Identification of elongation factor 1α from mouse liver

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EF-1α *Two-dimensional gel electrophoresis* *Immunoblotting* *Protein cataloguing*

1. INTRODUCTION

The bacterial protein elongation factors EF-Tu, EF-Ts and EF-G [1] are thought to have corresponding protein activities in eukaryotic cells now usually named EF-1α, EF-1β and EF-2, respectively. Preparations of EF-1α, the factor that carries aminoacyl-tRNA to the ribosome, have been obtained in various degrees of purity and activity from pig liver [2], rabbit reticulocytes [3], *Artemia salina* (brine shrimp) [4], yeast [5] and the fungus *Mucor* [6].

In this laboratory, we have recently begun an investigation of possible changes occurring in cellular enzymes during ageing [7–9], with particular reference to the enzymes involved in the translational apparatus. EF-Tu is a major protein in *E. coli* [10] as is EF-1α in rabbit reticulocytes [11]. Interestingly, a decrease in the specific activity of EF-1α with age has been reported for *Turbatrix aceti* [12] and for rat liver and brain [13]. Whether this change in specific activity is concomitant with a change in rate of synthesis or degradation or modification is as yet unknown. We are employing a combination of two-dimensional gel electrophoresis, SDS-PAGE and immunoblotting techniques to detect any alterations in the size or charge of EF-1α which may occur in this respect [14].

Here we report the localisation of EF-1α on two-dimensional gels using immunoblotting and the correlation of this information with currently available catalogues of mouse and human proteins [15,16].

2. MATERIALS AND METHODS

Friend leukemia cells were grown in suspension in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum and antibiotics (penicillin 100 IU/ml; streptomycin 50 μg/ml). The procedure for labelling with [^{35}S]methionine has been described [17].

Mouse liver homogenates were treated with DNase and RNAase, lyophilized and dissolved in dissociation buffer [18]. Non-equilibrium pH gradient gel electrophoresis (NEPHGE) was used as the first dimension of the two dimensional gel electrophoresis technique, which has been described in detail elsewhere [18,19]. Gels were processed for fluorography according to Laskey and Mills [20] and exposed to Kodak X-omat RP X-ray film.

Highly purified *Artemia salina* EF-1α and rabbit reticulocyte EF-1α were generously provided by Dr. W. Möller's group (Leiden) and Dr. W. Merrick's group (Cleveland), respectively.

2.1. *Antibody production*

Purified *Artemia salina* EF-1α in 20 mM Tris–HCl (pH 7.5), 10 mM KCl, 10% (v/v) glycerol was emulsified in an equal volume of complete Freunds adjuvant and injected intramuscularly into a 3 month old female rabbit. Two mg of protein factor were injected at 0 and 3 weeks. Periodic bleedings revealed a positive response by week 4. The rabbit was sacrificed on day 60.

The IgG fraction was purified on protein A–Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala) according to Ey et al. [21].

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2.2. *Immunoblotting*

Slab gel electrophoresis was conducted by use of the SDS-PAGE system of Laemmli [22]. Upon completion of the electrophoretic run, protein was transferred to nitrocellulose sheets essentially by the method of Towbin et al. [23]. Slab gels (8 cm wide × 5 cm long for one-dimensional SDS, 14 cm × 15 cm for two-dimensional gels) were placed onto wetted nitrocellulose paper and then covered with Whatman 3 MM paper and scouring pads on each side. The sandwich was placed between two 1 cm plastic sheets of an electrophoretic gel destainer. The entire assembly was slotted firmly into the electrode chamber. The chamber was filled with electrode buffer (25 mM Tris-base, 192 mM glycine, 25% methanol at pH 8.3) and electrophoresis was carried out at 20 V, 300 mA for 6 h with the anode on the nitrocellulose side of the sandwich. The temperature was maintained between 0–4°C. The nitrocellulose paper was stained with amido black (0.1% in 45% methanol and 10% acetic acid), destained (90% methanol and 2% acetic acid) and stored in water. If the blotted nitrocellulose papers were to be autoradiographed, they were dried between sheets of Whatman 3 MM filter paper and exposed to Kodak X-omat RP X-ray film at − 70°C. After autoradiography, the sheets were re-wetted in buffer 1 and processed for antibody binding as described below. The blots were incubated with 3% bovine serum albumin in saline (0.9% NaCl, 10 mM Tris–HCl, pH 7.4) for 1 h at 37°C and rinsed twice with buffer 1 (0.2% SDS, 0.5% Triton X-100, 0.5% BSA and 0.01% NaN_3 in saline).

