背景介绍

如今温室效应是人们面临的头号问题，受到各国政府的极大关注。CO_2大约占温室气体总量的2/3，是引起温室效应的最主要的气体[1]。因此各种控制二氧化碳排放的措施（如CO_2捕集）开始越来越受到重视。CO_2捕集的主要方法包括：溶剂吸收法、物理吸附法、膜分离法等。

溶剂吸收法主要是基于化学吸收法，一些化学吸收剂可以与CO_2反应形成化合物，并从流过含有吸收剂的烟道气中分离出CO_2。但是此方法也有以下几种缺点：溶液易氧化分解；溶液的腐蚀性较强，易腐蚀仪器，仪器维持反应成本较高；能耗大并且运行费用高。

物理吸附法是根据气体中不同组分对固体吸附剂的吸附特性不同将 CO_2捕集起来。但此方法需要大量的吸附剂来维持此过程的运行，且吸附剂的选择性较差，吸附容量低，效率低下，造成了运营成本很高，实际应用较少。

膜分离法是基于聚合薄膜对不同的气体组分的渗透率不同来达到分离不同气体组分的目的。所用的膜，大体可以分为有机膜和无机膜。其中有机膜对气体组分的选择性较强，装配简单，但其耐热性能和防腐蚀性能差；而无机膜正好相反，其耐热性能和防腐蚀性能好，但其装配较复杂。总的来看，这种方法捕集得到的CO_2的纯度不高，需要进行多次纯化，在工业上应用较少。

以上的几种CO_2捕集技术都具有成本较高、效率偏低、可循环性差的缺点，这些无法避免的缺点阻碍了其在生产生活中的应用。因此急需要新型的技术诞生，而碳酸酐酶（CA）捕集技术的出现弥补了其他方法的不足。

碳酸酐酶，是一种含有Zn^{2+}的金属酶，碳酸酐酶可以催化CO_2和H_2O生成HCO_3^-（如图1）。与其他类型的酶相比较，碳酸酐催化速率非常快。各种碳酸酐酶的催化速率的范围是每秒10^4到10^6个反应。在各种来源的碳酸酐酶中，人碳酸酐酶的催化效率最高。

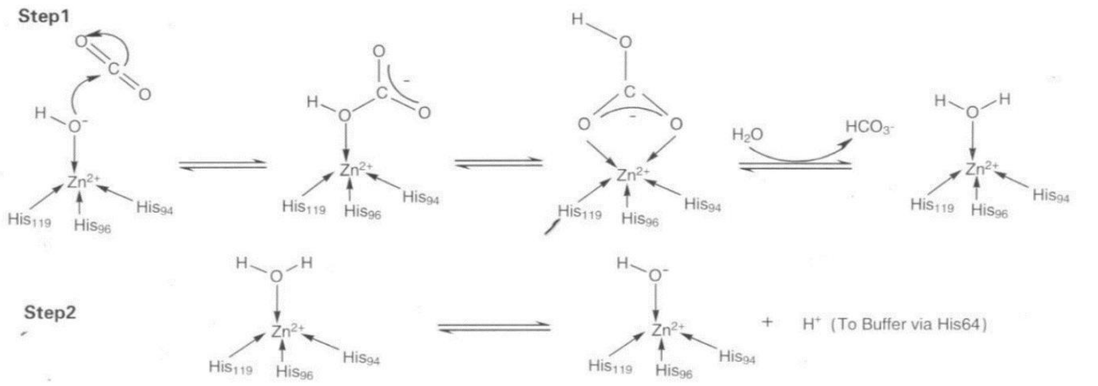

图 1　碳酸酐酶(CA2)的催化机理

碳酸酐酶的分子质量约为 30KDa，由单一肽链组成，包含有约 260 个氨基酸，每个酶分子含有一个 Zn^{2+}。其结构为椭球型，分子中部有一个袋空腔深约 1.5nm，腔口宽约 2.0nm，Zn^{2+}结合在空腔底部。目前，研究最多的一类碳酸酐酶为动物体内α-家族 CA，也被称作 CA2。它的主要二级结构位于其酶分子 10 个β-折叠中。正是由于它们的存在，酶结构分为两部分。酶分子中许多关键氨基酸残基位点与其活性有关。除了β-折叠之外 ，酶分子的表面还以α-螺旋结构分布，其通常是短小结构（如图 2）。

图 2　碳酸酐酶（CA2）结构 [2]

