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Proteomic Approaches for the Mapping of Human Erythrocyte Membrane Proteins

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Abstract

The absence of membranous organelles makes the study of the membrane proteins of human erythrocytes the ideal biological model to understand the structure and function of these molecules in eukaryotic cells, fact that also makes them a model for the development of new proteomic methodologies. The first proteomic studies were aimed at identifying as many proteins as possible. Currently, the focus is on continuing to increase the structural information regarding these proteins, in search of all possible interactions with each other, as well as the study of the identification of changes in expression levels, post-translational modifications and analysis of protein complexes for the proteomic study of the erythrocyte membrane. In this review we will carry out a compilation of the main methodological approaches described in the literature for the study of two important aspects such as the mapping of erythrocyte membrane proteins, highlighting some advantages and disadvantages of them.

Keywords: Proteomic; Membrane Protein; Erythrocytes

Introduction

The human erythrocyte has a unique biconcave shape that allows it to have an excess membrane surface close to 40% with respect to cell volume. This gives it elastic properties and allows it to deform in its passage through narrow blood capillaries, even those with a diameter less than 8 μm of the erythrocyte [1]. The identification of the structural proteins of the membrane and the way in which they interact remains a focus of interest in many areas of the biomedical sciences, such as aging, oxidative stress, interaction with pathogenic microorganisms, neurodegenerative diseases and exposure to environmental contaminants [2-5].

Therefore, the characterization of the erythrocyte membrane proteome can contribute to the identification of the molecular bases of important physiological and pathological processes. This characterization is carried out through the use of qualitative and quantitative proteomic methods aimed at determining changes in the level of expression of these proteins, their post-translational modifications and the ability to form protein complexes [6-9]. For this, we currently have proteomic methods based on mass spectrometry because they offer a wide repertoire of versatile, rapid and reproducible techniques for the identification of erythrocyte membrane proteins under different metabolic conditions [10-13].

In this way, in this review we will not focus on the main experimental approaches used for the identification of the erythrocyte membrane proteome, establishing considerable contributions in the description of detailed innovative methodologies to strengthen the area of structural and functional proteomics.

Erythrocyte Membrane Proteins

The erythrocyte membrane comprises an asymmetric lipid bilayer in which proteins, lipids and carbohydrates are commonly related to each other. The total lipid content is approximately $5.1x10^{10}$ mg per red cell, of which about 60% correspond to phospholipids, 30% to neutral lipids (mainly cholesterol) and the rest to glycolipids [14]. In the human erythrocyte phosphatidyl choline, phosphatidyl-ethanolamine, phosphatidyl-sphingomyelin and phosphatidyl-serine comprise about 90% of all phospholipids [15-18]. The interactions between lipids and membrane proteins are determinant for the maintenance of the biconcave shape of the red blood cell [15,19].

On the other hand, the membrane proteins of erythrocytes can be classified into two categories according to the ease with which they can be removed from the membrane. The first corresponds to those that are more freely associated, and are easily eliminated by changes in the ionic strength or pH of the extraction buffers, probably because they seem to be associated with a single face

(interior or exterior) of the membrane. This group of membrane proteins is called the "peripheral proteins" [20,21]. The second group corresponds to the integral membrane proteins, can be used to isolate such proteins from the membrane probably aggressive reagents (solvents, detergents) because they are tightly integrated to the lipid bilayer by hydrophobic domains within their amino acid sequences. The representative peripheral proteins are the α and β-spectrins, while the most typical examples of integral proteins are band 3 and glycophorin A and C [21,23].

Membrane proteins can also be classified into three categories according to their functional properties in the membrane ultrastructure. The first category includes cytoskeletal proteins, such as α and β spectrins, protein 4.1 and actin. These are associated with each other to form a protein mesh called the cytoskeleton network that is located just below the lipid bilayer. To the second category belong integral proteins such as band 3 and glycophorins, which are firmly embedded in the lipid bilayer. The third group contains anchoring proteins whose function is to connect the integral proteins with the cytoskeleton network [24]. Among these ankyrin and protein 4.2 are an example notable.

