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Interactions of nanoparticles with body proteins — improvement of 2D-PAGE-analysis by internal standard

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Abstract

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is the method of choice to investigate protein adsorption of blood proteins (opsonization) onto nanoparticular drug carriers. In general the reproducability of the obtained adsorption patterns is satisfying. However, direct comparison between the amounts of single protein spots from gels obtained in different runs is difficult, because 2D-PAGE is a multistep procedure. A possible solution of the problem is to establish a protein as internal standard. Therefore, selected proteins (Bio-rad) were under investigation. Due to its molecular weight and isoelectric point, soybean trypsin inhibitor (TI) does not interfere with plasma components. Therefore, a method was established to use TI as an internal standard protein to improve comparability between the 2D-PAGE gels obtained in different analytical runs.

Keywords: Two-dimensional electrophoresis; Internal standard; Protein adsorption; Nanoparticles; Drug targeting

Adsorption of blood proteins (opsonization) is regarded as the key factor determining the in vivo fate of intravenously (i.v.) administered colloidal drug carriers. For example, distinct differences in the protein adsorption patterns were found for i.v. injected surface-modified polymeric nanoparticles localizing in the bone marrow or circulating in the blood (Müller and Heinemann, 1989). In recent years the technique of two-dimensional polyacrylamide electrophoresis (2D-PAGE) was optimized for determining plasma protein adsorption onto polymeric nanoparticles by T. Blunk et al. (Blunk et al., 1993; Blunk, 1994). The protocol was successfully transferred to further drug carriers such as liposomes (Diederichs, 1996), superparamagnetic iron oxides (SPIO) (Thode et al., 1997) and parenteral fat emulsions (Harnisch and Müller, 1998).

However, 2D-PAGE is a multi-step-technique and the results are strongly depended on many factors, e.g. sample application, quality of the gels for the second dimension or staining duration. Comparison between the amount of correspond-

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ing protein spots on 2D-PAGE gels obtained in different runs is difficult, because deviation in the overall amount of detected proteins between different gels is possible.

Therefore, the aim of this study was to establish a procedure which enables the comparison of 2D-PAGE gels obtained from different runs.

All chemicals for 2D-PAGE were of analytical grade. Acrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), ammonium persulfate and piperazine diacrylamide (PDA) were purchased from Bio-rad (Munich, Germany). Carrier ampholytes 'Resolyte' pH 3.5–10 were from BDH (Poole, UK), cholamidopropyldimehtylhydroxypropanesulfonate (CHAPS) and Tris from Sigma (Deisenhofen, Germany). All other chemicals (Bjellqvist et al., 1993; Blunk et al., 1993; Görg et al., 1995) were either from FLUKA Chemie AG (Buchs, Switzerland) or Merck (Darmstadt, Germany). Ready-made immobilized pH gradients (IPGs) (pH 3.5–10, nonlinear) were from Pharmacia (Uppsala, Sweden). Human plasma was obtained from healthy male volunteers and stored at −20°C.

The proteins which were investigated with respect to their usefulness as to be internal standards were soybean trypsin inhibitor (TI) and rabbit muscle glyceraldehyde 3-phosphate dehydrogenase (GADPH) (Bio-rad) (Table 1), both purchased from Sigma (Deisenhofen, Germany).

A total of 30 μl human plasma and 10 μl of a solubilizing mixture containing 10% w/v sodium dodecyl sulfate (SDS) and 2.32% w/v dithioerythritol (DTE) were incubated for 5 min at 95°C, followed by 2 min cooling down at room temperature. Afterwards 490 μl of a solution containing DTE, CHAPS, urea, Tris and bromphenole blue was added according to (Bjellqvist et al., 1993).

Table 1
Molecular weight (MW) and isoelectric point (p*I*) of the selected proteins

Protein	MW (Da)	p*I* (pH)
TI	21 500	4.5
GAPDH	36 000	8.3–8.5

Soybean TI and GADPH was used in a concentration of 1 μg/μl (Hames and Rickwood, 1981). The total amount of protein applied to IPG-strips was 4–5 μg, respectively. Therefore, the proteins were treated corresponding to the procedure described above. This resulted in two different standard protein solutions (SPS).

