微生物学报
Acta Microbiologica Sinica
2017, 57(8): 1180-1188
http://journals.im.ac.cn/actamicrocn
DOI: 10.13343/j.cnki.wsxb.20170148

Mini-Review 小型综述

微生物嗜盐酶的研究进展

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摘要：嗜盐酶一般来自于嗜盐菌，它的主要特点是严格依赖体系中一定的盐离子浓度，可以在高盐环境中维持其结构稳定，并且能够抵抗高温、pH 和有机溶剂存在下的变性，因此在高盐、水/有机和非水介质环境的催化中具有重要的应用价值。本综述从盐对嗜盐酶活性和稳定性的影响、金属离子和有机溶剂对嗜盐酶的影响几个方面介绍了嗜盐酶的特点。在总结蛋白质数据库(PDB)中已有嗜盐酶的结构和特点的基础上，对嗜盐酶的嗜盐机制进行了分析，认为嗜盐酶不同于非嗜盐酶的特点在于盐桥和氢键明显增多，含有一些特殊的盐离子结合位点并且常以低聚体的形式存在，表面酸性氨基酸含量明显增多。最后对嗜盐酶的分子改造和应用进行了简要的介绍。

关键词：嗜盐酶，特点，结构，嗜盐机制，应用

嗜盐菌(Halophiles)指在高盐条件下生长的细菌，它主要生长在盐湖，比如中国的青海湖、美国大盐湖、死海、盐场等浓缩海水中，以及腌鱼、腌肉、泡菜等腌制品上。由于生长在高盐环境中，嗜盐菌产生的一些酶具有在高盐浓度下保持稳定和高活性的特点，所以称为嗜盐酶或耐盐酶，它们的主要特征是酶蛋白在高盐条件下的溶解度明显增高，酶的活性严格依赖反应体系中的盐离子的浓度，同时具有盐耐受性、热耐受性和对有机溶剂的抗性。由于嗜盐酶的这些特性，通过研究嗜盐微生物及其嗜盐蛋白的嗜盐机制对改造嗜盐酶和嗜盐微生物以及生产有用的生物活性物质都具有重要的意义。

1 嗜盐酶的特点

1.1 盐对酶活性的影响

盐的种类很多，嗜盐酶主要对钾离子和钠离子具有依耐性，研究最多的是钠离子。盐对酶活性的影响主要有三种情况，一是酶的活性依赖于钠钾离子的存在，没有这些离子存在就没有活性。比如极端嗜盐菌的苹果酸脱氢酶(Malate dehydrogenase)在 2.5–5.0 mol/L NaCl 中是稳定有活性的，但是在盐缺失环境中活性完全消失[1]；

基金项目：国家自然科学基金(31670069，31170068)

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收稿日期：2017-03-29；**修回日期：**2017-05-17；**网络出版日期：**2017-05-25

Haloferax volcanii 的二氢叶酸还原酶(DHFR)在 KCl 浓度低于 0.5 mol/L 时活性完全丧失[2]；*Natrialba magadii* 的胞外蛋白酶 Nep 在没有盐存在时发生解构和聚集沉淀导致酶活性完全丧失[3]。二是盐提高酶的活性，低盐时有微弱活性，但是蛋白没有正确的折叠结构。比如来自 *Haloferax alicantei* 的 β-半乳糖苷酶的活性随着盐浓度的增加而升高，在 4 mol/L NaCl 时活性达到最大[4]；来自 *Bacillus* sp. BG-CS10 的嗜盐纤维素酶 CelB 在 2.5 mol/L NaCl 或 3 mol/L KCl 中酶活性最高，最大酶活是不存在盐时的 10 倍，在 4 mol/L NaCl 或 3.5 mol/L KCl 的高盐浓度下活性仍保持高于 80%[5]；Miyashita 等使用紫外吸收、圆二色谱(CD)以及荧光光谱法研究了盐对二氢叶酸还原酶(HjDHFR P1)稳定性和酶功能的影响，以 NADPH 作为其催化反应的辅酶时，HjDHFR P1 在不存在 NaCl、pH 8.0 时没有 NADPH 的结合位点，而加入 NaCl (0–500 mmol/L)时，HjDHFR P1 形成明显的 NADPH 结合位点[6]。三是有些嗜盐酶在 KCl 中的活性显著高于 NaCl 中。如来自 *Haloferax volcanii* 的 3-羟基-3-甲基戊二酰辅酶 A 还原酶活性随着 KCl 浓度的增加而升高，而当 NaCl 浓度增加时活性则降低[7]；来自 *Haloferax volcanii* 的 DNA 连接酶(*Hv* LigN)活性完全依赖于 KCl 浓度，不存在该盐的情况下不能连接 DNA，在 3.2 mol/L KCl 中酶活达到了最大，但 NaCl 对 *Hv* LigN 催化活性基本没影响，即使高浓度 NaCl 也几乎没有影响[8]。