Another classification of the erythrocyte membrane proteins is based on either their association with the membrane surface (peripheral proteins) or their crossing the membrane (Trans-membrane proteins). All these components may interact with each other by Van der Waals bonds, ionic interactions and hydrogen bonds [25].

The membrane of the erythrocytes possesses membrane proteins bound to the outer surface by means of integral protein domains or by the anchor glycophosphatidyl inositol (GPI). Trans-membrane proteins such as band 3 protein, found in plasma as in cytoplasmic domains, are recognized in the structure of the membrane [21]. External domains include binding sites with immune complexes, trans-membrane channels, and signaling proteins. Since they are usually characterized by high glycosylation levels, they show a net negative charge at the surface level. Have been founded that the inactivation complement proteins involved in the decomposition of factors anchored to GPI (CD55) [26]. Some important proteins are related to the cytoskeleton such as spectrin, actin, protein 4.1 and Ankyrin [27-29]. Therefore, it has been established that the α and β strands are integrated to form heterodimers, binding actin and other proteins as band 4.1, capable of forming tetramers on the surface of the membrane [30,31]. On the other hand, the binding of spectra with trans-membrane proteins such as band 3 and glycophorin C, generating a biconcave and lax erythrocyte property [15].

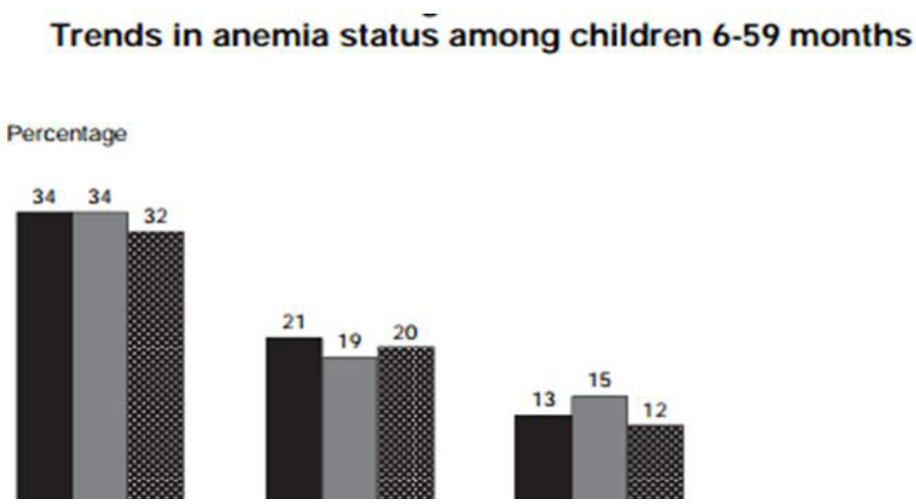

Figure 1: Representative diagram of the erythrocyte membrane

The structure of the human erythrocyte membrane with some proteins related to its morphology. In the Figure 1 described two complexes formed; left, (Ankyrin complex) and Right (4.1R complex). Authorized permit and Adapted from Mohandas N, & Gallagher PG [32].

Another series of proteins have been identified from the erythrocyte, obtaining specific data of a proteome that has been developed for decades. According to those described in Table 1, erythrocyte membrane proteins commonly reported by various authors are presented.

Protein	MW (kDa)	Peripheral or integral	Number of copies ($x10^3$/cell)	Function	References
α-spectrin	280	Peripheral	242	It unites the actin filaments in hexagonal form, joining the actin. Union of the actin with Ankyrin	[19,27]
β-spectrin	246.4	Peripheral	242		
Stomatin	31.7	Integral	200-400	It binds to the largest glucose transporter (GLUT 1), to the proteins of band 3 and AQP1. It plays a role in the regulation of ion channels in erythrocytes.	[33]

