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·综 述·

蛋白质聚集的三种途径和控制策略

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摘 要：蛋白质聚集在生物医药生产中是一个关键问题。在蛋白质的生产、运输和储存的过程中，多种因素都能导致蛋白质发生聚集。随着对蛋白质聚集这一现象的深入研究，发现蛋白质聚集的产生存在不同途径和各种影响因素，如理化因素、翻译修饰和蛋白质结构等。由于蛋白质的聚集对于蛋白质的活性和均一性具有重大影响，因此了解蛋白质聚集的途径以及研究如何控制聚集对获得均质蛋白是十分有意义的。本文主要阐述了 3D 结构域交换、盐桥的形成、氧化应激 3 种蛋白质的聚集途径，以及在蛋白质生产、运输、储存过程中控制蛋白质聚集的方法，这有助于减少由于蛋白质聚集体形成而造成的损失，并提高实验研究和商业生产中的蛋白质纯度和均质性。

关键词：蛋白质聚集；3D 结构域交换；盐桥；氧化应激；控制

Three ways for protein aggregation and the control strategies

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Abstract: Protein aggregation is a critical issue in the production of biopharmaceuticals. During protein production, transport and storage, various factors can lead to protein aggregation. With the in-depth study, different ways of protein aggregation and various influencing factors were identified. This includes physical and chemical factors, translation modifications and protein structure. Since protein aggregation exerts major impact on the activity and homogeneity of

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proteins, it is of great importance to study the ways of protein aggregation and how to control it to obtain high-quality proteins. The review focuses on three ways of protein aggregation, namely 3D domain swapping, salt bridge formation, and oxidative stress, as well as methods to control protein aggregation during protein production, transport and storage. This may facilitate reducing the loss caused by the formation of protein aggregation and improving the purity and homogeneity of protein in research and commercial production.

Keywords: protein aggregation; 3D domain swapping; salt bridge; oxidative stress; control

蛋白质聚集在生物医药生产中是一个关键问题。蛋白质聚集不仅会导致蛋白质在生产过程中收获率的降低，还会对蛋白质功能产生影响。在蛋白质的生产、运输和储存过程中引起蛋白质发生聚集的因素有很多，如热诱导、光胁迫、氧诱导、剪切诱导、表面活性剂、抑制剂、pH、金属离子、离子强度、表面电荷、二硫键、蛋白质浓度、蛋白质疏水性、蛋白质结构域[1]、蛋白质残基、蛋白质糖基化和磷酸化等。因此，了解蛋白质聚集的途径、影响因素和控制蛋白质聚集是十分必要的。

不同的因素以不同的作用形式和途径对蛋白质的聚集产生影响。例如热诱导和蛋白质浓度分别通过改变共价键的结合和静电作用力形式，从而导致蛋白质聚集倾向增加；表面活性剂十二烷基磷酸胆碱通过与 2 个膜蛋白(PagP)分子的极性顶端表面结合的方式介导了 PagP 折叠的非特异性聚集[2]；pH 的变化会改变蛋白质分子中酸、碱性氨基酸的解离程度，影响氢键、离子键的形成和稳定，从而导致蛋白质展开或部分展开促进蛋白质的聚集[3]。在蛋白质结构影响其聚集的研究中，发现一些特别的结构、区域以及修饰会对聚集产生影响，如细胞色素(cytochrome C, Cyt c)的“hot-spot” Met80 区域[4]、单克隆抗体的聚集倾向区(aggregation-prone regions, APRs)[5]及游离的 HC 链[6]、“TIM barrel-folding”的桶状结构[7]、N-末端的缺失、游离的硫醇基。尤其是蛋白质中的游离的硫醇基很容易形成共价键结合的二聚体。而且蛋白质中的半胱氨酸残基对以共价键形式的蛋白质聚集也十分重要[8]。除了上述结构外，一级结构中氨基酸的突变会对其聚集产生影响。在胰岛素二聚体的疏水腔中有一个 Phe24 残基，研究发现该残基被甘氨酸或丙氨酸取代产生的胰岛素二聚体稳定性降低[9]。此外，在蛋白质翻译过程中，蛋白质的谷胱甘肽化改变了蛋白质的四级结构，使蛋白聚体从十聚体变为更小的低聚体，而蛋白质的脱酰胺化则降低因 3D 结构域交换而形成的聚体[10-11]。而且具有较高翻译特点的蛋白质因其容易发生未折叠蛋白质反应导致聚集[12]。