相较于其他方法，碳酸酐酶捕集技术，具有专一性，可以从其他气体中特异的捕集 CO_2。且此方法比较环保，碳酸酐酶把 CO_2 转变成碳酸氢根，碳酸氢根可

以满足植物和微生物的生长需求[3]。当 CO_2 转变为碳酸氢根时，碳酸氢根可以与钙离子结合生成碳酸钙，稳定地储存在地下。

相较于其他方法，碳酸酐酶捕集技术，更高效。一般的 CO_2 捕集技术主要的限速步骤是 CO_2 的水合反应，而碳酸酐酶可以大幅度提高 CO_2 的水合反应速率，从而提高 CO_2 的捕集效率[4]。

碳酸酐酶捕集技术虽然具有高效性，但仍具有一定的限制。因为大多数天然碳酸酐酶对反应环境过于敏感，且不具有热稳定性，然而碳酸酐酶参与 CO_2 捕集时 ,环境的温度一般为 65℃,而天然碳酸酐酶在此温度下无法保持稳定，在多次循环之后酶活性丧失。碳酸酐酶的价格比较昂贵，若经常更换会使捕集成本大大提高，这限制了碳酸酐酶捕集技术的大范围推广。因此现在的关键是寻找热稳定性的碳酸酐酶。

为了寻找到高效催化和高稳定性的碳酸酐酶，在本项目中，我们利用人碳酸酐酶 2（以下简称 CA2）的高效催化特点，运用分子模拟手段对其氨基酸序列进行优化，设计出高活性且高稳定性的 CA2 突变体。项目包括以下几方面的内容：

1）分子模拟；

2）表达野生型和突变型 CA2 的大肠杆菌菌株的构建；

3）CA2 的表达与纯化；

4）CA2 的实际应用：CO2 捕集。

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2. Claudiu T. Supuran. Structure and function of carbonic anhydrases[J]. Biomolecular Biochemical journal, 2016, 473(14):2023-2032.

3. Lionetto M G, Caricato R, Erroi E, et al. Potential application of carbonic anhydrase activity in bioassay and biomarker studies [J]. Chemistry & Ecology, 2006, 22(sup1): S119-S25.

4. Migliardini F, De L V, Carginale V, et al. Biomimetic CO2 capture using a highly thermostable bacterial α-carbonic anhydrase immobilized on a polyurethane

foam [J]. Journal of Enzyme Inhibition & Medicinal Chemistry, 2014, 29(1): 146.

Background

Nowadays, greenhouse effect is the most important problem that people are facing, which attracts great attention from governments. CO_2 accounts for about two-thirds of the total greenhouse gas, and it is the most important gas which causes the greenhouse effect. As a result, various measures to control carbon dioxide emissions, such as CO_2 capture, are becoming more and more important. The main methods of CO_2 capture include solvent absorption, physical adsorption and membrane separation.

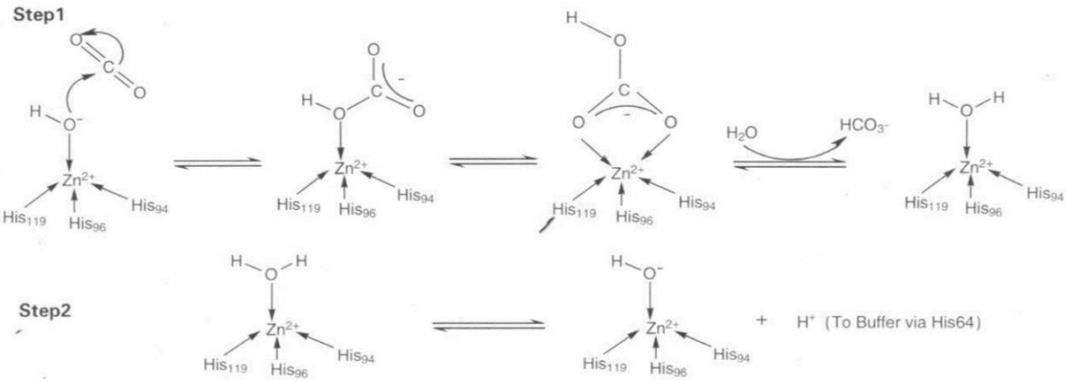
Solvent absorption is mainly based on chemical absorption. Some chemical absorbents can react with CO_2 to form compounds and separate CO_2 from the flue gas containing the absorbent. However, this method also has the following disadvantages: the solution is easy to oxidize and decompose; the solution is highly corrosive and easy to corrode the instrument; large energy consumption and high operation cost.

Physical adsorption method collects CO_2 according to the adsorption characteristics of different components of gas to solid adsorbent. However, this method requires a large number of adsorbent to maintain the operation of this process, and the adsorbent has poor selectivity, low adsorption capacity and low efficiency, resulting in high operating costs and few practical applications.