Tropomodulin	40.6	Peripheral	30	An actin-binding protein that regulates the elongation and depolymerization process of microfilaments.	[34]
Tropomyosin	32.9	Peripheral	70	Dimeric protein that works in red cells to stabilize and protofilaments limit F-actin to uniform segments measuring 37 nm the length of the Tropomyosin molecule.	[35,36]
Urea transporter	42.5	Integral	10-14	The Kidd glycoprotein forms the urea transporter in red blood cells, which works by transporting urea rapidly in and out of red blood cells as they pass through the high concentration of urea.	[37]
α-adducin	81	Peripheral	30	Membrane-skeletal protein localized at spectrin-actin junctions that binds calmodulin	[38,39]
β-adducin	80.7	Peripheral	30	Promotes the assembly of the spectrin-actin network. Binds to the erythrocyte membrane receptor SLC2A1/GLUT1 and may therefore provide a link between the spectrin cytoskeleton to the plasma membrane. Binds to calmodulin.	[38]
Aldoses A	39.4	Peripheral	20	An enzyme that participates in glycolysis. It catalyzes the reversible conversion of FBP to G3P and DHAP.	[40]
β-actin	41.6	Peripheral	500	Intervenes in the integrity, structure and motility	[18]
Ankyrin	206	Peripheral	120-160	It binds to the spectrin and band 3 and therefore forms the basis of the membrane-skeletal bond for the mechanical and viscoelastic properties of erythrocytes.	[41]
Band 3	101.8	Integral	1200	Anion transport protein, responsible for the exchange of Cl^- and HCO_3^-	[42]
Dematin (Band 4.9)	43.1	Peripheral	130	It is a widely expressed actin-binding protein that groups the actin filaments. binds to the cytoplasmic domain of GLUT1 glucose transporter	[43]
Glucose Transporter 1 (GLUT-1)	54.1	Integral	200-700	GLUT1 facilitates the transport of glucose. It transports DHA as it does with glucose.	[44]
G3PD	35.9	Peripheral	500	It catalyzes the conversion of G3P to G-1,3-bisphosphate	[45]
Glycophorin A	36	Integral	1000	Glycophorin A (GYPA, this protein) and B (GYPB) are the main sialoglycoproteins of the membrane of human erythrocytes that carry the antigenic determinants of blood groups	[46,47]
Glycophorin B	20	Integral	170- 250		
Protein 4.1R	66.4	Peripheral	240	Strengthens binding to α1 actin and β1-spectrin. It binds to β1-spectrin and actin forming a ternary complex. Joins the actin to the membrane through binding to band 3 or glycophorin C.	[38]
Protein 4.2	79.8	Peripheral	280	Interacts with the cytoplasmic domains of the band 3 protein.	[48]

Abbreviation described in Table 1: kDa: Kilodaltons; GYPA: Glycophorin A; GLUT1: Glucose Transporter 1; DHA: Docosahexaenoic Acid; G3PD: Glyceraldehyde-3-Phosphate Dehydrogenase; HCO_3^-: Ion Bicarbonate; Cl^-: Chloride Ion; PKA: Protein Kinase A, FBP: Foliate Transport Protein; G3P: Glyceraldehyde-3-Phosphate; DHAP: Dihydroxyacetone Phosphate; AQP1: Aquaporin 1; G-1,3-BP: Glucose-1,3-Bisphosphate.

Table 1: Some representative proteins present in the membrane of human erythrocytes

Methodology

For the purpose of writing this narrative review, literature published in English was identified by study of three online databases such as PubMed, ScienceDirect, and Google Scholar. The keywords used were 'membrane protein', 'erythrocyte' and 'mapping proteome'. In order to obtain recent updates about experimental studies related with mapping of erythrocytes membrane proteins, the databases were searched for published literature from 1985 -2018, with more emphasis on current articles published after 2004.

Experimental Approaches for the Mapping of Erythrocyte Membrane Proteins

Different methodological proposals have been involved in the identification and resolution of the proteome of human erythrocytes. In these series of investigations have focused on different points of view such as sample processing, separation and identification. Alike, it has been emphasized in the different experimental approaches in order to achieve exhaustive extraction through these methodologies; categorizing them into classical methods based on osmotic lysis and novel methods.

