Platelet Proteomics: Essentials for Understanding and Application

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Key Words

Platelets · Proteomics

Summary

Proteomics has become a promising technology for platelet research. The development of the last 10 years has provided a complex set of different separation tools and analytical techniques for proteins and their modifications. Several of these approaches have already been performed in platelet studies. Here, we present an overview of different methods used in the field of platelet proteomics, like, for instance, two-dimensional gel electrophoresis of proteins, proteolytic digestion of complete platelet lysates combined with subsequent high performance liquid chromatography (HPLC)-based peptide separation as well as strategies for the analysis of post-translational modifications. We will focus on methodical aspects of the different techniques as well as their potential and limitations in order to provide an orientation guide for further projects.

Schlüsselwörter

Thrombozyten · Proteomik

Zusammenfassung

Die Proteomforschung hat sich in den letzten Jahren als eine vielversprechende Methode zur Analyse humaner Thrombozyten etabliert. Die technologische Entwicklung der letzten 10 Jahre hat zur Verfügbarkeit einer großen Anzahl verschiedener methodischer Ansätze zur Trennung und Analyse von Proteinen und Proteinmodifikationen geführt. Einige dieser Methoden wurden bereits in mehreren Thrombozytenstudien angewandt. In dieser Arbeit geben wir einen Überblick über die wichtigsten Ansätze, wie z.B. die zweidimensionale Gelelektrophorese oder die HPLC (high performance liquid chromatography)-basierte Peptidtrennung nach vollständiger proteolytischer Spaltung sowie über Strategien für die Analyse posttranslati onaler Modifikationen. Besonderes Augenmerk gilt dabei den technischen Aspekten der einzelnen Methoden sowie deren Möglichkeiten und Limitierungen, um eine Orientierung für die Planung zukünftiger Projekte zu geben.

Introduction

The term ‘proteomics’ was introduced in 1995 by Marc Wilkins and Keith Williams to point out that the analysis of the protein level is the next task after the genomic era. The first breakthrough in proteomics was achieved by using appro-

priate analytical tools, such as high-resolution two-dimensional gel electrophoresis (2-DE) for protein separation and mass spectrometry (MS) for identification. Since 1995, proteomics has become one of the major new technology platforms in life science. During the last 10 years, many new approaches for the identification of proteins in complex mixtures [1], the analysis of post-translational modifications (PTMs) [2, 3] and the relative and absolute quantification of different samples [4–6] have been established. This enormous progress was possible due to the dramatic technical advance of MS instrumen-

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tation [7–9] and the automation of nano-high performance liquid chromatography (HPLC) separation [10] and other separation methods. Today, the analysis of complex sample mixtures and determination of protein content is possible within a fraction of time. Compared to the classical Edman sequencing [11] which was the protein analysis method of choice until the mid 1990s, modern proteome techniques allow a much faster analysis of proteins with considerably higher sensitivity and reliability.

The involvement of platelets in cardiovascular diseases, such as atherosclerosis, myocardial infarction and stroke, renders them highly relevant targets for current biomedical research. As platelets are anucleate and do not contain any genomic DNA, genomics is an inappropriate tool for studying the important processes of activation and aggregation. However, SAGE (serial analysis of gene expression) and microarray techniques are being applied for the identification of the transcriptome although there are ongoing discussions about whether the residual mRNA is really functional or just a relict of thrombopoiesis [12, 13]. Moreover, the rapid changes in platelet organization during activation and aggregation could not be affected entirely by gene expression. Therefore, proteomics is the method of choice for the analysis of these processes, describing minute alterations and dynamics on the protein level. In this review, we summarize the latest and most important proteome studies and their corresponding technological background in order to provide an insight into the different proteomic techniques as well as their potential and limitations.