1.2 盐对嗜盐酶稳定性的影响

已经研究发现嗜盐蛋白在高温下的稳定性是通过盐来调节的，嗜盐酶在高盐环境中一般都具有更好的热稳定性。对嗜盐纤维素酶 CelB 的热稳定性研究发现，没有 NaCl 存在时，酶在 50 °C 处理 15 min 后酶活性完全丧失，但是在 2.5 mol/L NaCl 体系中 55 °C 处理 30 min 仍能保持酶活不下降，说明盐显著提高了 CelB 的热稳定性[5]。极端嗜盐古菌 *Haloarcula japonica* 来源的嗜盐 2-脱氧-D-核糖-5-磷酸醛缩酶(DERA)在含有 2 mol/L NaCl 的缓冲液中表现出高的热稳定性，在 70 °C 加热 10 min 后仍保持 90%以上的活性[9]。为了研究 *Halothermothrix orenii* 来源淀粉酶 AmyA 的热稳定性与盐浓度之间的关系，Sivakumar 等在不同的盐浓度下通过 *CD* 检测 AmyA 的变性，发现 AmyA 的热稳定性随着 NaCl 浓度的增加而增加[10]。

1.3 有机溶剂对嗜盐酶的影响

有机溶剂破坏蛋白质亚基之间的氢键，并且通过影响活性位点处的临界水浓度来降低催化效率，高盐的存在也显著降低了水活度，研究表明大多数嗜盐酶具有机溶剂耐受性，已经发现嗜盐支链淀粉酶、纤维素酶、蛋白酶、淀粉酶、脂肪酶、酯酶、醇脱氢酶等多种酶具有机溶剂耐受性，适于在低水/非水介质中进行催化反应。如前文提到的嗜盐纤维素酶 CelB，DMSO、triton X-405 和异丙醇(5%)没有明显抑制该酶的活性，甘油、甲醇(5%)以及 0.025% SDS 可部分抑制 CelB 的活性，说明 CelB 可以在高渗环境中起作用[5]。还有嗜盐蛋白酶 EMB9 在亲水性有机溶剂中的耐受性显著增强，在 25% (*V*/*V*)甲醇和乙醇存在下，酶活性比对照提高了 1.5–1.6 倍，但是增加溶剂的浓度(高达 75%)引起酶活性逐渐丧失[11]。Tatsuya 等研究了甲醇、乙醇、乙腈或 DMSO 在 25 °C 温育嗜盐 2-脱氧-D-核糖-5-磷酸醛缩酶 DERA 来检测有机溶剂对酶稳定性的影响，孵育 10 min 后发现，即使 DMSO 和乙腈浓度高达 50%，酶仍保持了 80%以上的活性[9]。

也有文献利用圆二色谱和荧光光谱等研究了具溶剂稳定性的嗜盐酶的次级结构变化，Alsafadi 等通过荧光光谱研究了有机溶剂对嗜盐乙醇脱氢酶 *Hv*ADH2 活性和稳定性的影响机制，结果显示盐浓度对 *Hv*ADH2 折叠和构象有直接影响，降低盐浓度可导致嗜盐酶的解折叠，但是在低温下有机溶剂如 DMSO 可增加酶的结构稳定性[12]。通过圆二色谱检测溶解在 50% (*V*/*V*)正己烷和正癸烷中的 *Geomicrobium* sp.蛋白酶的二级结构，发现在 5% NaCl 存在时，蛋白 α-螺旋含量不受影响，但是没有盐存在时，α-螺旋明显减少，这说明盐对维持蛋白结构的稳定性具有重要作用[13]。