目前关于蛋白质聚集的问题，有多种应用方法可以解决。一些表面活性剂(如甘油)可以通过抑制蛋白质展开和稳定部分展开中间体来阻止蛋白质的聚集[13]；十二烷基硫酸钠(sodium dodecyl sulfate, SDS)、正烷基硫酸钠、N-烷基吡喃糖苷可以在亚胶束浓度和胶束浓度下的蛋白质减少其聚集[14]。其中 SDS 除了能减少聚集还被发现能诱导蛋白质的聚集，这种现象和所在溶液的 pH 相关。在表面活性剂的研究中表明，阴离子、阳离子表面活性剂在蛋白表面分布不同，推测可能是亲水性基团和烷基链对蛋白质表面的疏水裂缝的填充程度引起[14]，因此选择合适的表面活性剂抑制聚集也是需要考虑的。一些氨基酸和其他一些有机物也被用作抑制剂来抑制蛋白质的聚集，如精氨酸[15]、聚精

氨酸及其类似物聚鸟氨酸[16]、L-脯氨酸、L-异亮氨酸[17]、芳香萘醌-色氨酸杂交分子[18]、维生素 K3。在病理性淀粉样蛋白质聚集的研究中，黄酮类[19]、原花青素 B[20]、叔丁醇咪唑类离子液体[21]、高牛磺酸(homotaurine)[22]可以抑制 Aβ 淀粉样蛋白聚集。另有研究表明蛋白质的聚集和细胞代谢调控有联系，其中提到这种聚集依赖其他酶的作用，因此使用该酶的抑制剂便可以减少蛋白质的聚集[23]。

综上所述，不同的因素能够引起蛋白质聚集且聚集方式存在不同[15,24-25]，也会导致聚集途径中不同结构的中间体出现[26]。而且同一种蛋白也会以共价键和非共价键 2 种形式的聚集体同时存在[27]。甚至在一些研究中某些蛋白质单体和自身突变体的异源二聚体会降低突变体的聚集倾向，并且具有单体的活性[28]。所以蛋白质聚集的一系列过程是复杂的，且目前没有显著方法解决该问题。因此了解蛋白质聚集的途径和影响因素从而进行抑制，减少蛋白质生物医药生产中的损失是关键的问题。因此，本文综述了蛋白质的 3 种聚集途径和蛋白质聚集的控制策略，并且展望了抑制蛋白质聚集的 3 个方向：(1) 基于生物信息和计算机的计算，预测潜在的易于聚集的氨基酸序列和结构用于指导减少蛋白质聚集；(2) 稳定二聚体的结构防止其形成低聚体或者多聚体从而进一步形成纤维状体；(3) 抑制蛋白质聚集潜在的化学分子的发现和应用。

1 蛋白质聚集的途径

1.1 3D 结构域交换在蛋白质聚集中的作用

3D 结构域交换(3D domain swapping)是部分未折叠的 2 个或 2 个以上的单体蛋白质发生交换结构域的一种现象，且在理论上是所有蛋白质的一种普遍现象并且存在一定的可逆性[29-31]。3D 结构域交换首次在牛胰腺核糖核酸酶(图 1)的研究中提出，并且在后续的研究中被观察到[32-34]。在白喉毒素二聚体的研究中，3D 结构域交换最终得到证实[33]。3D 结构域交换常发生在蛋白质的 N-末端或者 C-末端，也会同时发生在 N、C-末端[34-35]。结构交换产生的聚体一般由蛋白质主体结构、铰链环、交换区