Two-Dimensional Gel Electrophoresis

2-DE was independently invented by O'Farrell [14] and Klose [15] in 1975 and is based on the subsequent separation of proteins by two independent physicochemical parameters. In the first dimension, proteins are separated according to their net charge in an isoelectric focusing step. Originally performed in tube gels and using carrier ampholytes which generate the desired pH gradient during the electrophoretic separation, Bjellqvist et al. [16] improved the handling of the 2-DE system by using immobilized pH gradient strips. For the second dimension, a common sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) is accomplished, separating proteins according to their molecular weight (MW). Since the isoelectric point (pI) and MW are independent parameters, the resolutions of both separation dimensions are multiplied, leading to a high separation capacity of up to 10,000 protein spots on a single gel.

MS-compatible Coomassie, silver or fluorescence staining procedures or Western blotting can be used for protein visualization. The simultaneous visualization of high numbers of proteins from a complex sample mixture on a single 2D gel as so-called proteome maps is one of the main reasons for the great success and acceptance of this technique. The first published

Fig. 1. Schematic overview of 2-DE-based proteomics approaches. **A** Purified platelets are lysed with sample buffer (8 M urea, 2 M thiourea, 2% CHAPS, 1% ampholytes). After first- and second-dimension separation, the 2D gels are stained, e.g. with silver as in this case. **B** Gel spots are excised, washed and tryptically digested, followed by MALDI-TOF PMF analysis or LC-ESI-MS/MS. **C** With MALDI-TOF PMF, peptides of one sample are displayed in a mass spectrum representing a unique pattern which is used for protein identification. **D** The LC-ESI-MS/MS approach uses LC for peptide separation prior to MS analysis. The MS-detected peptide ions are isolated and fragmented, yielding specific ion series. These spectra are used for peptide identification using a search algorithm.

platelet proteome study using the 2-DE approach was performed in 2000 by Marcus et al. [17]. In 2002 and 2004, respectively, O'Neill et al. [18] and Garcia et al. [19] of the Oxford Glycobiology Group published more extensive projects. While Marcus et al. [17] used broad pH range strips (pH 3–10) for isoelectric focussing, the others [18, 19] combined zoom strips with small pH ranges. This approach provides the advantage that resolution can be increased virtually by spreading the complete pH range over several gels, thereby allowing the detection of more discrete spots. Generally, for protein identification, visualized spots are excised and washed for removal of e.g. salts and detergents. Afterwards, the gel plugs are digested with a specific protease, such as trypsin, in order to generate

Table 1. Overview of published 2-DE proteome studies on platelets

	Marcus et al., 2000 [17]	O'Neill et al., 2002 [18]	Garcia et al., 2004 [27]
IEF pH range	3–10	4–5	5–11 (5–7/5–6/6–11)
Staining method	Coomassie G-250	fluorescence (OGT MP17)	fluorescence (OGT MP17)
Detected spots ^a , n	205 (annotated)	387	692/607/472
Used MS methods	MALDI-MS	ESI-MS/MS	ESI-MS/MS
Identified proteins, n	186	284	365/282/255
Unique proteins, n	123	123	472

^aNote the number of detected spots compared to the number of uniquely identified proteins. This is typical for classical 2-DE as many proteins are represented by more than one spot due to modifications, isoforms or degradation. O'Neill and Garcia applied zoom gels resulting in more detected spots and identified proteins. In the study of Marcus, the number of detected spots was not mentioned, but due to the use of Coomassie instead of silver or fluorescence staining, the total amount could be in the range of the reported 205 spots.