In this way, traditionally in the experimental stages for the isolation of erythrocyte membrane proteins it is convenient to develop optimized protocols that guarantee the achievement of protein extraction. Usually, methodologies based on extraction by osmotic

medium or hypotonic solutions have been implemented, which have proved to be of great application. The osmotic behavior by the hypotonic medium is responsible for an imbalance of concentration gradients in the cells that favor rupture. The mechanism is favored by an increase of the intracellular volume with the entrance of water until achieving the greater capacity of resistance of the membrane, obtaining finally the exit of the cellular components, which are applied in processes of fractionation of cellular components. Within the wide range of biological materials that can be processed by this methodology, we find blood cells, cell tissue cultures, among many others [49,50].

In this way, it is necessary to know in a broad way a series of research compendium that have allowed the study of erythrocyte membrane proteins. In Table 2, a concise description is made of different investigations involved in the study of the mapping of human erythrocyte membrane proteins, highlighting the methodologies of sample processing for protein isolation, as well as the different instrumental techniques for identification , establishing a detailed description of the results obtained from the proteome for each of the studies reviewed; equally, the characterization and quantification of each of the proteins according to different aspects such as its origin, location and function.

Methodologies	Methods of Identification	Description of Proteome	References
Lysis of erythrocytes with phosphate buffer, pH 8.0. Centrifugation at 13,000 g and again at 100,000 g. Resuspension in resuspension buffer (9 M, nonident P-40).	1D (IEF-SDS)	46 polypeptides without variability were identified. And 38 exhibited a normal combination and a polypeptide variant.	Rosenblum BB, *et al.* 1984 [51]
Lysis with 5 mM cold hypotonic phosphate buffer, pH 8.0, 1 mM EDTA, 1 mM PMSF. Centrifugation 17,000 g. continuous washes and centrifugation at 108,920 g. Resuspension in buffer (7 M urea, 2 M thiourea, 4% CHAPS).	1-D o 2-D (IEF-SDS) MALDI-TOF	102 proteins identified 59 different polypeptides and 43 isoforms.	Low TY, *et al.* 2002 [52]
Lysis in hypotonic medium (PBS), digestion with trypsin in the presence of tosyl phenylalanyl chloromethyl ketone, centrifugation at 2,000 g, subsequent increase in velocity (cytoplasmic). For those of the skeleton, 1 mM EDTA, pH 8.0, resuspension in 100 mM $NaHCO_3$, trypsin addition and incubation are used.	RP-HPLC, IT MS/MS; filtration in gel – LC-MS/MS	182 unique proteins (91 membrane proteins, 91 cytosolic proteins). metabolic enzymes, proteins associated with transporters and channels, adhesion proteins, cellular proteins of the ubiquitin-proteasome system, G proteins of the Ras family, kinases, chaperone proteins, proteases, factors of translation initiation	Kakhniashvili D, *et al.* 2004 [53]
Lysis by HEPES, followed by sonication and centrifugation to obtain the proteins	Enzymatic digestion with trypsin, MudPIT (SCX + RP) 2D-nano-HPLC-ESI-MS/MS	272 proteins (30 of them with 2 unique peptides). 47 membrane and cytoskeletal proteins, 79 secreted or extracellular, 30 cytoplasmic, 63 not classified.	Tyan YC, *et al.* 2005 [54]
Lysis with 5 mM phosphate buffer, pH 8.0, 1 mM EDTA, 0.4 mM PMSF, centrifugation and washed successively. Peripheral proteins extracted with Triton X-100 and with 100 mM NaOH, cold and pH 13. Membrane proteins extracted with n-hexyl-β-D-glucopyranoside, followed by Triton X-100 and deoxycholate	2-D bidimensional electrophoresis (IPG/SDS). MALDI-TOF	At least 500 protein spots were detected. Detection of filamentous proteins such as spectrin, ankyrin. Integrals as band 3, 4,1 and 4,2	Bruschi M, *et al.* 2005 [55]
Lysis with cold phosphate buffer 5 mM, pH 8.0 and centrifuged. Membrane extraction with 100 mM Na_2CO_3, pH 11. Elimination with EtOH in the presence of sodium acetate, pH 5.0. Cytoskeletal proteins were tainted with 0.1 mM EDTA, pH 8.0.	1-D (SDS), digestion, LC-ESI-MS/MS (Q-TOF y LTQ-FT MS)	566 proteins were identified. 340 membrane proteins, (105 were classified as integral proteins, 54 are those associated or linked, 5 are related as anchored to GPI, 40 as belonging to the cytoskeleton, 21 proteins from organelles, 41 are cytoplasmic and 20 extracellular.	Pasini E, *et al.* 2006 [56]
Lysis with 5 mM phosphate buffer, pH 8.0. Addition of 1 mM EDTA, 1 mM PMSF. Centrifugation and increase of revolutions to 17,000 g.	2D/ESI-MS/MS	395 spots were identified (day 0), 487 (7 days) and on day 14, 487 spots.	D´Amici GM, *et al.* 2007 [57]
In silico analysis	---	Determination of 751 proteins, based on previous studies.	Goodman SR, *et al.* 2007 [58]
Lysis of erythrocytes with 5 mM saline phosphate buffer, pH 7.4, PMSF + 1mM EDTA. Centrifugation at 36,000 g. Additional, NaCl and a Librar-1 and 2 column. Elution performed with TUC solutions (Urea, thiourea and CHAPS). UCA (urea, citric acid).	Libraries of combinatorial ligand (CLL) - ProteoMiner - 2-D (IEF-SDS), Orbitrap MS	1578 proteins of the cytoplasmic fraction.	Roux-Dalvai F, *et al.* 2008 [59]
Lysis buffer (5 mM Tris–HCl, pH 8.0, containing 0.1 mM EDTA. Centrifugation at 76,000×g for 30min at 4 °C. Treated with lysis buffer containing 0.05% (v/v) Triton X-100.	Tryptic digestion, MudPIT analysis, LTQ-MS/MS	275 proteins in the tree fractions	De Palma A, *et al.* 2010 [60]