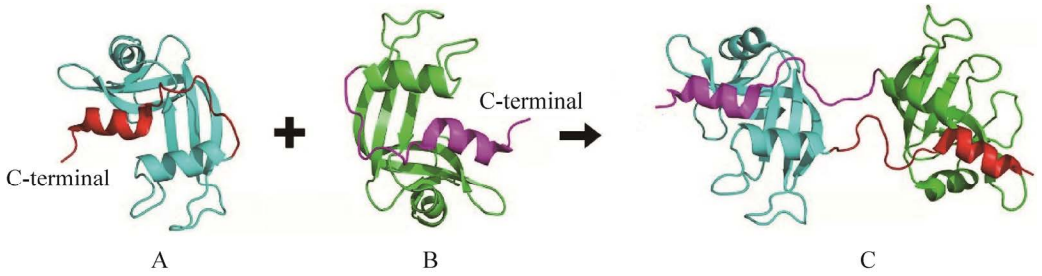

图 1 牛胰腺核糖核酸酶通过 3D 结构域形成二聚体的结构图[32-34]

Figure 1 Structure diagram of bovine pancreatic ribonuclease dimerization via 3D domain swapping[32-34]. The red and purple parts in monomers (A) and (B) represent the C-terminal exchanged structural region and hinge region of bovine pancreatic ribonuclease 3D domain exchange, and the exchange results are shown in (C). A and B, Method: X-ray diffraction; Resolution: 1.95 Å; PDB ID: 4RTE; https://www.rcsb.org/structure/4rte). C, Method: X-ray diffraction; Resolution: 1.99 Å; PDB ID; 3FKZ https://www.rcsb.org/structure/3fkz. The red and purple parts in A, B, and C are colored with Pymol 2.5.

构成，进而形成线性或者闭合环状聚体[36-37]。铰链环在 3D 结构域交换中具有特殊作用，其通过延展、转向等构象变化后，进而连接交换的结构域完成 3D 结构域的交换。铰链环的长度、弹性、铰链环中的脯氨酸残基的存在及其附近残基突变都会影响 3D 结构域的交换[38-39]。因此，铰链环的可塑性导致形成不同的 3D 结构域交换多聚体[40]。而且铰链环的构象变化可能会导致蛋白质聚体形成疏水簇，从而进一步促进二聚体的形成[41]。另有研究表明铰链环中含有丙氨酸、脯氨酸和聚脯氨酸等高螺旋倾向性的氨基酸将增加结构域交换的趋势[42-44]。而且铰链环中金属离子结合残基位点的存在也会促进蛋白质形成金属蛋白寡聚体，如组氨酸会和金属离子结合(Co^{2+}、Ni^{2+})[45]。

在已有影响 3D 结构域交换形成的聚集因素中，有研究表明乙醇的处理、冷冻干燥、盐缓冲溶液(离液序列高的和亲液序列高的盐)、pH、高浓度的蛋白质、蛋白质表面电荷也会促进蛋白质的结构域交换，并且各种环境因素介导 3D 结构域交换的作用形式也不相同[36,38,46-49]。

1.2 盐桥在蛋白质中聚体形成的作用

在以非共价键形成的蛋白质聚体的驱动力中(氢键、范德华力、离子相互作用、疏水相互作用)，盐桥(salt bridge)被认为是带相反电荷的残基(如 Asp、Glu、Tyr、Cys、His、Lys、Arg)之间的静电吸引力(图 2)[50]，主要由氢键和离子相互作用而成[51]。因此，一部分蛋白质因氨基酸残基形成链内盐桥导致聚体产生时易受到 pH 的影响[52]。水分子的参与、金属离子的结合都会对盐桥产生影响[53]，而且聚集体形成的链间盐桥比链内盐桥更稳定。盐桥的存在促进了“U”形肽的弯曲，进一步促进了平行结构的形成，从而促进了淀粉样原纤维形式的蛋白质聚集的形成[54]。在某些蛋白质中，盐桥参与了其构象状态的稳定(不同亚基之间的连接)和二聚体的形成[51,55-56]。盐桥在其所在的蛋白质结构位置中除了形成单独互相作用外，还存在