Antibodies and purified IgGs were diluted 1:200 in buffer 1 and were incubated together with the blots with gentle shaking at room temperature for 12 h. The blots were washed in buffer 1 for 30 min with 5 changes. The second antibody, swine anti-rabbit immunoglobins fluorescein isothiocyanate (FITC) labelled; DAKO (Copenhagen) was diluted 1:200 in buffer 1 and incubated with the blots for 4 h after which the blots were washed in buffer 1 for 30 min with 5 changes. The blots were photographed under UV light using a UV filter (Kodak wratten No. 2A).

2.3. *Silver staining*

Gels for silver staining were processed according to Morrisey [24].

3. RESULTS AND DISCUSSION

Functionally active *Artemia salina* EF-1α was co-run on a two-dimensional gel with a total protein sample extracted from Friend leukemia cells, radioactively labelled with [^{35}S]methionine (fig.1). The high purity of the *Artemia salina* EF-1α is evident from the silver staining of the gel shown in fig.1A. The amount of Friend leukemia cell proteins applied to the gel is too small to be revealed by the silver stain. The location of proteins in the extract of Friend leukemia cells is shown in the autoradiogram of fig.1B. In this way, the *Artemia salina* EF-1α was used as a eukaryotic EF-1α probe to attempt to locate the corresponding mammalian factor. The position of *Artemia salina* EF-1α as seen in fig.1, corresponds to NEPHGE 12 in current human protein catalogues [15] and NEPHGE 13 in current mouse kidney protein catalogues [16]. The M_r estimated from SDS-PAGE (not shown) is approximately 53 000, which is in good agreement with estimates of the M_r for EF-1α from other sources [2,3,6,11]. From the NEPHGE results, the isoelectric point of EF-1α under the non-equilibrium conditions employed lies between pH 9 and 9.5.

To determine the location of mouse liver EF-1α, unlabelled mouse liver total protein extract was co-run on a two dimensional gel with a small amount of [^{35}S]methionine labelled Friend leukemia cell protein as marker and the separated proteins then blotted electrophoretically onto a nitrocellulose sheet. The nitrocellulose sheet was stained, dried and autoradiographed to give the result shown in fig.2A. A comparison of fig.2A and fig.1B gives evidence of the high efficiency and reproducibility of the protein transfer on blotting.

After autoradiography, the nitrocellulose sheet was processed for antibody binding as described in Materials and Methods. The relevant portion of the stained nitrocellulose paper is shown both before (fig.2B) and after (fig.2C) treatment with the FITC labelled, second antibody. This confirms the location of mouse liver EF-1α as NEPHGE 12 or NEPHGE 13 in human and mouse kidney catalogues, respectively.

Two other polypeptides give a weak antibody reaction (indicated by small arrows in fig.2A). Whether these are degradation products of EF-1α is under investigation, but it appears that they may