peptides for subsequent MS analysis. For determination of protein identity, several MS techniques are available. In the study of Marcus et al. [17], a matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) was used for peptide mass fingerprint analysis (PMF) (fig. 1). This MS approach is fast because the complete set of generated peptides from a gel spot are measured in a single mass spectrum, resulting in a characteristic pattern of signals which can be used for automatic protein identification. The disadvantage of this system is the relatively poor success rate of 30–40% due to the fact that peptides are often modified or peptide patterns of two or more proteins are superimposed. The applied search algorithm cannot compensate these variations in the spectrum and produces false-positive results or fails to identify the corresponding protein. In contrast, O'Neill et al. [18] and Garcia et al. [19] analyzed their protein spots using an LC-MS/MS approach. After digestion, the generated peptides were separated by reversed phase HPLC (RP-HPLC) coupled online to an Electrospray (ESI) Quadrupole TOF mass spectrometer for identification (fig. 1). Thus, peptides of complex mixtures are separated, concentrated and eluted directly into the mass spectrometer where the individual peptide ions are isolated and fragmented. Resulting fragment ions are acquired in an additional MS spectrum called tandem MS or MS/MS. This leads to huge amounts of peptide spectra which are separately interpreted and assigned to the identified proteins. Compared to MALDI-MS, this method has better identification rates due to the concentration of peptides during HPLC separation, rendering the analysis more sensitive and less susceptible to impurities. Moreover, the analysis of single peptides by tandem MS also allows the identification of modifications. However, compared to MALDI-TOF, the LC-ESI-MS/MS approach is much more time consuming with regard to net measurement time and requires higher computing power for the interpretation of the data derived from the LC runs. O'Neill et al. [18] and Garcia et al. [19] modified the common nano-HPLC setup by removing the separation column and performing the runs only on the preconcentration column. On the one hand, analysis time was reduced because shorter gradients

could be applied. On the other hand, the use of a separation column increases resolution and peptide concentration which is mandatory for reliable identification of proteins with multiple and low abundant peptides.

Due to the splitting of the 3–10 pH range for isoelectric focussing into smaller areas (zoom gels), O'Neill et al. [18] and Garcia et al. [19] were able to detect a total of 2,300 different protein features compared to 205 annotated spots in the study by Marcus et al. [17]. Apart from the application of zoom gels, one major reason for these differences was that Marcus et al. [17] used Coomassie Brilliant Blue G250[®] (National Diagnostics Inc., Atlanta, GA, USA) for staining, which is much less sensitive than silver or fluorescence staining protocols [20]. From the 205 spots, 123 unique proteins were detected, whereas O'Neill et al. [18] and Garcia et al. [19] identified 123 and 472 open reading frames (ORFs), respectively, after removal of redundancies (table 1).

Although a comprehensive proteome map can be generated via 2-DE, the technique in general has major limitations which have to be taken into account. The isoelectric focusing step is very sensitive towards charged substances, such as salts, lipids and detergents. Therefore, all samples have to be desalted and purified before separation, for instance by trichloroacetic acid (TCA)/acetone precipitation. However, such preconditioning procedures can cause irreversible loss of protein. Additionally, the usage of strong ionic detergents, such as SDS, is not applicable, because due to their charge they would interfere with the isoelectric focusing. The relatively weak zwitterionic detergent CHAPS (3-[(3-cholamidopropyl)dimethylamino]-1-propanesulphonate) has become one of the routinely used detergents for isoelectric focusing. Although there are some developments to overcome these limitations, like the usage of stronger zwitterionic detergents or Triton X-100, no breakthrough has been achieved so far [21, 22]. Moreover, the proteins move to their isoelectric point during the focusing procedure, which per definition is the point of lowest solubility, increasing the tendency for precipitation [23]. Therefore, hydrophobic proteins, like receptors or other membrane proteins, tend to precipitate under these conditions. For this