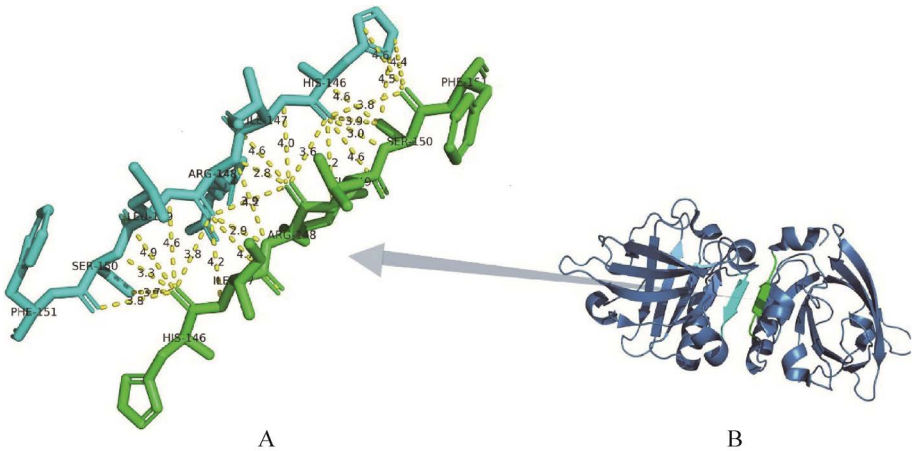

图 2 牛 β-乳球蛋白通过盐桥形成聚体[50]

Figure 2 Bovine β-lactoglobulin forms aggregates through salt bridges[50]. A: Salt bridge formed by negatively charged carboxyl (−CO) of His, Arg and Ser amino acids drawn with Pymol 2.5. The yellow dotted lines represent possible hydrogen bonds, and the numbers represent the interaction distances. B: A dimer of bovine β-lactoglobulin through a salt bridge. The blue and green parts represent the amino acid parts (His, Ile, Arg, Leu, Ser, Phe). Method: X-ray diffraction; Resolution: 3 Å; PDB ID: 2AKQ https://www.rcsb.org/structure/2AKQ).

不同位置的盐桥之间的复杂相互作用[57-58]。在MSH3 的研究中，其谷氨酸残基与 2 条链中的赖氨酸产生相互作用进而形成盐桥[59]。

盐桥很容易在内在无序性蛋白质(intrinsically disordered proteins, IDPs)中形成，因为其富含极性和大量带相反电荷的残基[60]。嗜热蛋白一般也含有较多的盐桥，以提高其热稳定性[58]。实验研究中要破坏盐桥形成的淀粉样蛋白的二聚化，则可以通过添加乙醇进行破坏抑制[61]。

1.3 氧化应激对蛋白质聚集的影响

氧化应激(oxidative stress)导致的蛋白质聚集，在体内和体外都有重大影响[62-64]。在神经细胞的研究中，线粒体受到抑制会导致活性氧的增加，促进蛋白质的氧化诱导聚集。在体外研究中，光照、辅料、冻干[65]等都有可能产生自由基或者加速蛋白质的氧化聚集。但是也有研究表明羟基自由基在诱导蛋白质聚集的影响很微小，反而空化导致的气泡表面积增加才是聚集的原因[66]。紫外辐射诱导的氧化应激通过将蛋白质中的甲硫氨酸氧化成甲硫氨酸-亚砜(met-sulfoxide)，影响 C-末端疏水簇和非淀粉样组分结构(non-amyloid components)之间的分子内相互作用以及扰动远程静电相互作用从而形成非纤维低聚物[64]。也有研究发现甲硫氨酸的氧化提高了 β-折叠的形成，促进了低聚物的产生[67]。并且发现 C-末端和 N-末端的甲硫氨酸的氧化难易存在显著差异[64]，推测可能与蛋白质中的甲硫氨酸暴露的位置和程度有关[67]。蛋白质中的硫醇基氧化形成以二硫键连接的聚体[68]，而且硫醇基、半胱氨酸以及甲硫氨酸的氧化可能会导致蛋白质发生剧烈的结构重排[69-70]。在一项研究中显示不同的蛋白质通过各自半胱氨酸氧化，可以形成以二硫键交联的异源二聚体[71]。因此，可以发现蛋白质氧化的位置常发生在甲硫氨酸和半胱氨酸 2 个残基上，且严重影响蛋白质的二级或三级结构。因此在富含甲硫氨酸和半胱氨酸蛋白质的处理上，要尽量避免氧化应激产生损害。而且不同的蛋白质也会表现出不同的氧敏感性，有些蛋白质很容易受到氧化应激的影响，形成细胞毒性聚集；有些则相反。

