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Review 综 述

微藻葡萄糖磷酸变位酶研究进展

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摘　要：葡萄糖-1-磷酸是光自养生物淀粉合成的前体物质。葡萄糖磷酸变位酶(phosphoglucomutase, PGM)属于磷酸己糖变位酶家族，具有较高的保守性，能介导葡萄糖-6-磷酸与葡萄糖-1-磷酸相互转化，调节植物和藻类细胞淀粉合成。与高等植物相比，微藻具有独特的光合系统，一些微藻藻株可以利用有机碳源进行异养培养或混养培养，这可能赋予微藻葡萄糖磷酸变位酶特殊的结构和淀粉代谢功能，调节微藻光合固碳、糖类代谢等通路的水平。本文综述了微藻 PGM 分子特性、生物学功能和调控 PGM 活性潜在机制及策略，阐明 PGM 调节微藻淀粉合成对胞内蛋白质、油脂等代谢通路的潜在影响机制，为微藻固碳和高值化开发微藻资源提供理论依据，助力我国“双碳”目标奠定理论基础。

关键词：葡萄糖磷酸变位酶；微藻；淀粉合成

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Research progress of phosphoglucomutases from microalgae

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Abstract: Glucose-1-phosphate is a key precursor for starch biosynthesis of photoautotrophs. Phosphoglucomutases (PGMs) belonging to the phosphohexomutase family have a high conserved characteristic and perform the interconversion between glucose-6-phosphate and glucose-1-phosphate to regulate the starch biosynthesis. Compared with the higher plants, microalgae possess unique photosynthetic systems. Additionally, some microalgae strains can utilize organic carbon sources to produce valuable biomass by heterotrophic or mixotrophic cultivation, which might endow PGMs with specific structural features and biological functions in starch metabolism to regulate the levels of carbon fixation by photosynthesis, carbohydrate metabolism, and other pathways in microalgae. This article summarizes the molecular characteristics, functions, and activity regulation of PGMs for microalgae. Moreover, this article elucidates the potential mechanisms by which PGMs regulate microalgae starch synthesis to influence intracellular protein and lipid metabolic pathways. This review lays a theoretical foundation for microalgae carbon sequestration and the value-added utilization of microalgae resources, contributing to the achievement of China's "dual-carbon" goals.

Keywords: phosphoglucomutase; microalgae; starch synthesis

微藻是一类典型的光自养生物，可合成蛋白质、多糖、多不饱和脂肪酸等多种活性物质，被广泛应用于食品、工业、能源、环境等领域[1-3]。与高等植物相比，微藻具有生长速度快、生物量产量高、不竞争耕地等优点，受到食品、医疗、化工等行业科技工作者的普遍关注[4]。因此，利用微藻开发高附加值生物制品，对推动我国食品、饲料、保健品等行业的高质量发展具有重要意义。

近年来，多项研究表明在微藻生长过程中，淀粉等多糖的积累调控其机体蛋白质与脂质的合成水平[5-9]。光合自养生物淀粉合成途径中，CO_2 通过卡尔文循环，经过果糖-1,6-二磷酸酶、磷酸葡萄糖异构酶、葡萄糖磷酸变位酶(phosphoglucomutase, PGM, EC 5.4.2.2)等关键酶的转化合成淀粉[9-13]。葡萄糖磷酸变位酶属于磷酸己糖变位酶家族，序列保守性高，对光合生物固碳和目的产物合成的调节起着至关重要的作用[10,14-16]。当前，关于高等植物 PGM 调节淀粉合成、蔗糖代谢、固定 CO_2 和植物生长发育的研究较多[10-11,17-18]。与高等植物不同的是，微藻作为一类低等光合生物，拥有独特的光合系统和淀粉代谢通路。此外，有些微藻藻株，如普通小球藻(*Chlorella vulgaris*)等，可以利用有机碳源(如葡萄糖)进行异养培养和混养培养，调节 PGM 活性可影响细胞内淀粉合成和蛋白质、油脂、色素等高附加值产物的代谢水平[6,19-22]。因此，本文根据微藻 PGM 分子特性、功能、和调控 PGM 活性对藻类淀粉合成的影响，阐释藻类淀粉作为

