TWO-DIMENSIONAL ISOELECTRIC FOCUSING ANALYSIS OF RARE AND SILENT ESTERASE D TYPES. DESCRIPTION OF A NEW ESD VARIANT PHENOTYPE

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INTRODUCTION

We have recently shown that the analysis of ESD phenotypes by one-dimensional isoelectric focusing (1-D IEF) under reducing and mild denaturing conditions offers high resolution in the separation of the different ESD allele products (Alonso et al. 1991).Furthermore a new two-dimensional isoelectric focusing method (2-D IEF) has been described that permitted the identification of the ESD subunits from the homodimeric and heterodimeric forms of five common ESD phenotypes.

In this report, we analyzed three ESD variant phenotypes (ESD 7-1, ESD 7-2, and a new ESD 1-VAR phenotype observed in a Spanish population) as well as two heterozygous phenotypes of a silent allele (ESD 1-QO and ESD 2-QO, found in a case of disputed paternity) by 1-D IEF under reducing conditions or under reducing and mild denaturing conditions and by 2-D IEF.

MATERIALS AND METHODS

One-dimensional separations as well as the first dimensional separation of the 2-D IEF method were carried out by IEF under reducing conditions(50mM DTT) or under reducing and mild denaturing conditions (50mM DTT and 1.5M urea) using a narrow pH gradient (Pharmalyte 4.5-5.4) in combination with two separators (HEPES and ACES). The second-dimensional separation was performed by focusing the first-dimensional ESD bands under denaturing conditions (9M urea) with a wide pH gradient (4-10.5), according to the method of Alonso et al. (1991).

RESULTS AND DISCUSSION

Figures 1a and 1b show the band pattern of different rare and silent ESD types (ESD7-1, ESD7-2, ESD1-VAR, ESD1-QO and ESD2-QO) analyzed by 1-D IEF under reducing conditions or under reducing and mild denaturing conditions, respectively. As previously described, the presence of low urea concentrations in the gel induces an inversion in the relative mobility of the ESD1 and ESD2 allele products. A similar inversion in the relative mobility of the ESD7-1 and ESD7-2 bands was observed in this study. Furthermore, figures 1a, 1b, and 1c show the 1-D IEF pattern of a "new" ESD1-VAR phenotype found in a Spanish population. The new variant which was tentatively named ESD1-Gijon (ESD1-Gij) is characterized by a very cathodal pattern. For identification it was necessary to use a broader pH gradient (a mixture of Pharmalyte 4-6.5 and Pharmalyte 4.5-5.4)(fig. 1c). Unfortunately, it was not possible to confirm the inheritance of this rare variant by family studies.

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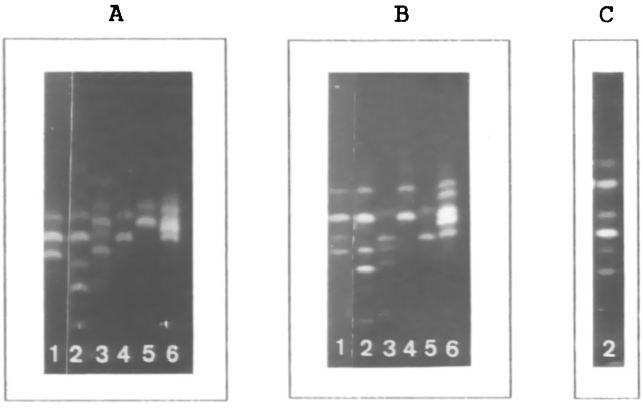

Fig.1. Rare and silent ESD types analyzed by 1-D IEF under reducing conditions. A: in the absence of urea, B and C: in the presence of 1.5 M urea. Samples: 1: ESD7-1, 2: ESD1-Gij, 3: ESD7-2, 4: ESD1-QO, 5: ESD2-QO, and 6: ESD2-1