reason, the important class of membrane proteins is generally underrepresented in 2D-PAGE-based studies. In addition, very acidic and basic proteins are not suitable either. A more general problem concerns the usage of complete cell lysates for separation. Platelets contain a very extensive cytoskeleton with high amounts of actin, tubulin, myosin and other cytoskeletal proteins [24, 25]. Along with thrombospondin and further cytoskeletal-associated proteins, such as filamin, these proteins represent the predominant majority of the protein content in platelets. Due to the limited dynamic range of each detection method, low abundant proteins can hardly be detected in the presence of these highly expressed proteins. Therefore, O'Neill et al. [18] and Garcia et al. [19] applied several different zoom gels covering a pH range of 4–11 in order to extend the dynamic range. However, they did not perform a prefractionation according to the pI range, which allows for the application of higher sample amounts. Nevertheless, 2-DE is a very important tool for proteomic research because of its capability to visualize complex samples and the possibility of computer-based image analysis for differential studies. Moreover, the method was recently improved due to the introduction of the differential gel electrophoresis application [26]. By using different fluorescent-labeled protein tags, samples can be multiplexed in one gel and used for differential display of two or more samples. This reduces the number of required gels, hence excluding the gel-to-gel variability and improving the comparability of experimental repeats.

In 2004, Garcia et al. [27] published the first differential platelet proteome study in which TRAP(thrombin receptor-activating peptide)-activated platelets were compared with non-activated platelets. This study revealed that the downstream adapter protein of tyrosine kinase 2 is regulated after TRAP treatment. Further studies on this protein demonstrated an involvement in the phosphorylation of the collagen receptor GPVI as well as integrin GPIIb/IIIa. The potential of proteome techniques in the identification of novel platelet proteins for functional research has been demonstrated by this work.

HPLC-Based Proteomics

While gel electrophoresis provides the highest resolution for the separation of proteins, RP-HPLC has proven to be well suitable for separation of peptides. HPLC has advantages in terms of automation, reproducibility and sensitivity compared to gel-based separation techniques. Additionally, RP-HPLC coupled online with ESI-MS provides a fully automatic and very sensitive system for high-throughput proteome analysis. Reduction of column size and flow rates to capillary or nano-HPLC systems with flow rates in the range of 200 nl per min yielded detection limits in the low femtomole range. Moreover, peptides feature less heterogeneity in their physico-chemical behavior compared to more complex proteins, making it easier to separate a broad range of peptides with a single

technique. Therefore, proteins which are normally not represented by 2-DE may be better accessible for separation on the peptide level. Furthermore, a cellular proteome comprises several thousand proteins which results in hundreds of thousands of peptides after digestion. This huge amount of analytes cannot be separated in a single step by any technique currently available, thus requiring multidimensional liquid chromatography (MDLC). Thereby, peptides are separated according to different orthogonal parameters similar to the classical 2-DE. Normally, ion-exchange chromatography is applied in the first dimension followed by reversed-phase chromatography in the second dimension which can be directly coupled to an ESI mass spectrometer. Already, several different applications have been designed for this approach [28, 29]. Another technique similar to MDLC named combined fractional diagonal chromatography (COFRADIC) was developed by the group of Joël Vandekerckhove [30, 31]. The proof of principle of this technology was first demonstrated in 2002 [30], and the application on complete platelet lysates was published in 2003 [31]. Before chromatographic separation, all cysteine residues and free amines (α - and ϵ -amines) were blocked by alkylation and acetylation, respectively. After chemical treatment, the sample was digested with trypsin and fractionated by C-18 RP-HPLC into 12 fractions. Then, each fraction was chemically modified with TNBS (2,4,6-trinitrobenzenesulfonic acid) in order to modify all unblocked peptide N-termini. Afterwards, the fractions were re-run under the same chromatographic conditions. While TNBS-modified peptides shifted to later retention times (hydrophobic shift), unaltered N-terminal peptides showed the same retention behavior as before and were collected for subsequent MS analysis and identification, respectively (fig. 2). Using this approach, sample complexity was reduced significantly, as only the N-terminal peptides of the original proteins were subjected to analysis. Although, unlike 2-DE, this method does not imply inherent restrictions concerning certain protein classes, in this particular case, the identification can only be based on a single peptide for each protein. This requires all acquired spectra to be of high quality so that reliable MS data interpretation can be achieved. In more and more proteomics groups, the MS-based identification of a protein is performed with at least two distinctively identified peptides. However, using this approach, most protein isoforms cannot be unambiguously distinguished, and if N-terminal peptides are too short or too long, they cannot be analyzed by MS, resulting in a loss of the respective protein information. Recently, the Vandekerckhove group [32] published a more comprehensive study based on the COFRADIC technique with two additional chemical modifications on methionine- and cysteine-containing peptides in order to overcome the restrictions mentioned above. By merging all these approaches together in a single study, 641 non-redundant platelet proteins were identified. Nevertheless, because many fractions have to be measured, the analysis of complex samples requires a prolonged

