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α -淀粉酶中某些氨基酸含量与其最适 pH 的关系

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摘 要 :针对生产中大多数 α -淀粉酶不能适应高酸性和高温条件, 需要改进才能适应工业生产的实际, 研究了 α -淀粉酶的最适 pH 与各种氨基酸含量的关系, 构建了 α -淀粉酶序列库和 pH 序列库. 通过对 α -淀粉酶 pH 序列库统计分析, 发现 α -淀粉酶的最适 pH 与某些氨基酸含量有一定的关系, 当丙氨酸(A)质量分数大于 5.8% 并谷氨酸(E)质量分数大于 5.0%, 苏氨酸(T)质量分数大于 6.5% 并酪氨酸(Y)质量分数在 4.3%~6.3% 时, α -淀粉酶的耐酸性能力较好.

关键词 : α -淀粉酶; 蛋白质序列; 氨基酸; 最适 pH; 生物信息学

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Relation between Percentage of Some Amino Acids and Optimal pH of Alpha-Amylases

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Abstract : Alpha-amylase is an enzyme of great significance for industry. As the enzyme used in the process, most alpha-amylases are unstable at lower pH and higher temperature. Consequently, there is a need to develop an enzyme that can operate at industrial conditions. For studying the relation between percentage of kinds amino acids and optimal pH, we built alpha-amylase's bank and alpha-amylase's pH-sequence bank. After analysis, we found that there were 2 kinds cases about relation between percent amino acid and optimal pH: as either (1) A(alanine)% > 5.0% and E(glutamic acid)% > 5.8%, or (2) T(threonine)% > 6.5%, and Y(tyrosine)% in range of 4.3%~6.3%, alpha-amylase had the higher anti-acid ability.

Key words : alpha-amylase; protein sequence; percent amino acid; optimal pH; bioinformatics

α -淀粉酶广泛应用于食品、纺织、洗涤剂轻工行业, 在国内外酶制剂市场占有重要地位, 已经发现的天然 α -淀粉酶大多需要改进才能适应苛刻的工业生产条件或提高生产效率, 研究的主要方法就

是对已知酶进行优化改造. 例如, 热稳定性较好的地衣芽孢杆菌 α -淀粉酶在低 pH 值条件下不稳定, 必须通过蛋白质工程改进其性能. α -淀粉酶的优化改造已成为近期的一个研究热点^[1~7]. 酶的优化改

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万方数据

造是通过对已知酶分子一级结构的改变来创造新的酶,这一技术实现,依赖于两大技术的发明,即基因工程中的定点诱变技术和生物信息学。为此,从大量的文献中收集序列结构数据,对其整理分析,寻找不同氨基酸含量对其最适 pH 的影响,为 α -淀粉酶的优化改造提供帮助。

1 材料与方法

1.1 数据收集与整理

首先从 SWISS-PROT(<http://www.expasy.ch/sprot>)收集与 α -淀粉酶有关的氨基酸序列数据 538 条,经过整理,得到了有 352 条序列的 α -淀粉酶序列库。再收集与 α -淀粉酶最适 pH 有关的文献^[1~48],从 α -淀粉酶序列库中进行检索,得到既有 α -淀粉酶最适 pH,又有序列 α -淀粉酶,称为 α -淀粉酶 pH 序列库。该库共有 50 条记录,分布为 pH 小于 5.5 的有 13 条,为耐酸;pH 在 5.5~6.5 范围的有 15 条,为弱酸;pH 在 6.5~7.5 范围的有 21 条,为中性;pH 大于 7.5 的有 4 条,为弱碱。

1.2 方法

计算 α -淀粉酶 pH 序列库中每一条序列不同氨基酸的质量分数,得到同一种氨基酸在不同序列(有对应 pH 数值)时的含量,用此数据作每一种氨基酸含量与 pH 的关系图。该图关系复杂,未能得出规律性的结论。于是,研究了不同氨基酸含量之间对上述 4 种类型 α -淀粉酶分布的影响。

2 结果与分析

运用上述方法,得到了 20 种氨基酸两两组合含量之间与 α -淀粉酶类型分布的关系图,共 190 幅,明显联系的有两幅,见图 1 和图 2。纵坐标和横坐标是对应氨基酸的质量分数,氨基酸种类以其对应单字母表示。

从图 1 中可以看出,当丙氨酸(A)质量分数大于 5.8% 并且谷氨酸(E)质量分数大于 5.0% 时,此区域为耐酸 α -淀粉酶所在区域。从图 2 中同样可以看出,存在着一个耐酸 α -淀粉酶所在区域,对应范围为苏氨酸(T)质量分数大于 6.5% 并酪氨酸(Y)质量分数 4.3%~6.3%。

α -淀粉酶最适 pH 与其氨基酸组成、结构应当有着必然地联系,只是影响因素太多,难以寻找。这

些统计结果可以运用到 α -淀粉酶的优化改造上。如果增加 α -淀粉酶的耐酸性,控制丙氨酸、谷氨酸、苏氨酸和酪氨酸含量应是有效的方法。

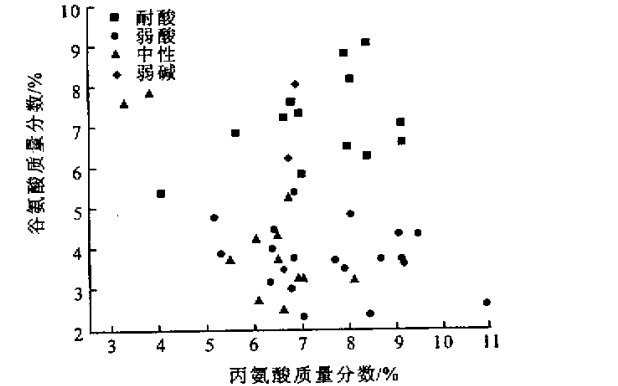


图 1 不同类型 α -淀粉酶与丙氨酸-谷氨酸对应的关系
Fig.1 Relation between types of alpha-amylase and couples of alanine-glutamic acid

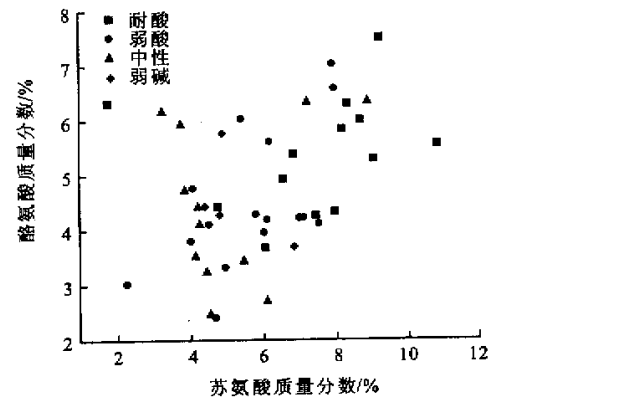


图 2 不同类型 α -淀粉酶与苏氨酸-酪氨酸对应的关系
Fig.2 Relation between types of alpha-amylase and couples of threonine-tyrosine

3 结 论

通过对 α -淀粉酶的最适 pH 与序列数据的分析处理,发现存在两个耐酸 α -淀粉酶所在区域:(1)丙氨酸(A)质量分数大于 5.8%,并且谷氨酸(E)质量分数大于 5.0%;(2)苏氨酸(T)质量分数大于 6.5%,并酪氨酸(Y)质量分数为 4.3%~6.3%。这些统计结果将会对提高 α -淀粉酶耐酸性提供有益的帮助。

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