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Enhancement of subunit contacts in human dipeptidyl-peptidase I

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PURPOSE OF THE ABSTRACT

The association of enzymes to form oligomeric structures is widespread in nature. The formation of homooligomers is a structural and functional advantage over the monomeric form, as it usually results in increased stability and can often lead to more complex regulation of activity [1]. A common functional property of many oligomeric proteins is cooperative substrate binding, which results from communication between substrate binding sites on different subunits. This allosteric information is transmitted through the complex in the form of conformational changes of different proportions [2]. Despite its benefits, not all homooligomeric enzymes exhibit cooperative behavior. For example, mammalian dipeptidyl-peptidase I (DPPI) is a homotetramer that belongs to a family of mostly monomeric enzymes, namely the papain-like cysteine proteases (PLPs). In humans, its most evident physiological function, in addition to nonspecific protein turnover, is the activation of granule-associated serine proteases in immune cells [3]. DPPI is synthesized as a preproenzyme that associates to form dimers. During processing into the mature, tetrameric enzyme, the internal propeptide is excised and each monomer is cleaved into three non-covalently bound polypeptide chains, i.e., the exclusion domain and the heavy and the light chains [4]. The resulting tetrameric complex is a dimer of dimers with two sets of isologous subunit interactions, namely, the head-to-tail and lateral interactions [5]. Despite initial findings of cooperativity in this enzyme, these results could not be reproduced by us or by other research groups [6–8]. Furthermore, we performed a computational analysis of the communication pathways between active sites on different subunits of DPPI and concluded that there are no cooperative communication pathways in the protein.

In DPPI, all subunits, as well as domains within each subunit, are linked by non-covalent interactions. Therefore, their association is reversible, and they will inevitably dissociate when sufficiently diluted. Thus, over a certain concentration range, an equilibrium exists between different oligomeric forms. The goal of our research group is to develop variants of DPPI with increased affinity between subunits by strengthening the interactions at subunit interfaces, thereby minimizing the probability of subunit dissociation. Moreover, we believe that enhanced interaction between specific amino acid residues could lead to the emergence of a communication pathway and thus cooperativity between subunits. In addition to developing improved enzymes, our research will advance the state-of-the art on the evolution of cooperativity in oligomeric proteins. This knowledge can then be applied to the design of oligomeric and cooperative enzymes from monomers.

In order to engineer a cooperative DPPI, we have been using rational and semirational protein engineering approaches. By calculating the correlation energies and the distances between interacting amino acid residues on the contact surfaces, we determined that the lateral interactions are weaker than the head-to-tail interactions [9]. We then aimed to enhance the lateral interactions by introducing disulfide bonds, hydrophobic interactions and salt

bridges into the contact surface. However, these mutant variants remain to be characterized in the future [9]. Unfortunately, rationally introduced mutations have an unpredictable effect on the folding ability of DPPI. Therefore, we opted for a semirational approach by randomly mutating predefined interaction hotspots on the contact surfaces. The resulting library is then screened using the commercially available bacterial two-hybrid system BACTH [10]. Experiments are underway to identify DPPI variants with increased affinity for association.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

dipeptidyl-peptidase I | oligomerization | cooperativity | protein engineering

BIBLIOGRAPHY

[1] d. s. goodsell, a. j. olson. annu. rev. biophys. biomol. struct. 2000, 29, 105-153.
[2] g. g. hammes, s. j. benkovic, s. hammes-schiffer. biochemistry. 2011, 50, 48, 10422-10430
[3] c. t. n. pham, t. j. ley. immunology. 1999, 96, 8627-8632.
[4] a. s. lamort, y. hamon, c. czaplewski, et al. int. j. mol. sci. 2019, 20(19), 4747.
[5] d. turk, v. janjic, i. stern, m. podobnik, et al. embo j. 2001, 20(23), 6570-6582.
[6] m. rebernik, b. lenarcic, m. novinec. protein expr. purif. 2019, 157, 21-27.
[7] m. rebernik, t. snoj, m. klemencic, m. novinec. arch. biochem. biophys. 2019, 675, 108121.
[8] v. santilman, m. jadot, f. mainferme. eur. j. cell biol. 2002, 81(12), 654-663.
[9] b. cigic, r. h. pain. eur. j. biochem. 1999, 264(3), 944-951.
[10] n. varda. dipl. delo. 2021.
[11] a. battesti, e. bouveret. methods. 2012, 58(4), 325-334.