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Differences between natural and recombinant interleukin-2 revealed by gel electrophoresis and capillary electrophoresis

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Abstract

High-performance capillary electrophoresis (HPCE) is shown to be useful for analysis of interleukin-2 (IL-2) in its native state under non-reducing conditions. The results obtained were compared with those from analysis of IL-2 by protein blotting after sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing and reducing conditions. In addition, resolution of the different glycosylated and non-glycosylated natural IL-2 species was achieved by HPCE. The HPCE electropherogram of native IL-2 could easily generate quantitative amounts of the different naturally occurring IL-2 species. For HPCE of IL-2 run times of less than 10 min are sufficient, and only extremely small amounts of IL-2 are needed. In this report, human IL-2 expressed in bacteria has been analysed by HPCE and the existence of two recombinant IL-2 forms was demonstrated.

1. Introduction

High-performance capillary electrophoresis (HPCE) is a method with great potential for the high-resolution separation of biological substances [1]. The aim of this study was to compare this technique with sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) for the characterisation of natural and recombinant interleukin-2 (IL-2).

Natural interleukin-2 (nIL-2) is a polypeptide which is synthesised and secreted by activated T-cells [2,3]. Previous investigations have demonstrated that the smallest nIL-2 component with a molecular mass of $15.0 \cdot 10^3$ is non-glycosylated, a $16.5 \cdot 10^3$ species is glycosylated and in addition mono-sialylated, and a $17.0 \cdot 10^3$ IL-2 component is glycosylated and di-sialylated [4].

In contrast, *Escherichia coli* derived recombinant interleukin-2 (rIL-2) is non-glycosylated. Additionally, in all genetically engineered IL-2 from *E. coli*, the cysteine residue at position 125 is substituted by a serine residue and the first amino acid alanine is missing [5].

2. Experimental

Human nIL-2 was prepared from the supernatants of human peripheral blood mononuclear cell (PBMC) cultures after activation with phorbol-12-myristate-13-acetate (PMA) and the calcium ionophore A 23187, and purified according to a scheme previously described [3]. Another nIL-2 was prepared from culture supernatants of concanavalin A (con A)-stimulated and propagated human PBMCs. The purification was done according to the same scheme. The rIL-2 used was produced by DNA technology. It was supplied by Euro Cetus (Frankfurt, Ger-

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many). The nIL-2 and rIL-2 samples used in the experiments were dissolved in 10 m*M* phosphate, pH 7.4, 0.9% sodium chloride. Capillary electrophoresis was performed in a Bio-Rad (Richmond, CA, USA) HPE 100, with data collection by a Hoefer Scientific GS 365 software package. Capillaries were supplied in cartridges with an integral flow cell for on-column optical detection. Proteins were introduced into the capillary by electromigration for 8 s at 8 kV. The samples were electrophoresed in a 100 m*M* pH 2.5 phosphate buffer at 10 kV in 20 cm × 25 μm I.D. coated capillaries.

SDS-PAGE was performed under reducing conditions using the method of Laemmli [6]. Stacking gel buffer: 0.062 *M* Tris, 0.062 *M* chloride, pH 6.7; separating gel buffer: 0.375 *M* Tris, 0.06 *M* chloride, pH 8.9; electrophoresis buffer: 0.05 *M* Tris, 0.38 *M* glycine, pH 8.3; sample buffer: 0.01 *M* Tris–HCl, 1% SDS, 5% β-mercaptoethanol, pH 8.0. For blotting, the slab gel was covered with a sheet of nitrocellulose membrane to which the proteins were electrophoretically transferred according to the procedure of Towbin et al. [7]. Transfer buffer: 25 m*M* Tris, 192 m*M* glycine, 20% methanol, pH 8.0. A voltage of 30 V for 16 h was used in a Trans blot cell at 10°C. To estimate the protein pattern after electroblotting of the gels, the transferred proteins were visualised by colloidal gold staining according to a modified method described by Moeremans et al. [8].

To check the reproducibility of the used methods all experiments were repeated three times.

