The MAIIA technology for rapid determination of protein isoforms

MAIIA and isoforms

MAIIA (Membrane Assisted Isoform ImmunoAssay) is a novel proprietary technology for rapid and sensitive measurement of protein isoforms in biological specimens.

Posttranslational modifications, like glycosylation, of a protein may result in a huge number of protein variants (isoforms) which might have high impact on its biological function.

There are numerous reports on protein isoform distributions related to clinical effects, but there is a lack of suitable methods for measuring isoforms occurring at low concentration in blood and urine.
The MAIIA technology seems to fulfil the requirements as a rapid and sensitive isoform determination method with ability to resolve and detect several types of posttranslational modified proteins even when they occur in femto-molar concentration.

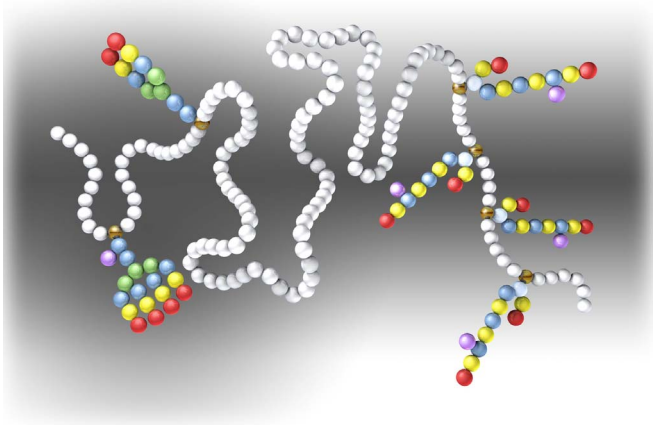

Fig. 1: A glycoprotein with the peptide chain and the protruding carbohydrate structures can host and transfer large amounts of information. The carbohydrate structures are the fine-tuning regulators of protein activity.

Technology

An ion-exchange or affinity chromatographic separation of protein-isoforms and a sensitive immunoassay detection are combined on a few cm^2 of a porous monolith chip.

Thin lines of immobilized antibodies are used for specific capturing of target molecules, which then can be detected by the reaction with antibodies bound to carbon black nanostrings or other suitable labels. The bound label is quantified by the use of an image scanner.

This technology can distinguish minor differences in protein carbohydrate structure and enables specific determination of proteins in a complex environment, requiring only a few femtogram of each isoform for its detection.

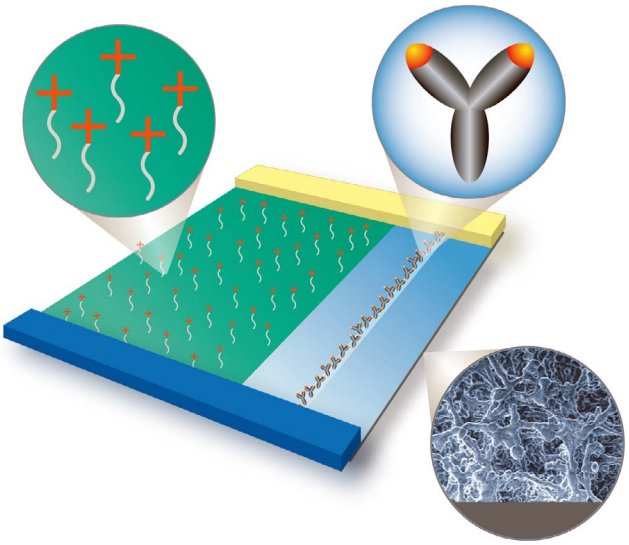

Fig. 2: An isoform separation zone containing ion-exchange or affinity ligands is combined with a capturing zone with immobilised specific antibodies. The separation and capturing process is performed on a few cm^2 of a porous monolith chip during some minutes.

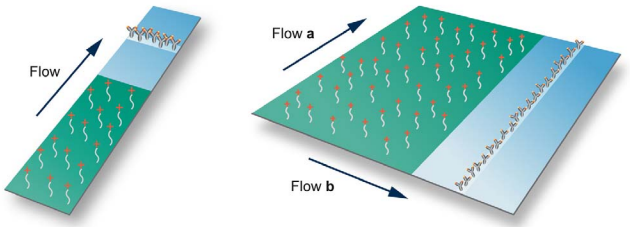

Fig. 3: There are different types of MAIIA chips. The 1D MAIIA (left) is a micro-column where isoforms interacting with the ligands in the separation zone are retarded. After having passed the separation zone, the weak binding isoforms will be captured and detected in the antibody zone. The result for the separation of EPO isoforms is shown in Fig. 5. The 2D MAIIA (right), with flow in various directions, can be used to measure isoform profiles as shown from the result for separation of transferrin isoforms in Fig. 6. The testing procedure takes only 10-20 min.