细胞内碳源转化为高附加值产物的合成机制，总结藻类 PGM 的研究进展，为进一步明确藻类淀粉合成机制和调控微藻高附加值产物合成提供参考。

1 微藻葡萄糖磷酸变位酶的分子特性

光合生物 PGM 主要在淀粉合成组织中发挥作用[23]。在高等植物中，基于 PGM 酶的亚细胞定位可分为质体 PGM (plastidial phosphoglucomutase, pPGM)和胞质 PGM (cytosolic phosphoglucomutase, cPGM)[14]。Herbert 等[14]通过$(NH_4)_2SO_4$ 梯度和二乙氨乙基(deaecellulose, DEAE)-纤维素离子交换色谱分离方法，分析 C_3、C_4 与具景天酸代谢途径的植物和小球藻中的 PGM 分布形式时发现，除 C_4 植物外，C_3 植物细胞和小球藻细胞的质体和胞质中均有 PGM；C_4 植物 PGM 有 3 种同工酶，成熟的 PGM 蛋白分子量约为 60 kDa。Li 等[18]从羽衣甘蓝(*Brassica oleracea* var. *acephala*)柱头组织中分离到 PGM2 和 PGM3 两种具有葡萄糖磷酸变位酶活性的蛋白质，其 cDNA 序列大小分别为 1 922 bp 和 2 066 bp，分子预测结果显示羽衣甘蓝 PGM2 蛋白由 583 个氨基酸组成，相对分子质量为 63.4 kDa；PGM3 蛋白由 582 个氨基酸组成，相对分子质量为 63.3 kDa。王海波等[24]通过同源序列比对方法克隆得到小桐子(*Jatropha curcas* L.) cPGM 与 pPGM 基因的全长编码序列，cPGM 基因 mRNA 长度为 2 214 bp，编码 582 个氨基酸，其蛋白分子量为 63.2 kDa；pPGM 基因 mRNA 长度为 2 347 bp，编码 637 个氨基酸，其蛋白分子量为 69.5 kDa。

一般认为，植物 PGM 所含氨基酸数目为 600 个左右，分子量约为 60 kDa[25-26]。不同的是，藻类 PGM 编码基因序列长度为 1 632−3 216 bp，编码的氨基酸数为 543−1 071，蛋白相对分子质量为 58.6−115.3 kDa (表 1)。在已报道的藻类 PGM 中，蓝藻的 PGM 氨基酸总数最少，分子量最小；相反的是，褐藻的 PGM 氨基酸总数最多，分子量最大，为 115.3 kDa (表 1)。这可能的原因在于，褐藻是真核藻类中较高级的一个类群，均为多细胞体，蛋白结构复杂，而蓝藻为原核藻类，结构简单，无细胞器，进化程度低。

由表 1 可知，不同属的微藻 PGM 的氨基酸数目存在明显的差别。例如，绿藻 PGM 蛋白氨

表 1 微藻 PGM 蛋白分子性质

Table 1 Molecular properties of microalgae PGM proteins

Species	GenBank ID	CDS (bp)	Number of amino acids (aa)	Molecular weight (Da)	Isoelectric point	Hydrophilicity (GRAVY)	Subcellular localization	References
Chlorophyta								
Dunaliella salina	ADD25038.1	1 815	604	65 084.14	8.01	−0.226	Chloroplast	[27]
Klebsormidium nitens	GAQ78653.1	2 421	806	87 710.80	6.89	−0.190	Chloroplast	[28]
Klebsormidium nitens	GAQ91740.1	2 046	681	73 633.40	6.41	−0.119	Chloroplast	[28]
Micromonas pusilla	XP_003056074.1	1 803	600	64 792.99	4.97	−0.176	Chloroplast	[29]
Micromonas commoda	XP_002507519.1	1 728	575	62 170.11	5.08	−0.222	Chloroplast	[29]
Ostreococcus tauri	XP_003083406.1	1 680	559	60 461.36	4.93	−0.179	Chloroplast	[30]
Scenedesmus sp.	KAF6254198.1	1 809	602	64 633.81	5.88	−0.097	Chloroplast	[31]
Chlorella sorokiniana	PRW59596.1	1 875	624	67 218.24	6.49	−0.187	Chloroplast	[32]

(待续)

(续表 1)

