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Proteinanalytics with Surface Plasmon Resonance Technique and Capillary Electrophoresis

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Abstract: Surface plasmon resonance technique and capillary electrophoresis are methods which are used in the Bioanalytics Group of the Chemistry Department at the University of Applied Sciences Basel (FHBB) in the attempt to characterize the integrity, purity, and function of relevant proteins in drug development, diagnostics or tissue engineering. Currently we are collaborating with Prionics AG in Zürich, ZLB Bioplasma AG in Bern and the Zürcher Hochschule in Winterthur.

Keywords: Bioanalytics · Protein · Biosensor · Biospecific Interaction Analysis · Capillary electrophoresis · Prion · BSE

With the Human Genome Project rapidly approaching closure, the products of transcripts of genomic DNA, the proteins, will become objects of increased interest also in applied Research and Development. Based on this view, the activities in proteinanalytics in the Department of Chemistry focus on two novel techniques: the Biacore chip-based biospecific interaction analysis technology and capillary electrophoresis. Using these methods we attempt to characterize the integrity, purity and function of relevant proteins in drug development, diagnostics or tissue engineering.

Biospecific Interaction Analysis

Biacore chip-based technology is based on the optical phenomenon of surface plasmon resonance, which detects changes in the refractive index of the solution close to the surface chip. The refractive index is directly related to the mass concentration in the surface layer and increases when analytes bind to an immobilized ligand, like an antibody, a receptor, a biomolecule or a small organic compound bound to a carrier protein. The experiments are performed under continuous, controlled flow conditions thus allowing observation – without an additional label – of the progress of the binding between specific molecules in real time. Consequently, the sensorgram reflects both the time course of the interaction and the amount of analyte bound (Fig. 1).

The use of Biacore chip-based technology for applied research was explored using two different approaches: on the one hand the binding of concanavalin A

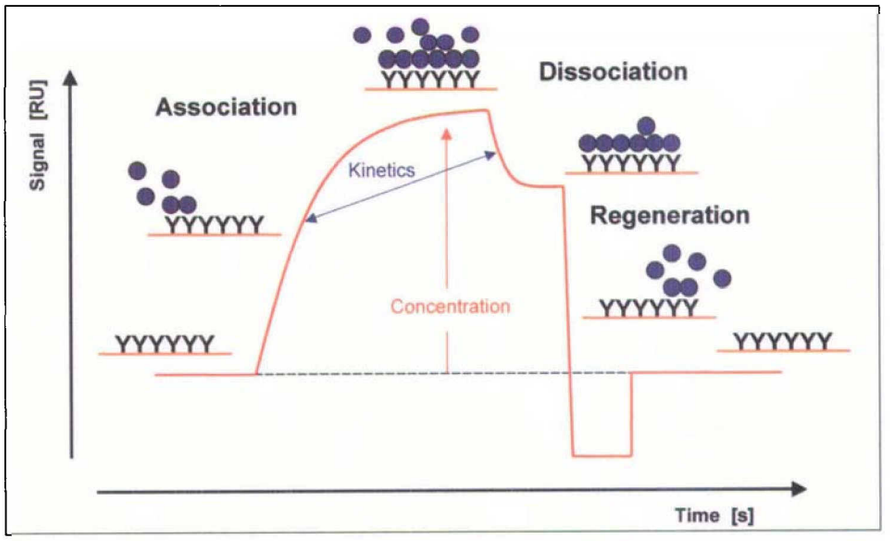

Fig. 1. Schematic representation of information that can be obtained from a real-time sensorgram