在抑制氧化应激引起的蛋白质聚集研究中，发现色氨酸、吡哆醇、酪氨酸或其同系物与其他抗氧化剂赋形剂结合，可有效保护治疗性蛋白质在氧化应激下免受聚集和氧化[74]。抗坏血酸、N-乙酰-L-半胱氨酸和 L-甲硫氨酸也可以作为抗氧化剂在保护蛋白质过程中使用[73]。

2 蛋白质聚集的控制方法

2.1 蛋白质结构和修饰的改造

已有研究发现，在关于蛋白质结构影响聚集的研究中，通过改变蛋白质结合区域(client-binding surface)的凹面表面静电势可以增强蛋白质的抗聚集能力[74]。Carballo-Amador 等[75]通过氨基酸定点突变改变蛋白质表面正电荷，从而提高蛋白质的溶解度，减缓蛋白质因浓度依赖的聚集，而且蛋白质表面正电荷区域的大小与蛋白质溶解度的关系也在其他实验中得到证明[76]。研究发现，通过模拟氨基酸定点突变改变该蛋白质的表面电势，降低了该蛋白质间因静电作用产生聚集体(结果未发表)。通过模拟 T27D 和 R131D 两个氨基酸位点的突变减少了 EPO 表面的正电荷，有潜在的可能提高其溶解度(图 3)。另有糖基化修饰不完整的糖链容易导致该蛋白质的聚集，而且在浓缩的过程中更容易产生聚体。可以通过对剪切应力、温度和保护剂浓度进行条件优化以减少聚集。

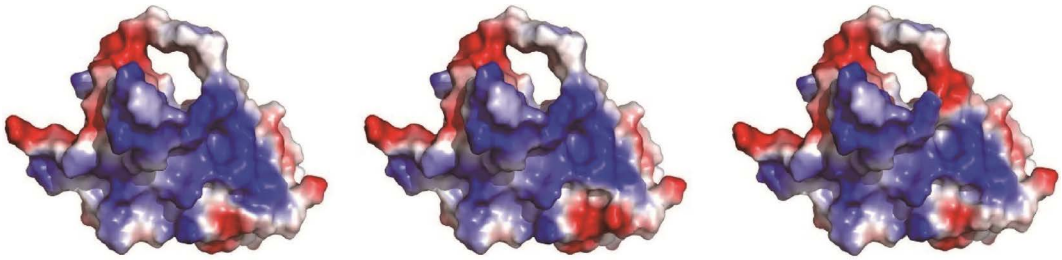

图 3 氨基酸定点突变导致 rhEPO 表面电荷的变化

Figure 3 Changes in the surface charge of rhEPO caused by amino acid site-directed mutagenesis. A: Non-mutant protein. B: Threonine mutation at position 27 to aspartic acid. C: Mutation of arginine at position 131 to aspartic acid. Positively charged patch area is reduced. Blue represents positive potential and red represents negative potential.

蛋白质糖基化修饰可以减少暴露的蛋白质疏水区域、展开发生的概率以及降低对氧的敏感性[77]。除了蛋白质糖基化修饰以外，也有研究表明二聚体蛋白质表面磷酸化会减低二聚体的稳定性[78]。因此，基于结构设计一种特殊的肽可以和蛋白质的聚集性部分折叠中间体(partially folded intermediates, PFI)进行结合，以抑制蛋白质早期聚集成核阶段[79]。研究发现聚脯氨酸-精氨酸(poly-proline-arginine, poly-PR)和聚甘氨酸-精氨酸(poly-glycine-arginine, poly-GR)的二肽重复序列，因为亲水的精氨酸残基的存在而相对不利于蛋白质形成聚体[80]。因此也可以尝试蛋白质中设计上述二肽重复序列以减少蛋白质聚集。

2.2 蛋白质生产和运输过程中聚集的控制

在培养基中，添加 $CuSO_4$ 可使游离醇含量降低，促进二硫键的生成，减少以共价键形成的蛋白质聚体[81]。在细胞培养过程中，细胞死亡后，内源性糖苷酶释放到培养基中可能会使培养基中的糖蛋白发生去糖基化，促使培养基中的蛋白质形成二聚体或多聚体[82]。可以通过添加糖苷酶的抑制剂或者采用灌流培养的方式减少糖苷酶的浓度。而且有研究发现，灌注培养的方式可以减轻线粒体功能障碍和内质网应激，从而改变了谷胱甘肽的还原型和氧化型的比例，减缓了蛋白的聚集[83]。