Species	GenBank ID	CDS (bp)	Number of amino acids (aa)	Molecular weight (Da)	Isoelectric point	Hydrophilicity (GRAVY)	Subcellular localization	References
Monoraphidium minutum	KAI8462587.1	1 875	598	63 782.74	7.09	−0.101	Chloroplast	[33]
Raphidocelis subcapitata	GBF95149.1	1 806	601	63 735.44	6.30	−0.044	Chloroplast	[34]
Auxenochlorella protothecoides	XP_011399073.1	1 698	565	61 191.86	5.13	−0.190	Chloroplast	[35]
Nannochloropsis gaditana	EWM21792.1	3 210	1 069	115 173.24	6.02	−0.126	Chloroplast	[36]
Polytomella parva	QKY14898.1	1 674	557	60 447.55	6.08	−0.205	Chloroplast	[37]
Cyanobacteria								
Xenococcaceae cyanobacterium	MDJ0573558.1	1 632	543	59 293.04	5.20	−0.216	Cytoplasm	[38]
Trichodesmium sp.	MDJ0518457.1	1 635	544	59 194.48	4.90	−0.305	Cytoplasm	[38]
Calothrix sp.	MDJ0621091.1	1 635	544	58 992.64	5.28	−0.200	Cytoplasm	[38]
Leptolyngbya sp.	PZV16368.1	1 635	544	58 760.67	4.87	−0.249	Cytoplasm	[39]
Shackletoniella antarctica	PZO44876.1	1 635	544	58 685.69	4.98	−0.233	Cytoplasm	[39]
Leptolyngbya sp.	PZU97200.1	1 632	543	58 601.42	4.98	−0.261	Cytoplasm	[39]
Oscillatoriales sp.	HIK31139.1	1 632	543	59 034.22	4.87	−0.237	Cytoplasm	[40]
Leptolyngbyaceae sp.	HIK46815.1	1 635	544	58 989.48	5.26	−0.179	Cytoplasm	[40]
Nodosilinea sp.	MBW4461093.1	1 635	544	58 639.38	4.88	−0.245	Cytoplasm	[41]
Trichodesmium erythraeum	MDT9339139.1	1 635	544	59 349.01	5.19	−0.284	Cytoplasm	[42]
Trichodesmium sp.	MDE5092974.1	1 635	544	59 403.05	5.20	−0.297	Cytoplasm	[43]
Hydrococcus sp.	NJP19473.1	1 632	543	59 383.86	5.33	−0.272	Cytoplasm	[44]
Phormidesmis sp.	NJM98782.1	1 632	543	58 781.89	4.71	−0.235	Cytoplasm	[44]
Trichodesmium sp.	MCH2048694.1	1 635	544	59 420.10	5.26	−0.301	Cytoplasm	[45]
Trichodesmium erythraeum	MBS9770748.1	1 635	544	59 334.99	5.20	−0.294	Cytoplasm	[46]
Pseudanabaena sp.	MCA6612058.1	1 635	544	59 268.60	5.16	−0.221	Cytoplasm	[47]
Tolypothrix tenuis	BAY99341.1	1 641	546	59 603.03	4.94	−0.238	Cytoplasm	[48]
Phormidesmis priestleyi Ana	KPQ33795.1	1 632	543	58 641.77	4.78	−0.250	Cytoplasm	[49]
Rhodophyta								
Porphyridium purpureum	KAA8499052.1	1 743	580	62 222.64	5.13	−0.115	Chloroplast	[50]
Gracilaria domingensis	KAI0560001.1	1 755	584	62 912.43	4.83	−0.211	Chloroplast	[51]
Chondrus crispus	XP_005717288.1	1 755	584	62 703.72	5.18	−0.147	Chloroplast	[52]
Diatoms								
Fistulifera solaris	GAX13287.1	3 180	1 059	114 682.51	4.97	−0.186	Chloroplast	[53]
Fistulifera solaris	GAX25814.1	3 180	1 059	114 659.54	4.97	−0.181	Chloroplast	[53]
Phaeodactylum tricornutum	XP_002185375.1	3 174	1 057	114 608.54	5.11	−0.185	Chloroplast	[54]
Chaetoceros tenuissimus	GFH52288.1	3 177	1 058	114 032.03	4.97	−0.118	Chloroplast	[55]
Phaeophyta								
Saccharina japonica	AIQ80989.1	3 216	1 071	115 362.10	5.19	−0.186	Chloroplast	[56]

Source of sequence information: NCBI. Protein molecular properties: Molecular weight, Isoelectric point, and hydrophilicity (GRAVY) date source from Expasy-ProtParam tool. GRAVY: Grand average of hydropathicity, a higher positive value indicates stronger hydrophobicity, while a higher negative GRAVY value indicates better hydrophilicity. Subcellular localization was analyzed by Plant-mPLoc.