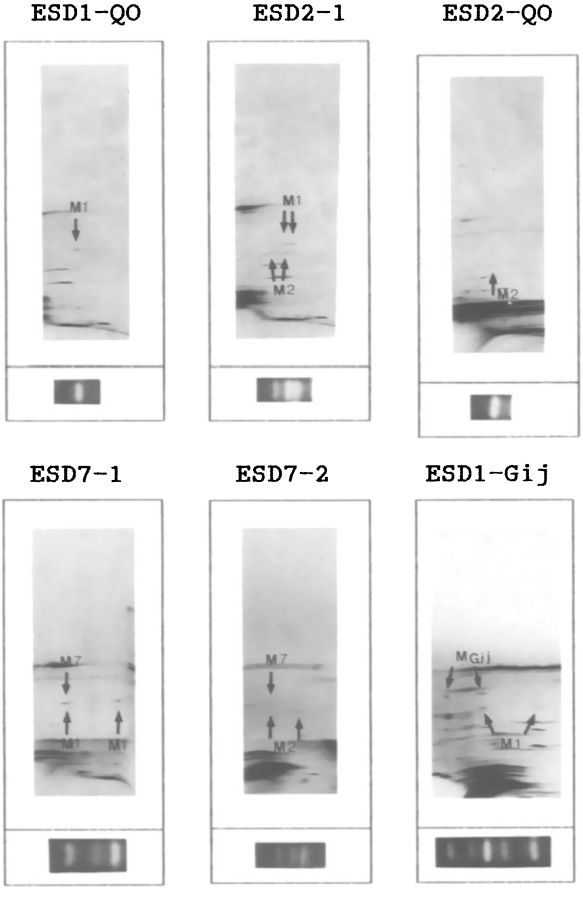

Fig.2. 2-D IEF analysis of silent and rare ESD types followed by silver staining. ESD monomers (M) are indicated with arrows

We have also analyzed rare and silent ESD phenotypes by a recently developed 2-D IEF procedure that consist of a first-dimensional separation of the different ESD dimeric forms by IEF under reducing and mild denaturing conditions using a narrow pH gradient capable of producing high resolution ESD bands, followed by a second-dimensional separation under denaturing conditions with a wide pH gradient. In this way we can correlate the focusing pattern of the dimeric ESD forms with the focusing pattern of its dissociated subunits. Figure 2 shows the result of these 2-D IEF analysis.

The 2-D IEF pattern of the ESD1-QO and ESD2-QO phenotypes were characterized by the presence of the monomeric band M1 (corresponding to the ESD1 one-dimensional dimeric band) and the monomeric band M2 (corresponding to the ESD2 one-dimensional dimeric band), respectively. No additional bands could be observed. This result allowed to rule out the possibility that the ESD*QO allele was a mutation that produced a polypeptide chain which was able to form dimers without enzymatic activity. The two remaining possibilities that explain the existence of a ESD*QO allele (a mutation that produces a polypeptide chain that is unable to form dimers or a mutation that leads to a lack of polypeptide product) could be distinguished by another two-dimensional electrophoretic analysis (Kondo et al. 1984).

The 2-D IEF analysis of the ESD7-1 and ESD7-2 phenotypes has permitted the identification of the ESD7 monomer (M7) as a band with similar isoelectric point found for ESD M1. This result suggest that the ESD*7 allele shows a neutral mutation that can be recognized by 1-D IEF because of a conformational change in the dimeric structure of the protein. It was also shown that the more cathodal one-dimensional bands of these phenotypes are heterodimeric forms composed of the M1 and M7 (ESD7-1 phenotype) or the M2 and M7 (ESD7-2 phenotype) monomers. We assume that cathodal to these heterodimeric forms is probably focusing the H 7-7 homodimeric form (Weidinger and Henke. 1988). However, this band has not been visualized in this study. Possibly there was a dissociation of the H 7-7 homodimeric form into its monomers by ageing.

Finally, the 2-D IEF analysis of the rare ESD1-Gij phenotype has permitted the identification of the ESD Gij monomer (M Gij) with a mobility cathodal to the M1 monomer.In addition the homodimeric (H Gij-Gij and H 1-1) and the heterodimeric forms (HE 1-Gij) of this phenotype, could be identified.

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