Journal of Chromatography A, 1217 (2010) 893–900

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Plucking, pillaging and plundering proteomes with combinatorial peptide ligand libraries

Pier Giorgio Righetti[a,*], Egisto Boschetti[b], Alberto Zanella[c], Elisa Fasoli[a], Attilio Citterio[a]

[a] Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Via Mancinelli 7, Milano 20131, Italy
[b] Bio-Rad Laboratories, 3, Boulevard R. Poincaré, 92430 Marnes-la-Coquette, France
[c] Hematology 2, Fondazione IRCCS Ospedale Maggiore, Mangiagalli e Regina Elena, via F. Sforza 28, 20122 Milano, Italy

ARTICLE INFO

Article history:
Available online 1 September 2009

Keywords:
Ligand library
Peptide ligands
Low-abundance proteome
Elution systems

ABSTRACT

Recent developments in the technique of combinatorial peptide ligand libraries, for enhancing the visibility of the low-abundance proteome, are reviewed here. Novel en bloc elution systems, allowing essentially complete proteome recovery in a single step, are reported here, particularly, en bloc elution with 3–5% boiling sodium dodecyl sulphate (SDS) or in urea–thiourea–CHAPS added with either 40 mM formic acid or 25 mM cysteic acid. Novel capturing systems are also discussed: in particular, although capturing at pH 7.2 in physiological saline has always been recommended, it is shown that capturing also at acidic (pH 3.8) and alkaline (pH 9.5) values substantially increments the total captured protein population. Some examples of detection of novel proteins by the described methodology are also discussed. In particular, in the case of venom proteins, where essentially all components had been detected and fully described by conventional means, the application of the ligand library technology allowed the discovery of two, previously unreported, trace enzymes necessary for the maintenance of the native structure of venom components, namely peroxiredoxin and glutaminyl cyclase. In the case of the red blood cell (RBC) cytoplasmic proteome, where a grand total of 1570 components of the 2% minority proteomes have been identified, these findings allowed to unravel the genetic defect of a rare RBC disease, called congenital dyserythropoietic anemia type II. The mutations are located in the SEC23B gene coding for the SEC23B protein, detected for the first time in the RBC proteome thanks to the peptide capturing technology.

Contents

1. Prologue

As odd as it sounds, pillaging, plucking and plundering (PPP) has been the favourite past time of all past "civilizations", to the point that the history of human kind is the account of all disasters provoked by infinite wars and the ensuing PPPs perpetrated by the winners onto the losers (unfortunately it does not seem that today we are doing any better!). Perhaps one of the most chilling PPP events took place in 1527 in Rome, and in fact it went down to history as "il sacco di Roma" (the sack of Rome); it is worth recalling some of the episodes here. In the 16 century Europe was shaken on its foundations by some major events, among which the fierce fighting between the French (François I) and the German (Charles

DOI of original article: 10.1016/j.chroma.2009.08.074.
* Corresponding author.
E-mail address: piergiorgio.righetti@polimi.it (P.G. Righetti).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.08.070

V) rulers for the control of the continent. In the long run, Charles V defeated the French King in Pavia in 1524, from where he was taken prisoner to Madrid. In an attempt at revenge, François I called upon the Sultan Suleiman to invade Hungary, a Province of Charles V empire. The Vatican had sided with the French and their policy so, as soon as Charles V could solve his problems at home, he let his general von Frundsberg and his terrifying army of landsknechts to march upon Rome to punish the ruling pope (Clemente VII). No troops could contrast their march. As the 10,000 ferocious soldiers, who had not received their salary for a whole year, hit Rome, havoc ensued. The population at large was massacred and soon 10,000 corpses paved the streets while another 2000 floated on the Tibur river. San Peter and the Vatican were savagely sacked and all roman palaces were pillaged too. Churches were transformed into army barracks and brothels and even the famous Stanze of Raphael in the Vatican used as stables. The barbarian troops even proclaimed pope Martin Luther and it was only by a miracle that the real pope, Clemente VII, survived, as he had barricaded himself in Castel Sant'Angelo, the only structure that the landsknechts could not conquer. Interestingly, the famous artist Benvenuto Cellini was among the guards defending the castle and he even claimed that he shot to death general von Frundsberg, the chief commander of the landsknechts. Attempts by the French and English armies to liberate the pope failed and Clemente VII himself had to escape from Rome taking refuge in Orvieto and then Viterbo. Basically, the pope was held hostage by the German emperor for two years; by the end of this disastrous pillaging, Rome had been left in total ruins.