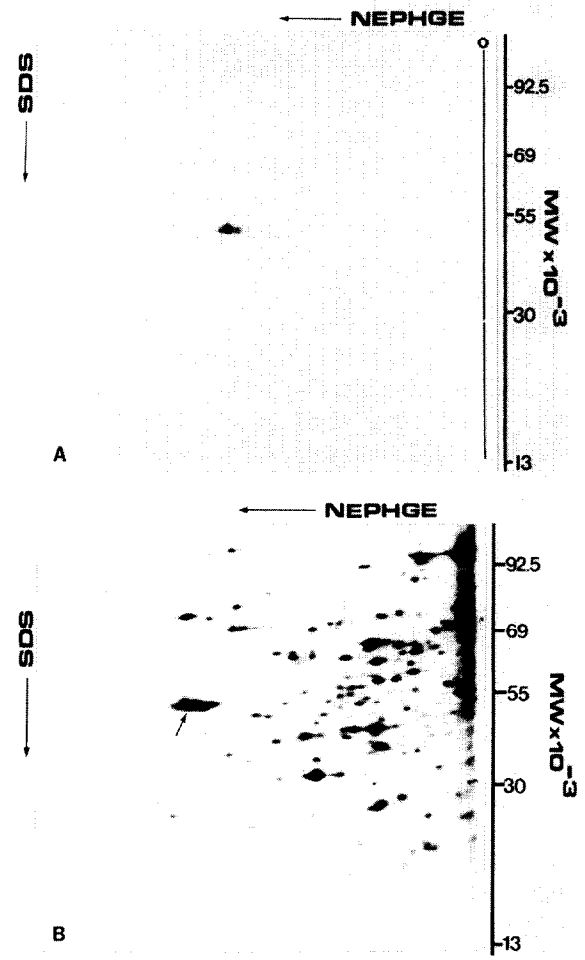

Fig.1. NEPHGE of highly purified *Artemia salina* EF-1α as revealed by silver staining (1A). [^{35}S]methionine Friend leukemia proteins have been co-run as marker and are revealed by fluorography (1B) after the silver staining step. 500 ng of purified *Artemia salina* EF-1α was applied to the gel. The Friend leukemia proteins are extracted from cells labelled to a high specific activity with [^{35}S]methionine [17]. Approximately 500 000 trichloroacetic acid precipitable cpm were loaded. The arrow indicates the position of EF-1α after superimposition of the fluorogram over the silver stained gel. Comparison of 1A and 1B with protein catalogues designates EF-1α as NEPHGE 12 on human catalogues [15] and as NEPHGE 13 on mouse kidney catalogues [16].

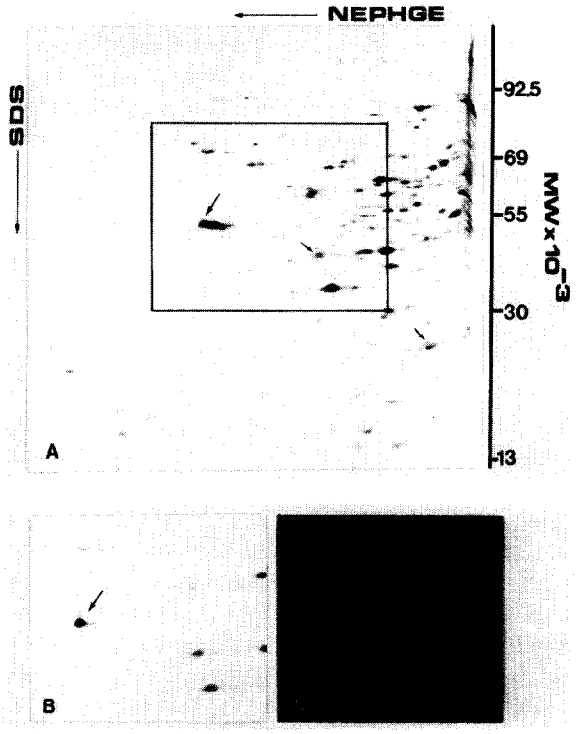

Fig.2. NEPHGE of mouse liver total proteins co-run with [^{35}S]methionine labelled Friend leukemia proteins as marker. The proteins have been blotted onto a nitrocellulose sheet and autoradiographed to give the result shown in fig.2A. Only the area shown in fig.2A by the thin lined rectangle containing the major antibody binding spot is reproduced from the amido black stained sheet to give fig.2B. Fig.2C shows the same area as in 2B but after FITC labelled antibody binding. The large arrows in fig.2A, B and C show the major binding spot. Weak binding spots are shown by the small arrows in fig.2A.

be associated with the heterogeneity of the liver since they do not occur in identical gels loaded with unlabelled Friend leukemia protein.