1.4 金属离子对嗜盐酶的影响

大多数嗜盐酶的活性都受到了金属离子的影响，一些金属离子能与酶活性中心的基团结合从而提高酶的活性，也有些金属离子会强烈抑制嗜盐酶的活性。Zhang 等研究了金属离子对嗜盐纤维素酶 CelB 活性的影响，发现在 2.5 mol/L NaCl 以及 5 mmol/L Hg^{2+}存在下，CelB 仍具有 45%活性，虽然在 CelB 序列中有 15 个色氨酸残基(Hg^{2+}可以氧化色氨酸残基的吲哚环)，但是在无盐条件下，Hg^{2+}完全抑制了酶的活性[5]。Ca^{2+}激活嗜盐古菌 *Halogranum rubrum* 来源蛋白酶的活性，而 Mn^{2+}和 Cu^{2+}强烈抑制了蛋白酶活性；而来自 *Halogeometyicum borinquense* TSS101 的蛋白酶活性仅由 Ca^{2+}激活[14]。

2 嗜盐酶的结构

由于嗜盐酶通常需要在高盐浓度下维持活性和稳定性，所以在大肠杆菌中异源表达纯化时通常需要在高盐下纯化以维持活性和正确的折叠。近年来已经通过在大肠杆菌中进行异源表达获得大量蛋白，通过蛋白结晶或者核磁共振技术解析了大量嗜盐蛋白及对应突变体的结构(表 1)，为了解嗜盐酶嗜盐机制奠定了基础。

通过比较各嗜盐酶的结构特点，可以发现嗜盐酶多以聚体形式存在、大部分会有相应的金属离子结合位点，而且几乎所有嗜盐酶的蛋白表面为较多的酸性氨基酸，仅有个别的蛋白表面为较多的碱性氨基酸或酸碱性氨基酸均一分布，这些结构特点与其特有的高盐环境是息息相关的。

3 嗜盐酶的嗜盐机理

极端环境生物学研究者一直想弄清楚嗜盐蛋白是如何在高盐的环境中维持稳定性和活性的，虽然到目前为止，对蛋白的嗜盐机制还没有确切的解释，但是随着许多嗜盐蛋白结构被解析，其结构中与嗜盐机理相关的结构特性也随之被揭示。本文在这里总结了几条目前研究者比较认可的嗜盐酶嗜盐机理，希望能够对研究嗜盐酶的研究者有一定的帮助。

3.1 嗜盐酶表面有很多酸性氨基酸

许多研究者通过对很多嗜盐酶的结构分析发现，几乎所有嗜盐酶的蛋白表面，酸性氨基酸的含量明显高于非嗜盐酶表面酸性氨基酸的含量，因此嗜盐酶这个十分保守的特性成为其主要耐受高盐环境的原因之一。嗜盐酶蛋白表面分布较多的酸性氨基酸，可以增加蛋白与环境中水的结合能力，使得蛋白表面形成的水化层更加牢固，从而防止高盐环境中蛋白发生聚集，丧失活性，同时也增加了蛋白在高盐环境下的溶解性。Madern 等通过对 26 种来自嗜盐菌的可溶蛋白进行统计学分析发现，这些蛋白含有很低含量的赖氨酸，从而使酸性氨基酸含量总体变高，同时他们还发现