2. Introduction

Notwithstanding the pernicious effects of PPPs, this procedure seems to draw proselytes from the four corners of the word in the field of proteomics. All of us in this arena dream of pillaging proteomes and running away with the booty, be it a biomarker for a given pathology, a membrane receptor, a novel key enzyme involved in an important biological pathway, a hidden allergen previously undetected. To that purpose, a host of techniques have been devised for ransacking proteomes and bringing to the limelight those low- to very-low-abundance species that constitute its vast majority [1–3]. One technique that is taking momentum is the combinatorial peptide ligand library that is the object of the present review. The story of a combinatorial peptide library commenced at the end of 1980s, when Furka et al. [4] presented the principle as a way to simplify the preparation of peptides. It was about the same period when combinatorial chemistry became successful as an enormous source of new molecules that could be screened for their potential therapeutic application. Within the same context, peptides and peptoids became also a focus for similar applications. With Lam et al. [5], however, peptides from combinatorial libraries were studied for the first time as a possible source of affinity ligands for the separation of proteins from complex mixtures. While the promise of discovering new effective drug candidates from combinatorial libraries was not as successful as hoped, the purification of peptide ligands was possible in a large number of cases. The use of similar libraries in proteomic investigations for detecting many more proteins and especially those with a concentration below the sensitivity of current analytical methods was described for the first time only in 2005 [6]. The interest of such an approach was seen as a novel way to detect not only hidden proteins from complex proteomes, due to the presence of highly abundant species, but even more importantly because it rendered possible the concentration of low- and very-low-abundance species. The interest of such a possibility is large not only for checking the function of rare species present in a given extract or cell organelle, but also for seeing if the genome is still active for proteins that are supposed to be repressed in their expression after the fetal stage. An additional benefit is to find markers of diagnostic interest as well as markers to follow the efficacy of a therapeutic treatment or to classify populations between respondents and non-respondents or even fatal outcome. The possibility of detecting traces of biomarkers permits to diagnose misregulated expressions at earlier stages than with current methods.

We have now applied the ligand library capturing technique to quite a number of biological samples, such as urine [7], serum [8,9], human platelets [10], red blood cells [11], bile fluid [12], chicken egg white [13] and yolk [14], recombinant DNA products [15,16] and snake venoms [17]. In some instances, the use of peptide libraries has enabled us to detect allergens in food stuff (notably maize and cow's whey) that had not been revealed up to the present [18,19]. In all cases, the increment in number of species detected was from two- to five-fold as compared to a control, non-treated sample. This substantial increment in detection applies mostly to low-abundance proteins, considering that they could not be detected in the untreated samples, where they should have been obviously present. In some cases, such as with the human red blood cell (RBC) cytoplasmic proteome, the increment was spectacular: a total of 1570 proteins could be identified [11], as opposed to 250 in the best and deepest proteomic analysis previously published [20]. We have also reviewed the field in quite a large number of articles, to which the reader is referred for a better insight on the technique [21–29]. In one instance, we have described a detailed protocol for solid-phase peptide library users [30]. In another couple of investigations, we have explored the capturing ability of these libraries as a function of the oligopeptide length [31] and even when using just single amino acids attached to the beads [32]. Therefore, in the present review, we will cover only the most recent advances not yet described in all of our previous reviews. Data presented are the result of the use of a commercially available library called ProteoMiner (primary amine terminal hexapeptides from Bio-Rad Laboratories, Hercules, CA, USA) and from a carboxylated library specifically chemically modified and that is not commercially available as we write.

3. General properties and behaviour of hexapeptide ligand libraries

As we have extensively described the properties and behaviour of hexapeptide libraries, we will here only briefly summarize their general characteristics. The solid-phase product is a mixture of porous beads on which hexapeptides are covalently attached. Each bead carries a large number (billions) of copies of the same peptide bait; the beads are thus different from each other, and all combinations of hexapeptides are present. Depending on the number of amino acids used, a library contains a population of millions of different ligands (*e.g.* 11, 24 or 64 millions starting, respectively, from 15, 17 or 20 different amino acids). When a complex protein extract is exposed to such a ligand library in large overloading conditions, each bead with affinity to an abundant protein will rapidly become saturated, and the vast majority of the same protein will remain unbound. In contrast, trace proteins will not saturate the corresponding partner beads, but are captured in progressively increasing amounts as the beads are loaded with additional protein extract. Thus, a solid-phase ligand library enriches for trace proteins, while concomitantly reducing the relative concentration of abundant species. In theory each bead that carries a single peptide ligand should interact with proteins that share the same epitope complementary to the peptide bait. However, because in a number of cases peptide ligands can differ from each other by just one amino acid, similar interactions can be found with more than one protein—the single interactions are governed in this case by differ-