3. Results and discussion

Fig. 1 shows the pattern of highly purified nIL-2 after separation by 15% SDS-PAGE, electroblotting and gold staining of the membrane. It shows the three different forms of nIL-2 migrating with different mobilities in SDS-PAGE, with molecular masses of $15.0 \cdot 10^3$, $16.5 \cdot 10^3$ and $17.0 \cdot 10^3$, respectively. The gold stain for protein blots has a very great sensitivity and therefore some artifactual bands are seen in the molecular-mass range $(40–70) \cdot 10^3$. These

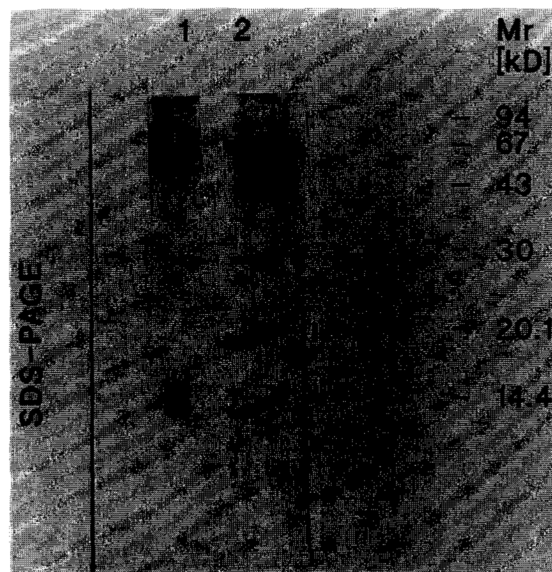

Fig. 1. Colloidal-gold stain on nitrocellulose of highly purified nIL-2. The protein was electrophoresed under reduced conditions. Lane 1: nIL-2; lane 2: molecular-mass marker proteins. An amount of 1 μg of the protein was applied per lane.

"ghost bands" simulate impurities, which could not be found in the electropherogram after HPCE (see below). To check further the purity and the composition of the nIL-2 preparations under non-denaturing conditions HPCE was used. The electrophoretic pattern of highly purified nIL-2 derived from A 23187/PMA-induced human lymphocytes is shown in Fig. 2. As

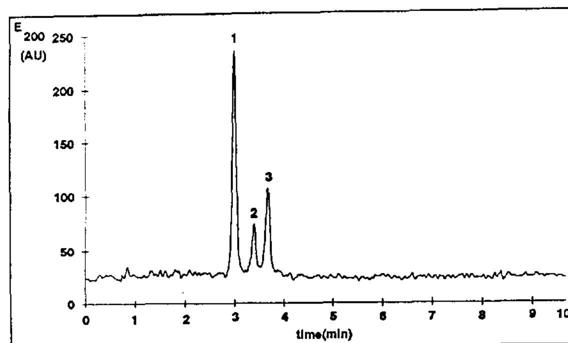

Fig. 2. High-performance capillary electrophoresis of nIL-2 (pIL-2 R435). Detection was by absorbance at 200 nm and the analog output to the collector was scaled 0.2 V and 0.005 AUFS.

can be seen, the three IL-2 species have different mobilities in HPCE. The first peak was detected at a retention time of 3 min, the second at 3.5 min and the third at ca. 4 min. Quantitation of the amounts of the three nIL-2 species was performed using the densitometer software GS 365. The percentage area for each peak is based on the total integrated area for the entire scan. For the first peak we measured 56% relative to the total area of the three peaks. The second protein peak takes 16% and the third protein peak takes 28% of the total peak area. Accordingly, in the nIL-2 preparation tested the proportion of the three peak areas was ca. 4:1:2. The ratio of the three IL-2 species was different in nIL-2 which was isolated from culture supernatants of A 23187/PMA-induced T-lymphoblasts which had been generated from T-lymphocytes by activation with concanavalin A 5–7 days prior to induction and purified using our standard procedure [3]. As shown in the electropherogram (Fig. 3), the first peak after 3 min represents 39% of the total protein, the second peak after 3.5 min 12% and the third peak after 4 min 50%, respectively. In this nIL-2 preparation the ratio of the three peak areas was 3:1:4. The results presented above demonstrate the high selectivity of the HPCE technique employed for separation of proteins with a single charge difference in polypeptides, like mono- and di-sialylated nIL-2. Fig. 4 shows recombinant IL-2 after 15% SDS-PAGE and electro-

