz, 1983). Thirdly, an amplifying cascade could exist, which means that one or more proteins, which are coded on chromosome 19, regulate the expression of one or more genes. The 1·5-fold amount of these proteins could then switch on (off) the transcription of other genes or increase (decrease) the transcription rate of some genes. This could lead to the loss (e.g. P22, PM26) or reduction of some proteins. A minimum of primary alterations of the protein pattern, not seen in the IEF-2D-PAGE pattern, could lead to decisive consequences in the protein pattern, which are detectable in IEF-2D-PAGE.

In trisomic plants it is possible to detect quantitative and qualitative alterations of the protein pattern with one-dimensional PAGE (Suh *et al.* 1977; McDaniel & Ramage, 1970). Klose & Putz (1983) found similar differences in various trisomies in the mouse. They divide these differences into two groups: (i) trisomic specific alterations; and (ii) those which were influenced indirectly through trisomies.

The differences in the protein pattern detected in human Ts 21 cells compared with normal cells were observed in cell cultures. Van Keuren *et al.* (1982) reported 12 proteins, and Weil & Epstein (1979) found four proteins which were influenced in human Ts 21 cells. Klose *et al.* (1982) have shown that the protein pattern varies more within Ts 21 cells than within normal fibroblast cells.

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