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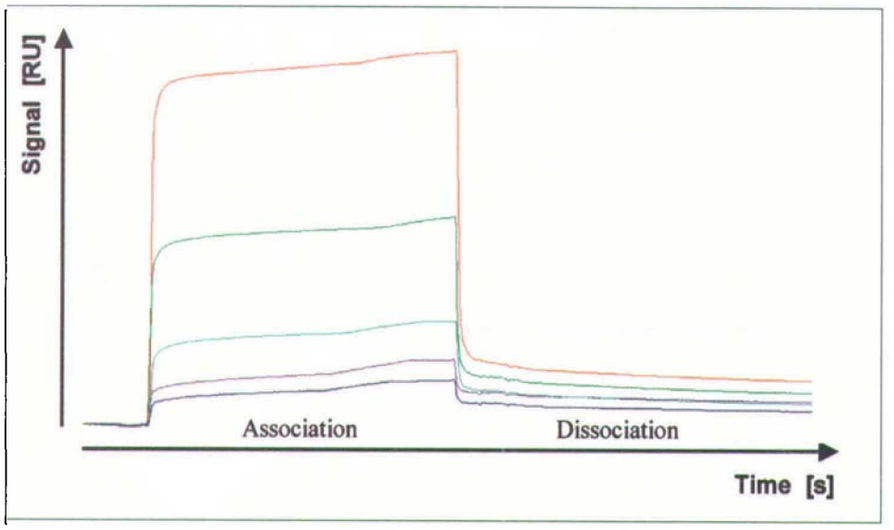

Fig. 2. Sensorgram of binding of invertase to immobilized concanavalin A.

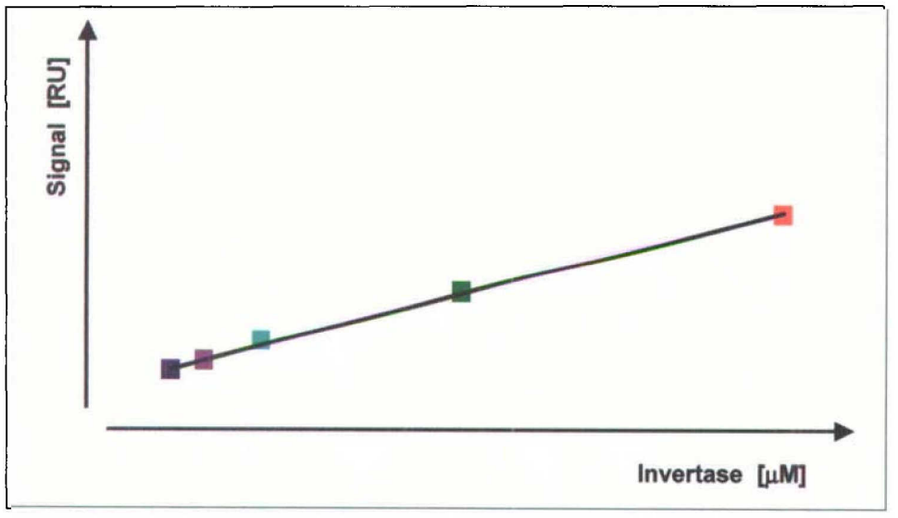

Fig. 3. Read-out (RU) after 200 sec in dependence of invertase concentration

to specific carbohydrate moieties and on the other hand the measurement of antibody-antigen interactions to select anti-prion protein antibodies based on its affinity.

Lectins are proteins which specifically bind or crosslink carbohydrates. The affinity a lectin displays for cells or macromolecular ligands is higher than that for dimeric carbohydrates [1][2]. Concanavalin A (Con A) belongs to the legume lectin family and selectively binds to α-mannopyranosyl and α-glucopyranosyl residues. Depending on the pH, Con A exists as a dimer with a molecular weight of 52 000 daltons in acid solution, whereas above pH 7 it is primarily a tetramer. Each subunit contains a carbohydrate binding site. Our interest in lectins is twofold. Firstly, because of their capacity to recognize oligosaccharide patterns, lectins are very useful probes for cell surfaces and secondly, because lectins can be used as a tool to mimic the drug receptor interaction on various chip architectures.

Concanavalin A was bound to one of Biacores's CM5 chips *via* the amine coupling technique. The interaction between the mannose moiety of invertase and immobilized Con A was estimated with different concentrations of invertase between 1.8 µM to 37.8 µM (Fig. 2). The resulting signals were proportional to the applied concentrations. From the different concentration curves the association and dissociation constants could be calculated with Langmuir model software of Biacore (Fig. 3). The association constant was 798 (1/Ms), the dissociation constant 0.0012 (1/s) and the affinity constant 6.66×10^5 (1/M).