表 1. PDB 中嗜盐酶结构的分析

Table 1. Analysis of the structure of halophilic enzymes in PDB

Halophilic enzymes	Source	PDB number	Structural resolution	Polymer or not	Optimal and tolerable salt concentration	Protein surface amino acids (AA)	References
Cellulase	*Bacillus* sp. BG-CS10	5EOC	2.35Å	Monomer/sometimes oligomers	Optimal 2.5 mol/L NaCl	Most acidic AA	[15]
Carbonic anlydrase	*Bovine*	4CNR	2.29Å	Tetramer	Tolerable 3.0 mol/L NaCl	Most acidic AA	[16]
Carbonic anlydrase	*Dunaliella*	1Y7W	1.86Å	Dimer	Tolerable 2.0 mol/L NaCl	Most acidic AA	[17]
Carbonic anlydrase	*Photobacterium profundum*	5HPJ	1.50Å	Monomer/dimer	Optimal 0.5 mol/L NaCl	Most acidic AA	[18]
Alkaline phosphatase	*Halomonas* sp. *593*	3WBH	2.10Å	Dimer	Tolerable 1.0–4.0 mol/L NaCl	Most acidic AA	[19]
Malate dehydrogenase	*Salinibacter ruber*	4CL3	1.55Å	Dimer		Most acidic AA	[20]
Malate dehydrogenase	*Haloarcula marismortui*	4JCO	1.70Å	Tetramer		Most acidic AA	Not published
Rnase H1	*Halobacterium salinarum*	4NYN	1.14Å	Dimer	Tolerable 3.0 mol/L NaCl	Most acidic AA	Not published
Nucleoside diphosphate kinase	*Haloarlula quadrata*	2ZUA	2.59Å	Hexamer	Tolerable 0.2–4.0 mol/L NaCl	Most acidic AA	[21]
Nucleoside diphosphate kinase	*Halomonas* sp.	3VGS	2.30Å	Dimer/tetramer/hexamer	Tolerable 2.0 mol/L NaCl	Most acidic AA	[22]
Malate synthase	*Haloferax volcanii*	3OYX	2.51Å	Monomer	Tolerable 3.0 mol/L KCl	Most acidic AA	[23]
Endonuclease	*Aliivibrio salmonicida*	2PU3	1.50Å	Monomer	Optimal 0.4 mol/L NaCl	Most basic AA	[24]
α-Amylase	*Halothermothnx orenii*	3BC9	1.35Å	Monomer	Optimal 0.9 mol/L NaCl	Most acidic AA	[25]
α-Amylase	*Halothermothnx orenii*	1WZA	1.60Å	Monomer	Tolerable 4.7 mol/L NaCl	Acid and alkaline AA	[10]
DHFR	*Haloferax volcanii*	2ITH	NMR	Monomer	Tolerable 3.5 mol/L NaCl	Most acidic AA	[26]
Cellobiohydrolase	*Heterobasidion annosum*	2XSP	1.70Å	Monomer		Most acidic AA	[27]

与非嗜盐蛋白相比这些蛋白脂肪族氨基酸含量减少，这一现象可能与高盐环境下蛋白的高溶解性有一定的关系[8]。同样地，通过对极端嗜盐苹果酸脱氢酶 hMDH 的序列分析发现，其酸性氨基酸含量为 19% (摩尔百分比)，而非嗜盐菌 hMDH 中酸性氨基酸只占 6%[28]。Jolley 等通过对 *Haloferaxv olcanii* 的二氢硫辛酰胺脱氢酶 K^+结合位点的研究，发现 4 个带负电荷的谷氨酸残基对该酶的嗜盐性有重要的影响：如果 2 个谷氨酸(每个亚基一对)被中性氨基酸取代，酶对盐的依赖性就会完全丧失，可见在高盐环境下嗜盐酶含有较多的酸性氨基酸是十分重要的[29]。

3.2 嗜盐酶的盐桥和氢键明显增多

研究者在比较嗜盐酶与同源的非嗜盐酶的结构时还发现，嗜盐酶中会形成更多的盐桥和氢键。大多数研究者认为嗜盐酶形成更多的盐桥可以清除盐离子的屏蔽效应，增加嗜盐酶的可溶性；形成更多的氢键有利于酶蛋白三级结构的稳定性，

从而使嗜盐酶可以耐受高盐环境。王四华等将 4 组有代表性的嗜盐酶进行分子动力学模拟，以便从原子层面了解嗜盐酶分子在高盐条件下的动力学行为，并从原子尺度来理解嗜盐酶的功能和稳定性。这 4 组嗜盐酶分别为二氢叶酸还原酶、苹果酸脱氢酶、碱性磷酸酶和核苷二磷酸激酶，研究结果发现，嗜盐酶中所形成的盐桥和氢键明显多于非嗜盐酶，嗜盐酶的溶剂可及性表面要比非嗜盐酶的小，而且嗜盐酶的结构较非嗜盐酶更具刚性[55]。因此能形成较多的盐桥、氢键，有较小的溶剂可及性表面和整体刚性结构，很可能是嗜盐酶在高盐环境中维持其结构稳定的另一个主要原因。