Fig. 2. Working scheme for the COFRADIC approach of N-terminal peptides. The isolation of N-terminal peptides from a complex mixture of thousands of peptides requires at least a 2-step separation approach. **A** Before digestion, the protein sample is reduced and alkylated (carbamidomethylated) to prevent disulfide bonds between cysteine residues which can interfere with the separation, and all free amino-groups are blocked by acetylation. **B** Afterwards, proteins are digested with trypsin, yielding only peptides with arginine at the C-terminus due to acetylation of lysine residues. **C** The tryptic peptides are separated by HPLC on a C18-RP column, and the eluates are fractionated. **D** Each fraction is incubated with TNBS which binds to free amino-groups. Only the N-termini of internal protein peptides remain unblocked and react with TNBS. **E** Rechromatography under the same conditions results in a shift to later retention times for each TNBS-coupled peptide. All other peptides are unaltered and must be N-terminal protein peptides.

analysis, especially if complementary COFRADIC approaches are used in parallel. However, the COFRADIC technology has a huge potential for complex proteome projects as well as for PTM analysis if appropriate chemical reactions specific for the modification of interest can be applied. Furthermore, the application can be performed in an almost fully automated way, as HPLC separation, fractionation and also derivatization reactions can be carried out by robot systems resulting in higher reproducibility.

Research on Platelet Sub-Proteomes

The latest published studies on platelet proteomics were focused on subcellular compartments or specific protein classes. This is a general tendency in the proteomics field for certain reasons. As sub-proteomes have a lower complexity and dynamic range, they are easier to analyze with the current technical equipment and provide better access to low abundant proteins increasing the yield of relevant proteins significantly. The analysis of sub-proteomes requires prefractionation or selective enrichment of the specific target proteins. For some tasks, a special analytical setup is favorable – for instance, phosphorylation analysis should be performed with bio-inert HPLC equipment and appropriate mass spectrometers which are specially designed for PTM analysis [33–36].

Phosphoproteomics

The phosphorylation of serine, threonine and tyrosine residues is a very important regulatory switch in cell signaling.

Because of their transient character, the phosphorylation pattern can change rapidly with regard to the site of phosphorylation and the stoichiometry between the respective phosphorylated and non-phosphorylated state. Due to the activity of kinases and phosphatases, only a minor amount of potential phosphorylation sites is quantitatively phosphorylated at a given time [37]. For this reason, the phosphorylated species have to be enriched and concentrated prior to MS analysis in order to overcome limitations concerning the dynamic range. Besides, the identification of phosphorylated peptides represents a challenging task for MS, because these peptides generally have a lower ionization rate compared to non-phosphorylated peptides in positive ion mode [38].

So far, only few phosphorylation studies on platelets have been performed [39, 40]. For instance, Maguire et al. [39] used the phosphotyrosine-specific antibody 4G10 for immunoprecipitation of thrombin-activated platelet lysates in order to enrich tyrosine-phosphorylated proteins (fig. 3). The eluates were separated by 2-DE and compared with control samples. Subsequently, 67 spots were detected after staining with the fluorescent dye Sypro® Ruby (Invitrogen, Karlsruhe, Germany) and image analysis. These spots were defined as dynamic tyrosine-phosphorylated signaling events after thrombin activation. 10 spots were analyzed by MALDI-TOF MS, and 8 proteins were successfully identified using different search algorithms. For 7 proteins, tyrosine-phosphorylation was determined by using MALDI-PMF analysis and the Net-phos algorithm [41]. A direct identification of the phosphorylation site by MS on the basis of peptide fragment spectra was not performed. Immunoprecipitation can only be applied for tyrosine-phosphorylated proteins as serine and threonine phosphates are not immunoreactive, and, hence, available anti-