Isolation of proteins in hypotonic medium, washing and successive centrifugations.	1D-GE (SDS), digestion, nanoHPLC-ESI-Q/IT-FTICR	271 proteins were identified present in the fractions. Pertaining to membrane proteins and vesicles formed.	Bosman G, *et al.* 2012 [61]
Hypotonic lysis buffer (5 mM, 1 mM EDTA, 0.1 mM DFP). Two resulting factions AOV and skeletal membrane.	LC-MS/MS	Proteins were identified in different fractions: WG-B (492), IOV-1 (303), IOV-2 (299), DCS (308), IOV-1 + DCS (417).	Pesciotta EN, *et al.* 2012 [9]
Osmotic lysis (5 mM phosphate buffer, pH 8.0, 1 mM EDTA) followed by high speed centrifugation, 24,000 x g for 10 min	1D-SDS PAGE, in-gel trypsin digestion, LC–MS/MS	41 proteins were identified by the Mascot database.	Kottahachchi D, *et al.* 2015 [62]
RBCs lysed employing 10 volumes of hypotonic lysis buffer (5 mM sodium phosphate, 1 mM EDTA, pH 8.0).	MED FASP, LC-MS/MS	Identification of 2650 proteins (1890 identified proteins at >100 copies per cell). Quantified 41 membrane transporter proteins.	Bryk AH and Wiśniewski, JR, 2017 [63]

Abbreviations referenced in Table 2: 1D: Electrophoresis in one Dimension; IEF: Isoelectric Focusing; SDS: Sodium Dodecyl Sulfate; MALDI: Matrix-Assisted Laser Desorption/Ionization; TOF: Flight Time; 2-D: Two-Dimensional Electrophoresis; MS: Mass Spectrometry; CLL: Combinatorial Ligand Library; LC: Liquid Chromatography; ESI: Q-TOF: Quadruple- Flight Time; LTQ-FT: IPG: IT-FTICR: HPLC: High Resolution Liquid Chromatography; RP: Reverse Phase; EDTA: Ethylenediaminetetraacetic Acid; CHAPS: 3 - [(3-cholamidopropyl) dimethylammonium] -1-propanesulfonate; PMSF: Phenylmethylsulfonyl Fluoride.