3.3 嗜盐酶含有一些特殊的盐离子结合位点，并常以低聚体形式存在

大多数嗜盐酶的亚基表面会存在一些特殊的盐离子结合位点，在高盐的环境下，嗜盐酶会与溶液中盐离子结合，进而促进嗜盐酶形成低聚体，在一定程度上使得其结构在高盐环境下保持稳定。Mevarech 等通过超速离心以及光散射检测极端嗜盐古菌 *Haloarcula marismortui* 的苹果酸脱氢酶 hMDH 的分子质量研究盐对其结构稳定性的影响，结果发现在 4.0 mol/L NaCl 中，hMDH 是四聚体，但是当盐浓度降低到 2.0 mol/L 以下时，酶直接解离成单体[30]。也有文献报道了盐对嗜盐乙醇脱氢酶 *Hv*ADH2 四级结构的影响，在 2.0 mol/L KCl 中，*Hv*ADH2 以四聚体形式存在，但当盐浓度降到为 1.0 mol/L 时，酶解离成二聚体[12]。从 *Halothermothrix orenii* 中分离的淀粉酶 AmyA，其蛋白表面不存在过量的酸性氨基酸，而是带正电荷和带负电荷的氨基酸均匀分布，但它表面含有高亲和力的离子结合位点，在高盐的环境中会形成低聚体，同时表面还有很多暴露的盐桥，从而使其能在高盐环境下保持结构的稳定，即使是在 4.7 mol/L NaCl 浓度，AmyA 仍可保持其最佳活性的 90%[10]。

4 嗜盐酶的分子改造和应用

4.1 嗜盐酶的分子改造

嗜盐酶的分子改造研究不多，主要集中在低盐环境的热稳定性和高活性两个方向的改造。在极端嗜盐菌 *Hbt*. sp. NRC-1 的半胱氨酰 tRNA 合成酶(CysRS)中插入肽段后，提高了在低盐浓度下酶的稳定性[31]。对极端嗜盐古细菌(*Halobacterium salinarum*)的核苷二磷酸激酶(HsNDK)进行突变研究发现，突变体 G114R 碱性二聚体单体之间的缔合增强，在低盐溶液中表现出提高的热稳定性；突变体 D148C 的碱性二聚体单体之间形成二硫键，也具有提高的低盐溶液热稳定性[32]。对嗜盐 α-淀粉酶 *k6* 通过同源建模，确定 Na^+结合位点上的氨基酸残基，并对相应位点进行定点突变，结果表明，跟野生酶相比，突变酶更加嗜盐，其最适 NaCl 浓度由 2.0 mol/L 增加到 3.0 mol/L，酶活力为 4831 U/mg，提高近 4 倍[56]。

4.2 嗜盐酶的工业应用

到目前为止，工业应用主要利用来自嗜温和嗜热生物体的酶。然而，因为嗜盐酶的高活性、广泛的底物特异性和在苛刻条件下的稳定性，越来越多的注意力已经转向在高盐环境中以嗜盐酶作为催化剂，使得它们在生物燃料生产，纺织品加工，废物处理以及作为洗涤剂添加剂等领域都具有潜在的应用。如嗜盐 α-淀粉酶在高盐环境下依然能保持稳定的结构和高效的生物催化能力，这在调味剂、食品腌制等的工业生产及盐碱地的

改造等多方面都具有开发利用价值；嗜盐脂肪酶 LipBL 因为可以耐受有机溶剂，被认为是生产多不饱和脂肪酸的优良选择[33]；丝氨酸蛋白酶在水/N-N′-二甲基-甲酰胺系统中是合成甘氨酸肽的良好选择，具有很大的肽合成潜力[34]；核酸酶 H 可以降解 RNA 和 DNA 产生 5′-单核苷酸用于产生 5′-鸟苷酸和 5′-肌苷酸，生产商业调味剂[35]；琼脂水解酶对寡糖生产是重要的，并且有助于维持海水中红藻的低度污染[36]。表 2 总结了目前部分嗜盐酶的种类和应用领域。