The bovine spongiform encephalopathy-specific form of PrP^{BSE} is a disease-specific marker which is independent of clinical signs and pathology. Even more importantly, this form of PrP is present before the appearance of clinical signs [3]. The goal of current research and development in this area will be to improve existing tests for rapid and reliable measurement of PrP^{BSE} primarily with regard to sensitivity. Improved assays might enable measurement of PrP^{BSE} in live animals. In particular, an *in vivo* assay for the detection of infected cattle based on a test which is able to detect PrP^{BSE} in blood or spinal fluid is highly demanded for a systematic screening of the cow populations of BSE-contaminated regions.

The comparative determination of kinetic and affinity parameters provides data for the evaluation and selection of antibodies used in diagnostics. In collaboration with Prionics AG, in Zürich and ZHW (Zürcher Hochschule Winterthur), we compared the binding characteristics of two monoclonal antibodies against the cellular prion protein (PrP) immobilized to the carboxylated dextran matrix of a sensor chip. The sensorgrams of the two antibodies depicted in Fig. 4 clearly demonstrate that the two antibodies bind the prion with different on- and off-rates. From this data it was calculated that the prion binding affinity of mAb1 is roughly two orders of magnitude larger than that of mAb2. Some key parameters characteristic of this type of experimental protocol are the small amount of ligand immobilized to the chip (2.9 ng/mm^2), the low sample volume (45 µl) and the short assay running time (400 sec).

To summarize, the Biacore-chip-based biosensor allows the determination of the kinetic binding constants of the interaction between the prion protein and monoclonal antibodies. Thus, this method is generally an interesting tool for the screening and selection of novel antibodies.

Capillary Electrophoresis

Capillary electrophoresis (CE) is a highly efficient separation technique based on principles of electro-osmosis and electrophoresis. It is a very attractive mode of separating proteins and peptides because of its simplicity, its relatively high resolving power in separation and its flexibility in manipulating separation parameters by static and dynamic coatings [4].

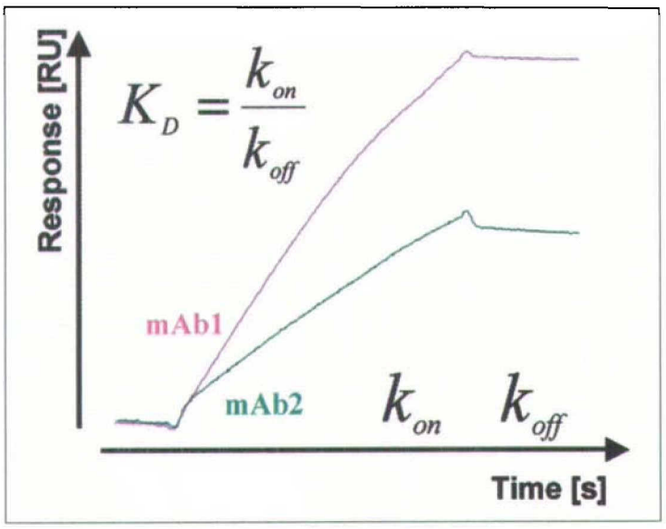

Fig. 4. Comparison of relative kinetics of two prion binding monoclonal antibodies (mAb1: $K_D = 2.6 \times 10^{-11}$ M and mAb2: $K_D = 4.5 \times 10^{-9}$ M)

