研究简报

Selecting EGF-binding Clones From a p Ⅷ-based Phage Display Library

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Abstract Angiogenesis-related diseases involving EGF include acherosclerotic plaques, haemangioma, angiofibroma, tumor growth and arthritis. EGF may serve as a drug target and its antagonists may have important clinical applications. Peptide phage display libraries have been successfully applied in areas of finding ligands for enzymes, receptors, and many other molecules. A p Ⅷ-based peptide phage display library was panned with the cytokine EGF and several EGF-binding clones were selected based on ELISA and micropanning assays. The selected EGF-binders from peptide phage display library may be utilized in affinity chromatography in EGF downstream processing and even act as potential antagonists of EGF if their affinity is further improved through secondary library strategy.

Key words phage display, peptide, EGF, drug target

CLC number Q75, Q78

Cytokines, as important modulators of homeostatic processes, can be viewed as double-edged swords, benefiting the host when their production and actions are regulated, but posing a threat to the host when their production and actions are unregulated. Such imbalances can contribute to the pathogenesis of diseases. Angiogenesis-related diseases involving EGF (epidermal growth factor) include acherosclerotic plaques, haemangioma, angiofibroma, tumor growth and arthritis[1]. The action of cytokines can be inhibited by agents at several levels, including agents which binds soluble cytokines. EGF may serve as a drug target and its antagonists may have important clinical applications. Screening peptide phage display libraries has been demonstrated as an effective new approach to finding ligands for targets of various types[2]. For this study, the library strategy was adopted to select EGF-binding clones and these EGF-binders may be antagonists candidates of EGF if their affinity is further improved through secondary library strategy.

The library used in this study contains a randomized peptide insert (XCX_{15}) expressed at the N-terminus of p Ⅷ, a major coat protein of filamentous phage[3]. Biopanning was performed in 96-well, flat-bottom ELISA (enzyme-linked immunadsorption assay) plates (Corning). Microwells were coated with 3 μg of EGF (expressed in *E. coli* and purity > 97%) overnight at 30 ℃, in 50 μl of 0.05 mol/L $NaHCO_3/Na_2CO_3$ (pH 9.1) before the library was biopanned as described[3]. After four rounds of biopanning, clones from round 2, 3 and 4 were selected and four clones were confirmed by ELISA and micropanning assays.

Phage ELISA was performed as follows: microtiter wells were coated and blocked and washed as described in reference [3]. Twice PEG-purified phage (10^9 TU) was added to each EGF-coated well of a microtiter plate and incubated for 1 h at 37 ℃ before the well was washed three times in PBS[4]. Anti-phage antibody-HRP conjugate (Pharmacia) was added to each well and incubated for 1 h at 37 ℃.

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Plates were washed three times for 4 min a time in PBS. 100 μl of 3, 3′, 5, 5′-tetramethylbenzidine (TMB) substrate solution was added to each well. The reaction was stopped after 5~ 20 min with 50 μl H_2SO_4 (2 mol/L) and the A_{450} was determined (Fig. 1). ELISA signal is calculated as average of two duplicates.

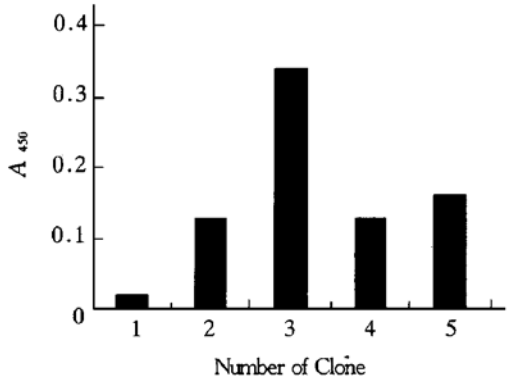

Fig. 1 Comparison of ELISA signal between negative phage peptide and positive phage peptides

Clone 1 is a negative control clone; clone 2 (RCGMESRMMDCHPLRSL), clone 3 (ICESRHYQVRLPPRPSM), clone 4 (LCRITNEEVTPGLTTTD), clone 5 (RCENLRHVNLPSPQRNR) are positive clones.

Micropanning was done as follows: microtiter wells were coated (or block-only) and blocked and washed as noted in library biopanning[3]. Twice PEG-purified phage in PBS (5×10^8 TU) were added to each well, and incubated for 1 h at 37 ℃. Wells were washed three times for four minutes in PBS and then eluted in elution buffer at room temperature for 10 min. Phage were then diluted and titered for tetracycline-resistance transducing units[3]. The yield of each micropanning can be calculated by dividing the output by the input while the yield ratio can be calculated by dividing the yield of EGF-coated well by the yield of block-only well (Fig. 2).

Although a dozen of phage display libraries had been screened against EGF (data not shown), only in the pⅧ-based phage display library were several positive clones selected and confirmed by ELISA and micropanning assays. Among the four confirmed clones, three of them have the consensus sequence PXR and all of them are rich in Pro, Ile or Val which are supposed to impose additional constraint[5]. It suggests that EGF-binding clones may be selected more possibly from multivalent and constrained peptide phage display libraries than from monovalent and linear ones. These peptide leads may be EGF antagonist candidates if their affinity is further improved by the secondary library strategy[6].