表 2. 嗜盐酶在生物技术方面的应用

Table 2. Application of halophilic enzymes in biotechnology

Halophilic enzymes	Organism	Application	Reference
Cellulase	*Aspergillus terreus* UniMAP AA-6	In situ saccharification of ionic liquids treated lignocelluloses	[37]
Amylase	*Halococcus* sp.	Starch hydrolysis in industrial processes in saline and organic solvent medium	[38]
Lipase	*Candida* sp.	Organic synthesis related to food/feed processing, pharmaceuticals or cosmetics	[39]
Protease	*Pseudomonas* sp.	Peptide synthesis	[40]
Xylanase	*Bacillus* sp.	Xylan biodegradation in pulp and paper industries	[41]
Chitinase	*Virgibacillus* sp.	Bioconversion of chitin from fish, crab or shrimp; treatment of chitinous waste	[42]
2-deoxy-D-ribose-5-phosphate aldolase	*Haloarcula japonica*	A potential biocatalyst for the production of a variety of stereo-specific materials	[9]
Malate dehydrogenase	*Haloarcula marismortui*	Catalyzed malic acid to produce oxaloacetic acid	[4]
Alcohol dehydrogenase	*Rhodococcus* sp.	Enantioselective oxidation of sec-alcohol and the asymmetric reduction of ketones	[43]
Alkaline phosphatase	Antarctic bacteria strain HK47	Radioactive end-labeling of nucleic acids	[44]
β-galactosidase	*Arthrobacter* sp.	Lactose hydrolysis at low temperature, production of ethanol from lactose-based feedstock	[45]
Glutaminase	*Micrococcus* sp.	Flavor-enhancing in food industries, anti-leukaemia agent	[46]
Cholesterol oxidase	*Pseudomonas* sp.	Organic synthesis	[47]
Lipase	*Marinobacter* spp.	Hydrolysis of fish oil into free eicosapentaenoic acid	[48]
Lipase	*Pseudoalteromonas* spp.	Detergent formulations and bioremediation of fat-contaminated aqueous systems	[49]
Dihydrofolate reductase	*Haloarcula japonica* strain TR-1	Catalytic folic acid to produce dihydrofolic acid	[6]
Lipase	*Marinobacter* sp.	Hydrolysis of fish oil into free eicosapentaenoic acid	[50]
Nuclease	*Micrococcus* sp.	Production of the flavoring agent 5′-guanylic acid	[51]
Pectinase	*Pseudoalteromonas* sp.	Enhancing extraction yield, clarification, and taste of fruit juices	[52]
Serine hydroxymethyltransferase	*Aphanothece halophytica*	Involved in photo respiratory pathway of oxygenic photosynthetic organisms	[53]
β-galactosidase	*Bacillus* sp.	Synthesis of N-acetyl-lactosamine	[54]

5 总结和展望

总的来说，随着越来越多的嗜盐酶被发现，嗜盐酶基因被克隆和异源表达，盐和金属离子对嗜盐酶活性和稳定性的影响研究也越来越多，因为嗜盐酶的高活性、广泛的底物特异性和在苛刻条件下的稳定性使得它们在生物燃料生产、纺织品加工、废物处理以及洗涤添加剂等领域都具有潜在的应用。随着越来越多嗜盐酶的结构被解析，嗜盐酶的结构特点分析发现嗜盐酶有着不同于普通酶的结构：盐桥和氢键明显增多，含有一些特殊的盐离子结合位点并且常以低聚体的形式存在，表面酸性氨基酸含量明显增多。基于理性设计的嗜盐酶分子改造，提高比酶活和稳定性将是以后嗜盐酶发展的研究方向，利用异源高效表达系统，获得低成本的嗜盐酶产品将是未来工业化发展的方向。

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Advances in microbial halophilic enzymes

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Abstract: Halophilic enzymes are derived naturally from halophilic bacteria that survive in high salt environment, as it can maintain the structural stability of the enzyme only in high salt environment. These enzymes can withstand high temperature, pH and organic solvents, so they can be widely used for their catalytic activity in high salt, water/organic and non-aqueous environment. In this review, we address the effect of salt on the activity and stability of halophilic enzymes, and the role of metal ions and organic solvents on the halophilic enzymes. In addition, molecular modification and the application of halophilic enzymes were introduced.

Keywords: halophilic enzyme, characteristics, structure, halophilic mechanism, application

(本文责编：张晓丽)

Supported by the National Natural Science Foundation of China (31670069, 31170068)
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Received: 29 March 2017; Revised: 17 May 2017; Published online: 25 May 2017