Isoelectric focusing and the following equilibration were carried out using IPGs, (Bjellqvist et al., 1993). For the second dimension the strips were sealed at the top of the slab gels. Upper and lower electrode buffer of the second dimension were prepared according to Görg et al. (1995). The gels used in this study were cast with a gradient from 9 to 16% using 0.4% w/v PDA as cross-linker. After proceeding the second dimension the gels were silver stained according to (Bjellqvist et al., 1993). The stained gels were scanned with a laser densitometer and data were processed by using MELANIE II software (Bio-rad, Munich, Germany). The protein spots were identified by matching the gels to the master map of human plasma (Anderson and Anderson, 1991; Golaz et al., 1993) and to Bio-rad (Bio-rad).

Proteins used as internal standard for investigations by 2D-PAGE employing human plasma or serum for incubation should suit following demands:

1. Analysis by 2D-PAGE should yield only to a single spot on the gel.
2. Due to its molecular weight (MW) and its isoelectric point (p*I*) the spot should not cover other proteins.
3. Reasonable price.

Fig. 1 shows the protein spots obtained from soybean TI and GAPDH, Fig. 2 shows the protein pattern of human citrate plasma including both proteins:

The total protein amounts obtained by the SPSs were in the same order of magnitude. Application of pure SPSs (5.3 volume units (VU), standard deviation (S.D.) = 2.4) or SPSs mixed with plasma (4.7 VU, S.D. = 2.7) revealed no considerable differences. The percentage of the standard proteins in plasma was 2.3% (S.D. = 1.2).

TI appeared as a distinct single spot at a position in the gel where no plasma proteins were detected.

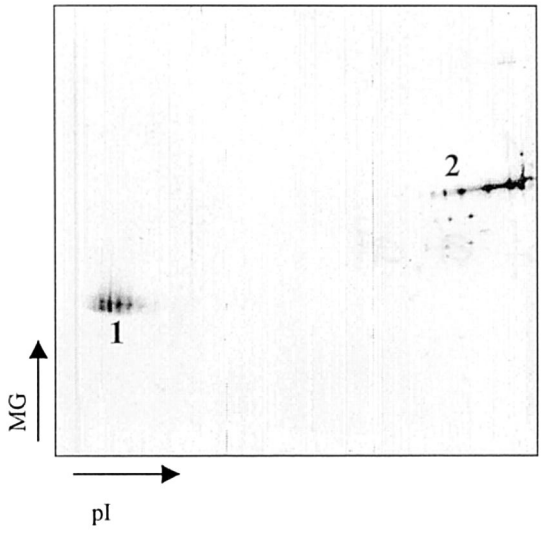

Fig. 1. Spots of trypsin inhibitor (TI) (1) and glyceraldehyde 3-phosphate dehydrogenase (GADPH) (2) of an amount of 5 μg each, obtained with two dimensional-polyacrylamide gel electrophoresis (2D-PAGE). For explanation cf. Fig. 2.

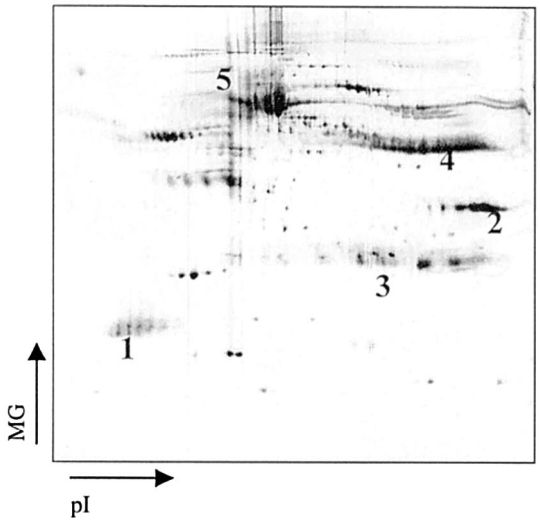

Fig. 2. Protein pattern of human plasma containing soybean trypsin inhibitor (TI) (1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (2). *x*-axis, isoelectric point (p*I*), pH range from 3 to 10; *y*-axis, molecular weight (MW) 6–200 kDa; 1, soybean TI; 2, GAPDH; 3, Ig light chains; 4, IgGγ-chains; 5, Albumin.

GADPH showed a chain of spots. Due to its p*I* and molecular weight, the spot chain was next to immunoglobuline G-γ- and fibrinogen-β-chains, so differentiation between the protein chains could be difficult.

In conclusion soybean TI in an amount of 5 μg per gel was found to be appropriate for internal standard protein used in 2D-PAGE analysis and working with human plasma or serum. After 2D-PAGE analysis it showed a distinct single spot which does not overlap any other spots of the 2D pattern of human plasma or serum, respectively. The price of the protein is reasonable. Therefore, soybean TI complies with all requirements for an internal standard for 2D-PAGE analysis.

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