Fig. 3. Overview of two published platelet phosphoproteomics approaches. **A** After radioactive labeling, cells are lysed and subjected to 2-DE. Phosphorylated proteins can be visualized directly by autoradiography. Sample analysis requires subsequent staining and matching of both images. **B** In contrast, immunoprecipitation with the anti-phosphotyrosine antibody 4G10 does not require radioactive substances but instead adequate conditions to increase selectivity and sensitivity. Enriched proteins are visualized by immunodetection with the corresponding phosphotyrosine-antibody or by standard protein staining if the amount of purified protein is sufficient. However, for protein identification, the gels have to be stained and matched with the corresponding Western blot.

bodies are sequence-specific and lack specificity. Moreover, phosphotyrosines make up less than 1% of all O-phosphorylation sites. Consequently, the number and quantity of tyrosine-phosphorylated species is quite low.

Another approach to the analysis of phosphorylation in platelets was published in 2003 by Marcus et al. [40] who used radiolabeled ^{32}P -orthophosphate for incubation. In this study, the pattern of phosphorylated proteins in thrombin-stimulated platelets was compared to control samples using the 2-DE approach. However, the use of ^{32}P for labeling of phosphorylation motifs enabled access to serine- and threonine phosphorylations as well (fig. 3). Additionally, the detection of phosphorylated proteins by autoradiography on 2D gels is quite a sensitive method. However, complete cell lysates were used in this approach, resulting in a relatively low amount of phosphorylated species per definition and making the analysis of these spots very challenging. Nonetheless, radioactive labeling provides the advantage that phosphorylated peptides can be detected and isolated after HPLC separation due to Cerenkov radiation [42]. Nevertheless, application of non-radioactive methods is preferable, because cellular stress caused by radiation might change the phosphorylation pattern. Furthermore, the application of non-radioactive methods for phosphorylation analysis has been developed further. For instance, immobilized metal affinity chromatography is used to enrich phosphorylated peptides [43–46]. However, these applications have not yet been applied to platelets. Other approaches are based on the elimination of the phosphate group in order to add a chemical moiety for higher ionization efficiency and consequently improved MS analysis. Furthermore, affinity tags were combined with these moieties to enable additional purification and concentration proce-

dures [47]. But in general, chemical derivatization strategies lack sufficient sensitivity for most low abundant phosphorylated proteins.

Membrane Proteomics

The plasma membrane of platelets contains important receptors for initiating activation and aggregation. Integrins as well as G-protein-coupled receptors represent important drug targets for the pharmaceutical industry. Therefore, the identification of membrane and membrane-associated proteins on platelets represents a first important step towards basic understanding of platelet function, which has had an important impact on the development of more specific drugs for platelet-associated diseases. Recently, the most detailed platelet membrane proteome study yet was published by our group [48]. The project combined classical membrane purification strategies, such as density centrifugation, with state-of-the-art proteomic techniques (fig. 4). We used the 16-BAC(benzyl-dimethyl-n-hexadecylammoniumchloride)/SDS-PAGE system for separation of hydrophobic proteins and analyzed the isolated species from excised protein spots by highly sensitive nano-HPLC-ESI-MS. Recently, 16-BAC/SDS-PAGE has gained importance in the separation of membrane and hydrophobic proteins [49–51]. The technique is based on the consecutive separation of proteins by means of electrophoretic mobility using two different detergents, the cationic 16-BAC in the first and the anionic SDS in the second dimension [52]. Because separation patterns of both dimensions are slightly different, proteins are finally separated in an elliptic area around the diagonal axis. Compared to classical 2-DE, the res-