超滤浓缩前的搅拌及其引起的空化使蛋白质在气液界面暴露其疏水区域进而发生聚集[15,84]。在超滤浓缩过程中因为膜表面浓度高于原溶液浓度[85]而出现的“浓度极化(concentration polarization)”的现象(图 4)，进而容易产生浓度依赖性的聚集。而且机械应力和水流切向力导致蛋白质聚集且在溶液中聚集分布不均[81,87-87]。而在以透析获取蛋白质的方法中，因为唐南效应导致溶液 pH 值出现很大偏移，也会引起蛋白质的聚集[77]。因此，在蛋白质浓缩过程中，目标蛋白质和杂蛋白质在膜表面或膜内部形成的聚集不仅降低了膜的通量还增强了蛋白质间的短程静电吸引导致更严重的聚集[88]。解决上述问题可以通过研发具有不同结构的超滤膜、膜转化改性以及通过添加表面活性剂减少蛋白质颗粒的形成[89]。在色谱层析过程中，蛋白质的饱和结合导致局部层析柱表面蛋白质浓度升高也会引起聚集。精氨酸因为可以削弱蛋白质的结合作用而成为常用的洗脱保护剂[90]。而且一项研究表明，在流动相中添加钠基盐和钾基盐会对特定蛋白质产生影响[91]。因此对于特定蛋白的流动相成分的选择需要认真研究。

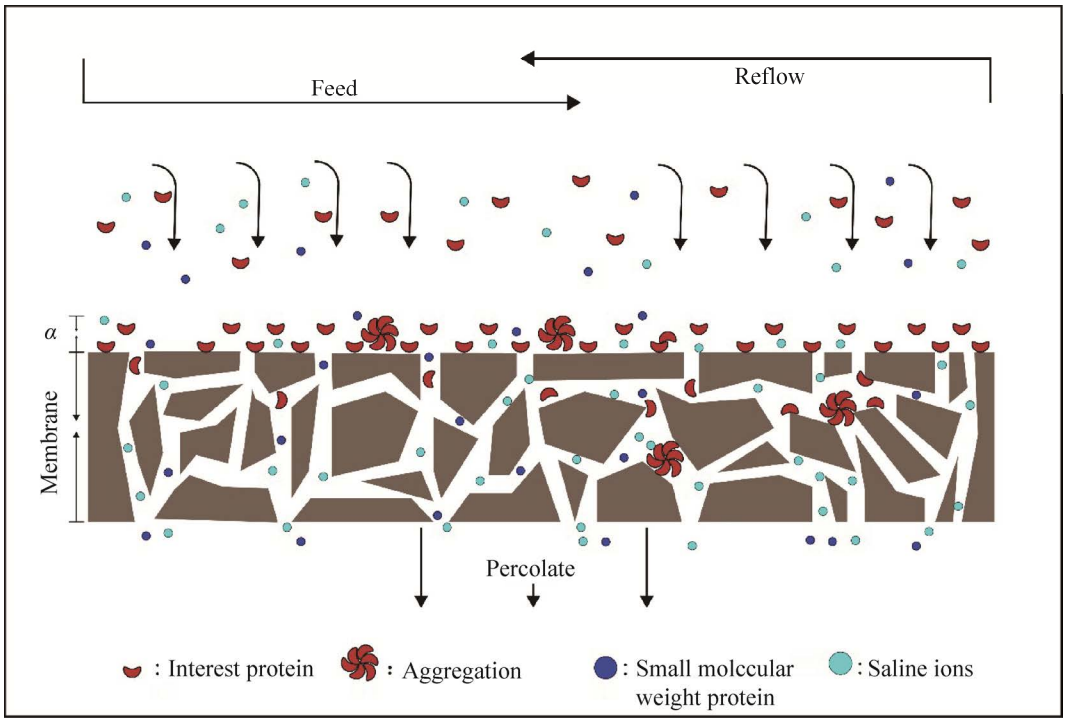

图 4　蛋白质在超滤浓缩过程中局部浓度依赖性产生的极化现象

Figure 4　Local concentration-dependent aggregation of proteins during ultrafiltration concentration. During the ultrafiltration concentration process of proteins, the surface protein concentration on the membrane surface (α) will be higher than that in the bulk solution, resulting in concentration-dependent aggregation of proteins on the membrane surface, causing membrane blockage and reduced membrane flux, further leading to the formation of aggregates.