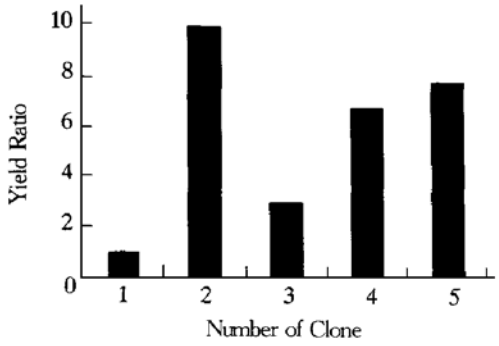

Fig. 2 Comparison of micropanning yield ratios between negative control and positive clones

Clone 1 is negative phage peptide and its yield ratio is about 1. Clone 2, 3, 4 and 5 are positive phage peptides and their yield ratios ≥2.

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从噬菌体文库中筛选与表皮生长因子结合的多肽.

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摘要 与许多疾病相关的血管生成作用是由一些血管生成

因子介导的，其中就包括表皮生长因子．在肿瘤生长、关节炎等疾病中，表皮生长因子参与了其中的血管生成作用，拮抗表皮生长因子介导的血管生成就有可能对与其相关的疾病起到治疗作用，因此，表皮生长因子的拮抗剂可能具有重要的临床价值．拮抗表皮生长因子的作用可以通过许多途径，其中之一就是找到能与表皮生长因子结合并能干预其与受体结合的分子，因而表皮生长因子可作为药物靶分子．从噬菌体文库中筛选药物靶分子的拮抗剂和激动剂已被证明是一种有效的方法．以表皮生长因子作为药物靶分子，从多肽噬菌体文库中筛选与表皮生长因子结合的噬菌体多肽，这些潜在的表皮生长因子拮抗剂先导分子经过优化可能具有重要的临床价值．

关键词 噬菌体展示，多肽，表皮生长因子，药物靶分子
学科分类号 Q75，Q78

高分子络合树酯固定化多酚氧化酶的研究

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摘要 为探索新的固定化酶方法，以漆酚-酪氨酸树酯为固定化酶载体，与 Cu^{2+} 络合制备成高分子络合剂，对多酚氧化酶固定化，实验结果表明，这种固定化方法是可行的．固定化多酚氧化酶的适宜 pH 值为 6.64 和 7.17，在 60 ℃放置 25 min 后活力保留 50.7%，以邻苯二酚为底物的米氏常数为 1.49×10^{-2} mol/L，较游离酶略小．根据实验结果提出了固定化酶模型．

关键词 高分子络合树酯，多酚氧化酶，固定化
学科分类号 Q55

我国固定化酶的研究比较活跃，出现了一些独特的固定化技术[1]，常用的物理吸附法、包埋法、交联法、共价法现已有许多研究[2,3]，但也存在一些缺点，需要探索新的固定化酶方法和开发高效固定化酶载体．漆酚 (urushiol) 是天然产物生漆的主要成分，是 3 位有 15 个碳原子的邻苯二酚化合物，我们曾以漆酚为原料合成了一系列功能基聚合物，能作为络合树酯络合金属离子和固定化酶的载体[4,5]，为进一步改进载体的性能，探索高分子络合剂对酶的固定化方法，本文将酪氨酸接枝到漆酚树酯上，制备成漆酚-酪氨酸铜高分子络合剂，对多酚氧化酶进行固定化，并探讨这种方法的可行性．

1 材料与方法

1.1 主要试剂与仪器

漆酚：将生漆用沸程为 60 ℃～ 90 ℃的石油醚萃取而得，酪氨酸为生化试剂，硝化纤维为 5% 乙醇-乙醚溶液，其余试剂均为分析纯．721 分光光度计：上海第三分析仪器厂．

1.2 漆酚-酪氨酸接枝树酯的合成

取适量漆酚 (urushiol) 石油醚溶液，称取聚合催化剂，研细后加入反应瓶中，20 ℃恒温搅拌反应，预聚 1 h 后，加入酪氨酸和硝基苯溶剂，温度升至 50 ℃继续反应 2 h，加阻聚剂使催化剂失活，蒸出溶剂，用热水反复洗涤，80 ℃干燥．将粗产品装柱，用沸水回流洗涤 2 h，再用乙醇回流洗涤 1h，除去聚合物中的小分子物质，然后干燥，得到漆酚-酪氨酸功能基树酯 (urushiol tyrosine resin, UTR)．

1.3 多酚氧化酶的提取

取市售香蕉果肉约 300 g，用捣碎机捣碎成糊状，移入烧杯，加入缓冲溶液，充分搅拌，抽滤，滤液中加入硫酸铵，使之浓度为 0.35 g/ml，抽滤，滤液中再加硫酸铵至饱合，沉淀过滤，为进一步纯化酶，将沉淀用蒸馏水溶解，再加丙酮，沉淀抽滤，所得凝胶状沉淀即为多酚氧化酶，低温保存备用[6]．

1.4 活性碳对多酚氧化酶的固定化

称取 1 g 粉末状活性碳和 1 g 多酚氧化酶，一同装入一小瓶内，加入约 5 ml 磷酸盐缓冲溶液，充分振荡 10 h．过滤，水洗，得活性碳固定化多酚氧化酶．

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