In further characterization and search for similar properties of eukaryotic EF-1α and prokaryotic EF-Tu, we have used an immunocross-reactivity test, the results of which are shown in fig.3. For this test, we raised antibody to a sample of *Artemia salina* EF-1α. Using one-dimensional gel electrophoresis (fig.3), we observed that anti-*Artemia salina* EF-1α showed strong cross-reactivity with mouse liver cell extract at the appropriate molecular weight region, and gave a strong cross-reaction with one band of the rabbit reticulocyte EF-1α sample from W. Merrick's group. In addition, there was a weak cross-reaction with protein from a yeast cell extract but none with *E. coli* EF-Tu. In

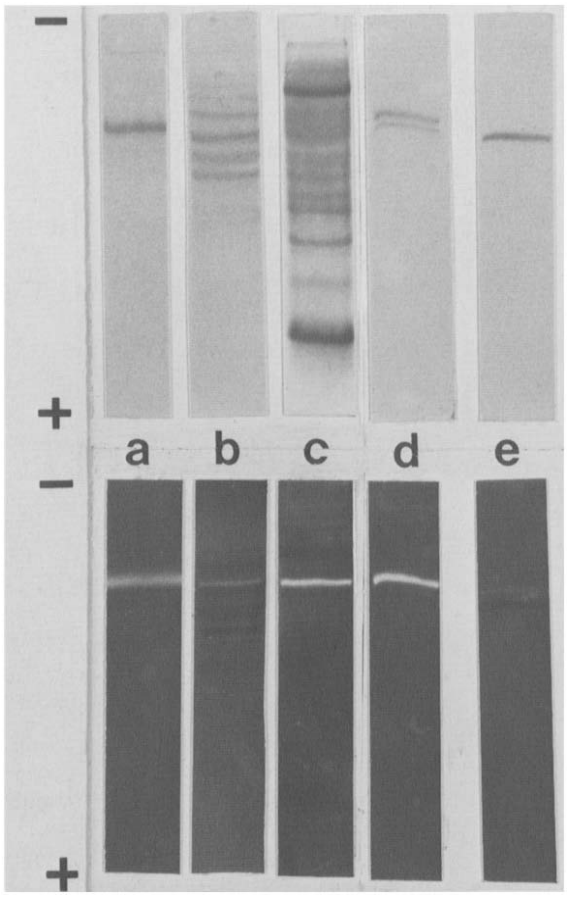

Fig.3. Cross-reactivity of antibodies raised against *Artemia salina* EF-1α with EF-1α from other sources. Lanes a–e show proteins after blotting onto a nitrocellulose sheet (upper) and after FITC-labelled antibody binding (lower). The lanes shown: (a) purified *Artemia salina* EF-1α; (b) yeast total protein extract; (c) mouse liver total protein extract; (d) purified rabbit reticulocyte EF-1α; and (e) *E. coli* EF-Tu.

support of the lack of cross-reactivity with the prokaryotic EF-Tu, we did not observe cross-reactivity in a reciprocal experiment between mouse liver EF-Tu and anti *E. coli* EF-Tu (kindly provided by G. Schatz's group, Basel).

4. CONCLUSION

We have localized mouse liver EF-1α in two-dimensional gel electrophoresis patterns and correlated the position with existing mouse protein catalogues [16]. This is a step towards the identification of such a component of protein biosynthesis made in in vitro systems. Our further aim will therefore be to identify mouse liver EF-1α mRNA for the production of its cDNA as a probe for EF-1α gene organization.

Functional characteristics of the mouse liver EF-1α also remain to be worked out. Interestingly, we have found that there is conservation of antigenic properties among various eukaryotic EF-1α preparations. This conservation of some structural parameters does not appear to extend from eukaryotic EF-1α to its prokaryotic bacterial counterpart EF-Tu.

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