Table 2: Studies of membrane proteins of human erythrocytes

Discussion

The objective of detecting more proteins between associated membrane proteins and integral proteins, establishes different extraction methods to greatest information on proteins, among them additional stages have been established in addition to conventional methods. Ethanol is included to perform membrane delipidation processes; carbonate, in order to obtain a considerable proportion of low abundant proteins and avoid membrane vesiculation, although risk of loss of association proteins; the implementation of protocols with saturated carbonate, where a satisfactory extraction is achieved although with risk of loss of interaction with ionic proteins; likewise, the inclusion of EDTA in experimental trials has indicated that it can stimulate the cytoskeleton's uncoupling [4,9,64,65]. Some researchers have pointed out ethanol treatment and extraction methodologies using alkaline solutions based on sodium carbonate in different concentration levels. In the same way, together with the traditional method based on osmotic lysis, another series of agents that facilitate extraction have been incorporated, including surfactants, chaotropic agents, and solvents such as trifluoroethanol (TFE), ethanol and methanol. These agents have been reported a capacity to improve the solubility of proteins, on the other hand the use of certain agents breaks the interactions of proteins with the entire membrane complex, and like they are able to achieve the destabilization of proteins. Tertiary and quaternary structures of proteins constitutes an option to extract membrane proteins [56,66-68].

To date, there are several investigations based on the use of these methods with results of efficiency and compatibility with various techniques applied in the field of proteomics. Thus, it has been shown that a greater utility of these strategies has been achieved with the mechanisms of the cellular system because they notably favor the interruption of the lipid bilayer of erythrocytes. Nowadays they are reproducible methodological options and lead to the achievement of isolation objectives [54]. In this way, several authors have been referred to the application of the procedures of Pasini, *et al.* Álvarez-Llamas *et al.*, And Low, *et al.*, whichever have led to studies of mapping erythrocytes membrane protein applying the extraction methods in the phenomenon of hemolysis in the hypothetical medium using a buffer at the concentration levels between 5 and 10 mM [56]. According to the use of these buffered media of 5 mM cold phosphate (Na_2HPO_4) at pH 8, containing chelating agents such as 1 mM EDTA, it allows cell breakdown, promoting washing and elimination of hemoglobin, which for an undesired factor and a contaminant during the extraction process [9,52,69]. Likewise, the increase in the centrifugation speed has been increased to obtain a consistent sediment and the improvement of the yield values [52,69]. In the same way, comparative studies that use only the traditional method of osmotic reading with phosphate buffer as often as possible will get results with the lowest results, as well as other technologies based on the function of sodium carbonate as wash agent and phenol as precipitant [70]. Likewise, the application of this type of isolation methodologies in combination with instrumental techniques such as LC-MS/MS, nano-LC-MS, LTQ and MALDI-TOF, have allowed the identification of the characteristics of the membrane characterized as: α- spectrin, β-spectrin, ankyrin, aducin, tropomodulin, protein band 3, protein band 4.1, band 4.2, band 4.9, actin, band 6, aducin, tropomyosin, stomatin, dematin, flotilin 1 and 2, α-glycophorin, protein the membrane of erythrocytes of 55 kDa. Also, the detection of proteins of around 20 kDa, such as cofilin, an important protein that is able to bind to actin F and G involved in the control of reversible polymerization [52,65,69].

In contrast, Pasini, *et al.*, in combination with ethanol, in the case of carbon solutions (Na_2CO_3 or $CaCO_3$) in combination with ethanol, are effective tools in the isolation of proteins. The saturated carbon solutions have been used in the 100 mM parameters, separation profiles with better resolution have been shown [56]. Ciana, *et al.*, with the use of alkaline solutions such as Na_2CO_3 at 100 mM, pH 11, increases the level of protein detection infrequent, given by the effect of the elimination of weakly bound cytosolic proteins to the membrane, which are capable of to be solubilized under these conditions [66,71]. According to studies established by Pasini, *et al.* The inclusion of a treatment with alkaline media and ethanol for the isolation and elimination of interferences in membrane proteins, has accompanied the identification of 340 membrane proteins of which 105 they were classified as integral

proteins, 54 associated or linked, 5 are related as anchored to GPI, 40 as belonging to the cytoskeleton, 21 proteins of organelles, 41 are cytoplasmic (8 involved in processes such as glycolysis) and 20 extracellular and other people [57]. In addition, many of these proteins are related to the binding systems, others with the catalytic processes, they can also function as transporters and transduction markers or act as modifiers of structural activity. Similar studies established the application of the alkaline extraction method led to an increase in the proportions of integral proteins as band 3 [66,72].