Fig. 4. The platelet membrane proteome study. **A** Purified platelets were lysed by ultrasonication and the lysate loaded onto a discrete sorbitol step gradient. After ultracentrifugation, the relevant membrane vesicle phase was located at the 1.0 and 1.8 M interphase. The fraction was collected and washed twice with carbonate buffer to convert the vesicles into beta sheets, thus releasing internal proteins. After carbonate extraction, the membrane proteins were isolated by differential extraction with Triton X-114. Hydrophobic proteins were located in the detergent phase and require TCA/acetone precipitation for recovery. Purified membrane proteins were separated by either SDS-PAGE or 16-BAC/SDS-PAGE and visualized by silver staining. **B** For SDS-PAGE, the complete lane was cut into 1-mm slices, yielding approximately 60 fractions. **C** After 2D separation, all visible spots were excised, resulting in a total of approximately 200 samples. Samples were washed, digested with trypsin and analyzed by nano-LC-ESI-MS/MS, yielding 297 protein species.

olution is inferior but still much better than in a one-dimensional gel. Additionally, proteins are focused within spots, increasing their local concentration, as opposed to one-dimensional gels where proteins are localized in broad bands. The improved resolution reduces the number of different proteins in single spots, which leads to a better accessibility of low abundant proteins in the sample by MS. We complemented the analysis by identifying proteins from conventional SDS gels, yielding a total of 297 unique proteins. Compared to already published 2-DE studies, we achieved a 6-fold increase in membrane-related proteins. In addition, we identified several new potential receptors or hypothetical proteins with putative transmembrane domains. Moreover, this study demonstrates that the application of different separation methods enables the access to complementary subpopulations of proteins.

Platelet Microparticles

In 2005, the group of Donald F. Hunt published the first project on platelet-derived microparticles using SDS-PAGE followed by nano-HPLC and high-speed scanning using linear ion-trap MS [53]. The platelet microparticles (PMPs) were generated by incubation in 10 $\mu\text{mol/l}$ adenosine diphosphate and platelet depletion by centrifugation. Afterwards, the PMPs were further purified by fluorescent-activated cell sorting (FACS) with a fluorescent-labeled annexin V conjugate. The purified PMPs were separated on a SDS gel, and 26 fractions were excised, digested and analyzed by nano-LC-MS. Surprisingly, Garcia et al. [53] were able to identify 528 unique proteins with this analytical set-up indicating the improved sensitivity and capability of the used MS equipment. Additionally, this paper compared the identified proteins with the 2-DE projects of the Oxford Glycobiology Group and revealed that 380 proteins were newly identified. The technical equipment demonstrates the improved performance and speed of state-of-the-art technology which will become a standard in future proteome projects.

Conclusion

Proteomic research on platelets has just begun. While the required and appropriate tools for a comprehensive analysis of the platelet proteome are not yet completely developed and evaluated, the technical progress and the variety of available approaches gives a promising outlook for the future. So far, due to the high complexity of the platelet protein content, the presented methods can only provide a specific extract of the complete proteome. This has to be taken into account for future projects. The data from all the reviewed studies demonstrates that a single technique cannot represent the complete platelet proteome because of its complexity and dynamic range. Several groups have presented their technical approaches of handling the complete platelet proteome or subproteome. In terms of better comparability, these results have to be standardized in future projects [54].

The dynamics of the platelet proteome will become a future focus, especially with regard to clinically relevant and comparative studies. New quantitation and multiplexing technologies will facilitate the realization of complex proteomic strategies. The vast advance of the analytical equipment increases the speed of acquisition as well as data analysis, providing higher sample throughput in the same period of time. Today, the analysis of post-translational modifications, such as phosphorylations or glycosylations, has significantly improved compared to just a few years ago, and therefore more advanced studies regarding these important topics will have to be carried out in the near future.

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