Membrane separation method is based on the polymer film to different gas components of different permeability to achieve the purpose of separation of different gas components. The membranes used can be divided into organic membranes and inorganic membranes. Among them, the organic membrane has strong selectivity for the gas components, simple assembly, but poor heat resistance and corrosion resistance. The inorganic membrane, on the contrary, has good heat resistance and corrosion resistance, but its assembly is complex. In general, CO_2 captured by this method is of low purity, requiring multiple purification and less application in industry.

The above CO2 capture technologies all have the disadvantages of high cost, low efficiency and poor circulability. These unavoidable disadvantages hinder their application in production and life. Therefore, new technologies are urgently needed, and the technology of carbonic anhydrase (CA) capture makes up for the shortage of other methods.

Carbonic anhydrase, a metal enzyme containing Zn2+, can catalyze CO2 and H2O to produce HCO3- (as shown in figure 1). Carbonic anhydride catalyzes faster than other types of enzymes. The range of carbonic anhydrase catalytic rates is 104 to 106 reactions per second. Among various sources of carbonic anhydrase, human carbonic anhydrase has the highest catalytic efficiency.



Picture 1 Catalytic mechanism of carbonic anhydrase (CA2)

The molecular weight of carbonic anhydrase is about 30KDa, which is composed of a single peptide chain and contains about 260 amino acids. Each enzyme molecule contains one Zn2+. The structure is ellipsoid, with a pouch cavity in the middle about 1.5nm deep and a cavity opening about 2.0nm wide. Zn2+ binds at the bottom of the cavity. At present, the most studied type of carbonic anhydrase is cosine-family CA, also known as CA2. Its main secondary structure is in its enzyme molecule 10 pali-fold. It is because of their existence that the enzyme structure is divided into two parts. Many key amino acid residues in enzyme molecules are related to their activity. In addition to extension-folding, the surface of the enzyme molecules is also distributed in the form of an icy-helical structure, which is usually a short structure (picture 2).

Picture 2 The structure of carbonic anhydrase (CA2)

Compared with other methods, carbonic anhydrase capture technology is specific and can capture CO2 from other gases. In addition, this method is environmentally friendly. Carbonic anhydrase converts CO2 into bicarbonate, which can meet the growth demand of plants and microorganisms. When CO2 is converted to bicarbonate, bicarbonate can combine with calcium ions to form calcium carbonate, which is stably stored underground.

Compared with other methods, carbonic anhydrase capture technology is more efficient. The main rate-limiting step of general CO2 capture technology is the hydration reaction of CO2, while carbonic anhydrase can significantly increase the hydration reaction rate of CO2, thus improving the CO2 capture efficiency.

Although carbonic anhydrase capture technology has high efficiency, it still has some limitations. Because most natural carbonic anhydrase environmental sensitivity in the reaction, and do not have heat stability, carbonic anhydrase in CO2 capture, however, the environment temperature is 65 ℃, and natural carbonic anhydrase in the temperature cannot remain stable, after many times circulation loss of enzyme activity. The price of carbonic anhydrase is relatively expensive, and frequent replacement will greatly increase the cost of capture, which limits the wide spread of carbonic anhydrase capture technology. So the key now is to look for the thermal stability of carbonic anhydrase.

In order to find the high efficiency catalysis and high stability of carbonic anhydrase, in this project, we use the high efficiency catalysis characteristic of human carbonic anhydrase 2 (hereinafter referred to as CA2), use molecular simulation method to optimize its amino acid sequence, and design the CA2 mutant with high activity and high stability. The project includes the following aspects:

1) molecular simulation;

2) construction of escherichia coli strains expressing wild-type and mutant CA2;

3) expression and purification of CA2;

4) practical application of CA2: CO2 capture.

1. Rahman F A, Aziz M M A, Saidur R, et al. Pollution to solution: Capture and sequestration of carbon dioxide (CO2) and its utilization as a renewable energy source for a sustainable future[J]. Renewable & Sustainable Energy Reviews, 2017, 71:112-126.
2. Claudiu T. Supuran. Structure and function of carbonic anhydrases[J]. Biomolecular Biochemical journal, 2016, 473(14):2023-2032.
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4. Migliardini F, De L V, Carginale V, et al. Biomimetic CO2 capture using a highly thermostable bacterial α-carbonic anhydrase immobilized on a polyurethane foam [J]. Journal of Enzyme Inhibition & Medicinal Chemistry, 2014, 29(1): 146.