However, some authors suggest the adaptation of joint osmotic lysis procedures to detergents capable of causing rupture of the erythrocyte membrane. In these protocols, buffered solutions of 5 mM PBS, pH 7.4 with detergents such as ASB-14 and ASB-16 in concentration between 1 and 100 μM, which have been shown to be efficient in the rupture of erythrocyte membranes [73,74]. These procedures constitute an important contribution in the processes of membrane lysis, due to the inclusion of agents as surfactants in which a high hydrophobic interaction between the acyl chains of the ASB-16 surfactants and the membrane components of the surfactants has been demonstrated. erythrocytes causing high solubilization [75]. On the other hand, the inclusion of protocols based on traditional methods with cold phosphate buffer in combination with agents such as saponin has been considered as alternatives to induce cell membrane lysis conditions. It has been demonstrated with previous studies that when erythrocytes are incubated with saponin in an isotonic buffer, the membranes increase their permeability achieving the entry and exit of the cytoplasm of small molecules [75].

On the other hand, methodological variations for the extraction of proteins require the use of surfactants for the solubilization of the lipid component of the membranes, it has been shown that a certain volume of lysis buffer solutions combined with detergents such as Triton-X100 in concentrations of 0.05%, have established an optimal isolation of proteins [13,43,51]. Likewise, the remarkable influence of other quaternary ammonium detergents such as cetylpyridinium chloride (CPC), a detergent that has the ability to interact with the lipids of the erythrocyte membrane, promoting a cell lysis effect, has been reported. Other novel methods have been based on the extraction of membrane fractions, under the enzymatic digestion processes with the use of trypsin modified with tosyl phenylalanyl chloromethyl ketone.

Conclusions and Perspectives

The future perspectives from the proteomic point of view regarding the study of the mapping of membrane proteins of human erythrocytes revolve around the achievement of completely efficient and versatile methods, with a high degree of sensitivity; thus, the purpose of determining new proteins involved in various processes of the human erythrocyte physiology, the search for new methods for the isolation of proteins; as well as the importance of the analysis of post-translational modifications resulting from phenomena related to oxidative stress, physiological alterations and pathologies caused by an external agent or from a functional anomaly of organisms. Therefore, it is necessary to obtain exact and robust methods that allow a correct identification and quantification. The general purpose can also be focused on the obtaining and analysis not only of peptides subjected to treatment stages, but that it can perform a complete analysis from the native protein and expand the knowledge with contributions of new methodologies with a greater registry of proteins identified, the relationship with the functioning of the human erythrocyte; similarly, establish the application of this new alternative methodologies in search of an understanding of the phenomena related to the behavior of proteins and their structural changes.

It is important to consider that the human erythrocyte represents a complex structure for its study in proteomics and specifically in the study of membrane proteins. For many epochs there have been many contributions originated to give answers and to construct the conceptual bases of what is known today; for this reason, it has focused on making a tour of the different approaches for the study of these proteins, from the point of construction and development of membrane protein mapping and on the other hand the consolidation of the study of post-translational modifications, making different experimental approaches and parallel emphasis has been placed on the description of traditional, alternative and innovative methods with the purpose of establishing a contribution in the enrichment of knowledge in relation to the proteomic study of the erythrocyte. In the same way, to show how these methods have been oriented to offer answers more reliable, exact and robust through the technical development and the strengthening of the experimental bases, either in the normal study or related to alterations caused by different processes that could affect the functional status of the erythrocyte as a study matrix.

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