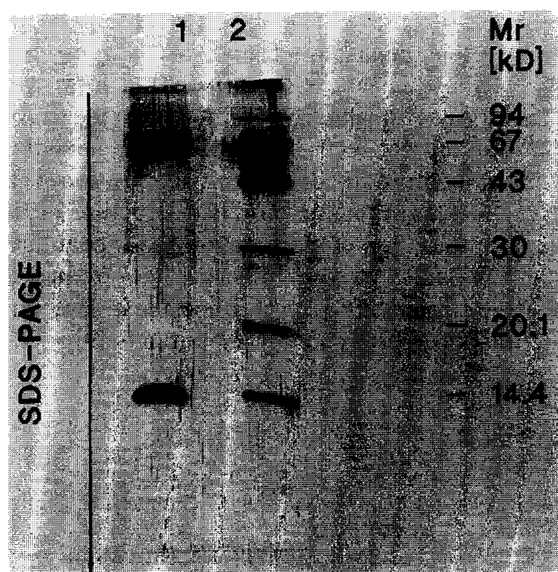

Fig. 4. Colloidal-gold stain on nitrocellulose of highly purified rIL-2. The protein was electrophoresed under reduced conditions. Lane 1: rIL-2; lane 2: molecular-mass marker proteins. An amount of 1 μg of the protein was applied per lane.

blotting onto membranes. Under these denaturing and reducing conditions only one protein band was detected with a molecular mass of $15.0 \cdot 10^3$. Additionally, we again found ghost bands like those detected in the highly purified nIL-2 (Fig. 1). When the rIL-2 preparation was analysed by HPCE (Fig. 5), surprisingly, two protein peaks were detected instead of one, with approximately the same electrophoretic mobility as peak 1 in the electropherogram of nIL-2 in

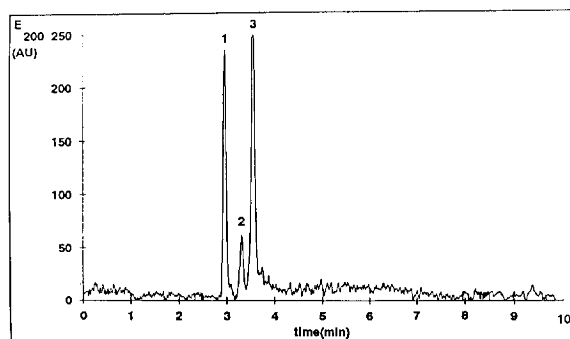

Fig. 3. High-performance capillary electrophoresis of purified nIL-2 from propagated cells. Detection was by absorbance at 200 nm and the analog output to the collector was scaled 0.2 V and 0.005 AUFS.

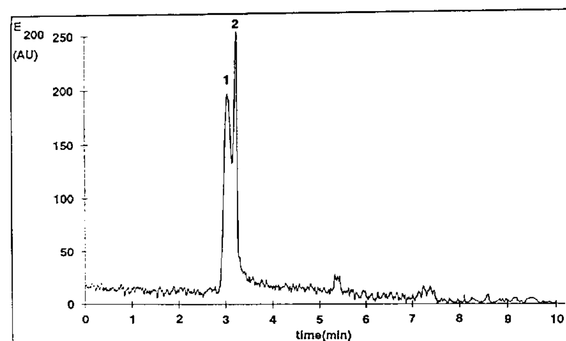

Fig. 5. High-performance capillary electrophoresis of rIL-2. Detection was by absorbance at 200 nm and the analog output to the collector was scaled 0.2 V and 0.005 AUFS.

Fig. 2. Up to now, it is not clear why in HPCE two protein peaks are found. We suggest that one of those peaks represents a rIL-2 form with a conformation slightly modified compared to the normal $15.0 \cdot 10^3$ species. More analytical work is required to confirm this supposition. One may speculate that the modified structure in the rIL-2 preparation could function as a neoantigen and thus may be the cause of the frequently observed relatively high antibody response during immunotherapy in cancer patients [9].

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