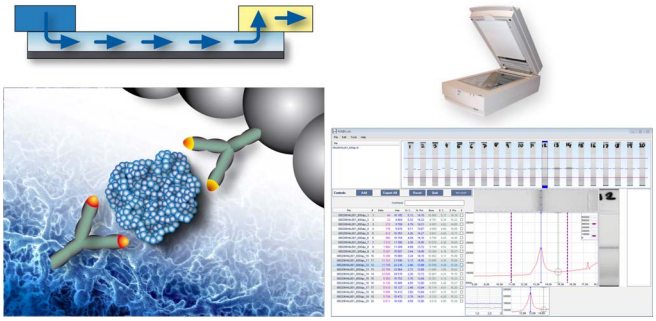

Fig. 4: The components for the MAIIA tests, besides the chip with separation and capture zone, are chromatography using capillary flow; sandwich immunoassay with the use of antibodies bound to carbon black nano-strings as label; scanner for detection of bound carbon black intensity; and specially designed software.

Applications

Several interesting analytes appear in low concentrations in biological specimens with extensive micro-heterogeneity. Determination of selected isoforms should give much higher diagnostic specificity.

Prostate specific antigen (PSA), about 100 pM in serum, occurs mainly in complexes with other proteins. It appears as degradation products and as isoforms with differences in the carbohydrate chains and should be a better marker for prostate cancer if the aberrant glycosylated isoforms could be determined.

Cardiac troponin I (cTnI), about 3 pM in serum, is released into the circulation after myocardial injury (MI). It appears in complexes with other proteins, and may undergo oxidation and phosphorylation as well as proteolysis after its release. The isolated measurement of recently released non-degraded isoforms should make possible earlier and more specific diagnosis.

Erythropoietin (EPO), about 0.3 pM in urine, is a glycoprotein hormone with about 40% carbohydrate and several hundreds of isoforms appearing in samples from normal individuals. Methods for identification of aberrant EPO isoforms are useful for detection of doping with recombinant EPO, as the recombinant and endogenous isoforms have different types of glycosylation.

1D MAIIA - measures the weak binding population.

For EPO doping tests it has been found that the 1D MAIIA test (see Fig. 3), using a MAIIA micro-column, rapidly can distinguish different types of EPO subpopulations. The separation zone can be a zone with anion exchange groups (see results in Fig. 5) or a zone with ligands like the lectin wheat germ agglutinin.

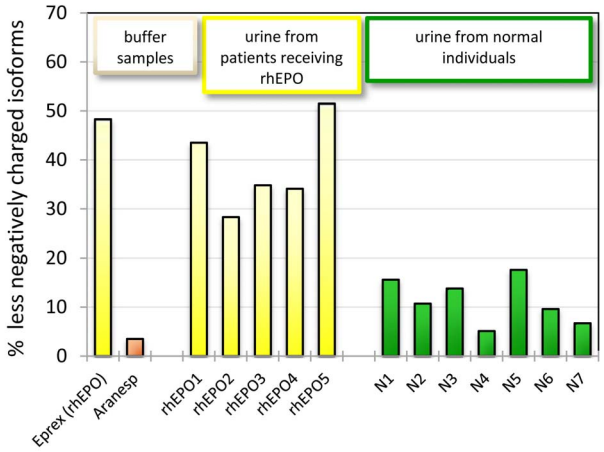

Fig. 5: A rapid and easy-to-use EPO doping test has been developed that can reveal the presence of aberrantly charged glycosylated EPO isoforms, like recombinant EPO, even when they appear in the low concentration range (down to 0.5 ng/L) of EPO found in urine specimens.

2D MAIIA - measures the isoform profile

The Carbohydrate deficient transferrin (CDT) population with asialo-, monosialo- and disialotransferrin constitutes about 3 % of the total transferrin population, but will increase after repeated too high alcohol intake. The major isoform population is tetrasialotransferrin which makes up 70-75% of all isoforms. The method, transferrin anion exchange MAIIA, with consequtive liquid flow in more than two directions (2D MAIIA), can efficiently separate the different transferrin isoforms in a chip of a few cm^2. The results can be seen in Fig. 6.

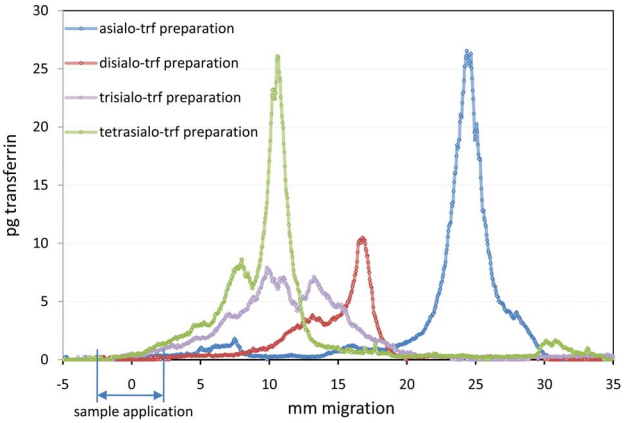

Fig. 6: Partially purified transferrin isoforms were separated and specifically quantified by the anion-exchange chromatography MAIIA technology in less than 15 min.

Summary

Posttranslational modification (PTM), like glycosylation, results in a huge number of protein variants (isoforms). The distribution of isoforms seems to have high impact on the biological function as well as being an indicator of pathological conditions, but the lack of suitable methods hampers the clinical use of isoform based diagnostics. The combination of chromatographic separations and a sensitive immunoassay detection miniaturised in a small chip constitutes a promising isoform determination methodology.