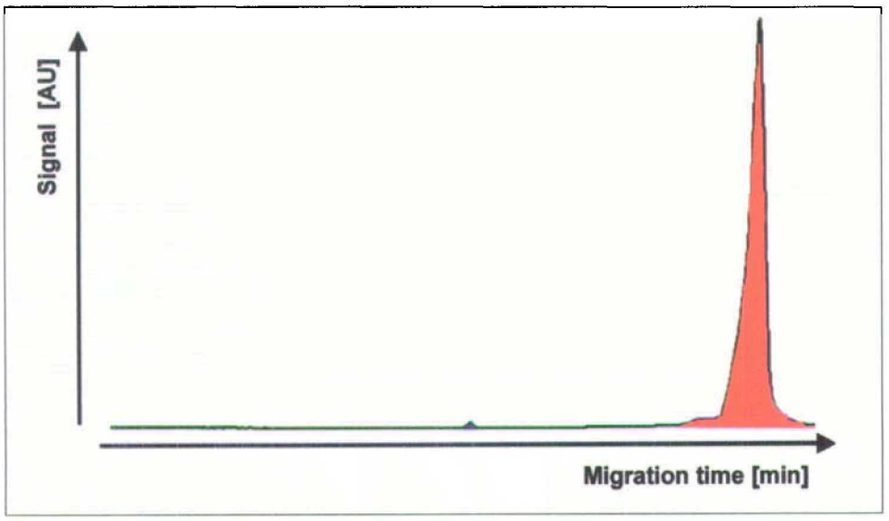

Fig. 5. Separation of normal human serum by capillary electrophoresis. Precision (N = 10); Albumin (migration: 1.62% CV, %-area: 2.51% CV and mobility: 0.78% CV), Gamma (migration 1.62% CV, %-area: 3.14% CV and mobility: 1.85% CV)

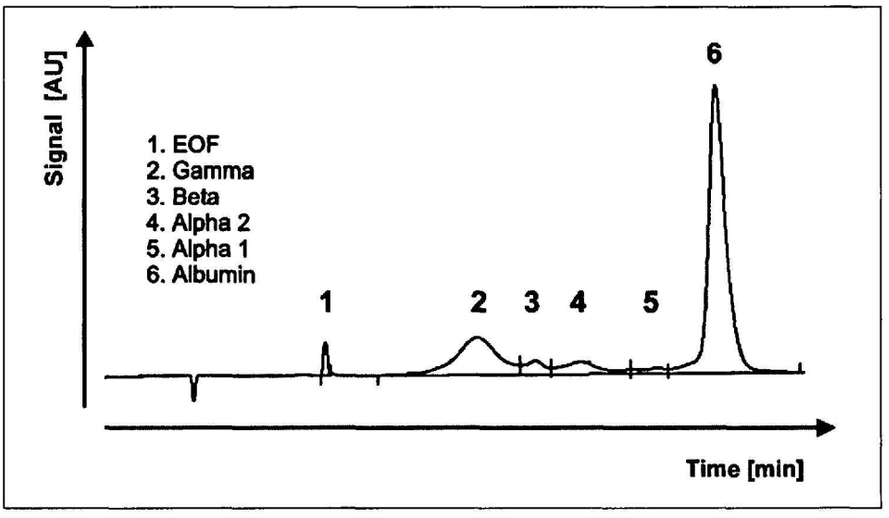

Fig. 6. Electropherogram of purified human albumin from blood acquired with the capillary electrophoresis MDQ system from Beckman Coulter using uncoated silica capillary

For ZLB Bioplasma AG in Bern capillary electrophoresis could be an alternative method for the quality control of blood derived proteins and might be able to deliver comparable results to the acetate electrophoresis method already approved by the FDA. The purpose of our investigation was to demonstrate comparability of data arising from acetate electrophoresis and capillary electrophoresis using normal serum and purified albumin from human blood.

With a novel CE method (Fig. 5) normal human serum protein could be separated into its major components (gamma-, alpha 1- and 2-, beta-globulin and albumin). Two of them, namely, albumin and gamma, could be measured with good precision. Furthermore, the results agree well with those obtained by acetate electrophoresis. The quantification of purified albumin from human blood by CE [5] shows good correlation with conventional cellulose acetate paper electrophoresis; with regard to precision, speed, and automation, CE is even superior. Fig. 6 shows the electropherogram of human albumin. In order to assess assay reproducibility, key performance parameters were determined in twelve consecutive runs. The mean ratios of the area under the signal intensity curves of human serum albumin to the impurity was 1 513.7 kAU to 1.1 kAU with coefficients of variation of 1.62% and 7.91%, respectively. The purity of the human serum albumin was >99%. The mean value of the migration time for human serum albumin was 6.8 min with a coefficient of variation of 0.65%.

In conclusion, the new CE methods presented could be the basis for the quality control of blood derived proteins.

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