在冻融胁迫条件下，可以使用甜菜碱稳定并抑制蛋白质的聚集，但是这种抑制是在低浓度蛋白质下发挥作用[92]；甘油通过抑制蛋白质展开和稳定部分展开中间体来阻止蛋白质的聚集；添加高浓度的组氨酸可以屏蔽蛋白质表面电荷，减少远程和短程静电力以抑制聚集[93]。

在运输保存的过程中，为提高蛋白质产品的储存时间，有时会需要添加抗菌防腐剂，如间甲酚、苯酚、苯甲醇、苯氧乙醇、氯丁醇。然而这些防腐剂也被证明可以导致蛋白质局部结构失稳引起聚集[94]。在以玻璃瓶、不锈钢等其他容器储存蛋白质时，甘露醇、甲硫氨酸、泊洛沙姆 188 以及吐温等赋形剂或表面活性剂通过降低水面张力减缓蛋白质在容器表面的吸附和聚集趋势[95]。而且当容器内部表面有液滴落在蛋白质溶液表面时，会产生空化气泡并随后坍塌，在坍塌的同时会在局部产生了较高的温度和压力[96-97]，导致蛋白质发生聚集。这种现象可以通过添加聚山梨酯 80 降低溶液表面张力，减少蛋白质的聚集[84]。但是一些研究发现，聚山梨酯 80 和蛋白质疏水表面相互作用在一定的化学计量比下发挥作用[98]。对于一些蛋白质二聚体，使用适当浓度的乙醇溶液进行抑制的效果更好[99]，因为乙醇作为一种两亲性溶剂可以和蛋白质分子的疏水残基和极性残基发生显著作用，从而改变表面自由能增加聚体解离概率。在一项关于稳定促红细胞生成素的研究中，使用甘氨酸、谷氨酸和吐温 20 组合降低

了其产生聚集和降解[100]。因此可以添加不同的中性、酸性、碱性氨基酸组合以稳定对环境胁迫较敏感的蛋白质聚集以及保持蛋白质的活性。除了上述常见的抑制剂，各种具有抑制蛋白质聚集的分子和材料被发现和应用，如一种半胱氨酸反应小分子 ebselen 能够有效地促进含有二硫键的形成从而减少共价键形成的聚集体[101]；吲哚衍生物和乙二醛对蛋白质进行修饰，起到了稳定蛋白质构象作用[102]；化学伴侣 4-PBA 可以剂量依赖性地缓解蛋白质的聚集[103]；两性离子聚合物的纳米凝胶可以保护蛋白质的高级结构抑制由热诱导引起的蛋白质聚集[104]。

3 总结与展望

蛋白质的聚集在生物医药研发和生产过程中是关键的问题。因为在整个治疗性蛋白质的研发和生产中，蛋白质的纯度和均一性表征是受到严格要求和检测的。因此，解决蛋白质的聚集问题具有重要意义。在生物医药生产中，由于工艺周期较长，容易出现多种因素导致目标蛋白质发生聚集。因此，解决蛋白质的聚集问题需要考虑到多种因素的影响。各学科交叉的研究也为蛋白质聚集研究提供新的角度和方向，如基于生物信息和计算机的计算可以预测并了解蛋白质易于聚集的序列和结构信息，用于指导减少蛋白质聚集的理论和方法研究[105]。因此，通过计算机计算和氨基酸定点突变改变蛋白质疏水性、静电斥力和主链氢键的相互作用，从而干扰或抑制蛋白质聚集以及 3D 结构域形成是很有潜力的[106]。在淀粉样成纤维蛋白聚集的过程中，成核过程是该类型聚集的初始阶段。如果可以稳定二聚体的结构防止其形成低聚体或者多聚体从而进一步形成纤维状体[107]也会是潜在的方向。近些年，潜在的抑制剂化学分子的发现和应用也会促进蛋白质聚集问题的解决。

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