基酸数目在 557−1 069 之间，蓝藻 PGM 蛋白氨基酸数目在 543−546 之间，红藻 PGM 蛋白氨基酸数目为 580−584 之间，褐藻与硅藻的 PGM 蛋白氨基酸数目均大于 1 000 (表 1)。

关于微藻 PGM 的等电点，杜氏盐藻(*Dunaliella salina*) PGM 为碱性蛋白(pI=8.01)[27]，其余藻类PGM均为酸性蛋白(表1)，这与高等植物 PGM 特性相似[57-58]。亚细胞定位显示，真核藻类 PGM 蛋白均定位于叶绿体中(表 1)，蓝藻 PGM 分布在胞质中[59-60]。这些结果可表明，真核藻类合成淀粉与高等植物相同，主要在叶绿体中进行。

使用 TMHMM-2.0 和 SignalP-5.0 在线分析分别预测微藻 PGM 跨膜结构和信号肽结构，结果表明微藻 PGM 普遍为亲水蛋白且无跨膜结构(表 2)。这与曹丽等[58]研究的拟南芥(*Arabidopsis thaliana*)和水稻(*Oryza sativa* L.)的 pPGM 与 cPGM 结果相似。尽管微藻 PGM 在等电点、亲水性、跨膜结构特性上相差不大，但物种间 PGM 氨基酸数和分子量有较大差异，这表明微藻 PGM 在物种内保守性较高，但在种间仍有差异。PGM 二级结构比例上，微藻 PGM 的二级结构中 α-螺旋与无规卷曲占比最高，分别为 32.96%−38.86%和 37.32%−44.07%，而 β-折叠与延长链结构相对较少(表 2)。

为了阐明微藻 PGM 序列的进化关系，以源于拟南芥的 3 个 PGM 蛋白序列[pPGM (NP_19995.1)和 cPGM (NP_177230.1 和 NP_173732.1)]为 BLASTp 模板，选择绿藻门、蓝藻门、红藻门等 6 个门类的微藻 PGM 蛋白，构建了微藻 PGM 系统发育树及其保守结构域编码区模型(图 1)。多序列比对 39 个微藻 PGM 蛋白序列，结果显示微藻 PGM 系统发育树可为 3 个分支：(1) 在第一分支中，蓝藻门单独聚为一支，可能是由于蓝藻属于原核生物，无成熟的细胞器且进化程度低，在 PGM 进化上差异较小，这表明微藻 PGM 在进化保守的同时，真核藻类与原核藻类的 PGM 进化是存在差异的(图 1)；(2) 在第二分支中，红藻门与绿藻门聚为一支，PGM 进化存在亲缘关系(分支节点自展值 100)，推测 2 个物种 PGM 进化上存在一个共同祖先，其基因组在物种分化过程中发生了基因丢失或突变导致了 PGM 的区别；(3) 在第三分支中，褐藻门、硅藻门、绿藻门中的山蜡梅克里藻(*Klebsormidium nitens*, GAQ78653.1)以及微拟球藻(*Nannochloropsis gaditana*, EWM21792.1)共聚为一支，这些微藻 PGM 蛋白都有共同特征，即在 N 端上游具备独特的保守基序，例如：山蜡梅克里藻(*Klebsormidium nitens*, GAQ78653.1)的 DNA 断裂-重新连接酶保守基序属于 DNA_BRE_C 超家族，主要功能为通过酪氨酸的亲核攻击切割 DNA 双链，以产生 3′-磷酸酪氨酸蛋白-DNA 复合物；褐藻门、硅藻门、微拟球藻(*Nannochloropsis gaditana*, EWM21792.1) PGM 蛋白基序的N端上游存在UTP-葡萄糖-1-磷酸尿苷酸转移酶功能区间，主要负责催化 MgUTP+葡萄糖-1-磷酸和 UDP-葡萄糖+MgPPi 的相互转化(图 1 和表 3)。这些酶虽然也具备 PGM 的保守结构域，但其 N 端上游还具备其他功能的结构。这意味着：(1) 这些酶有可能属于复合蛋白[61]；(2) 在进化的过程中，这些藻类 PGM 编码序列在基因组上可能发生了位移，导致与其他蛋白的编码基因发生了连锁表达[62]。系统发育树和保守结构域预测分析显示，微藻 PGM 蛋白保守结构域氨基酸数目为 539−598，均属于磷酸己糖变位酶超家族成员(cl38939) (表 3)，催化糖底物上可逆的分子内磷酰基转移。

表 2　微藻 PGM 蛋白二级结构和信号肽预测

Table 2　Secondary structure and signal peptide prediction of microalgae PGM proteins

GenBank ID	α-helix		Extended strand		β-turn		Random coil		Signal peptide	Secretory protein
	Amino acids number (aa)	Proportion (%)	Amino acids number (aa)	Proportion (%)	Amino acids number (aa)	Proportion (%)	Amino acids number (aa)	Proportion (%)		
Chlorophyta										
ADD25038.1	211	34.93	97	16.06	38	6.29	258	42.72	0.001 0	No
GAQ78653.1	289	35.86	132	16.38	62	7.69	323	40.07	0.001 4	No
GAQ91740.1	239	35.10	118	17.33	36	5.29	288	42.29	0.000 6	No
XP_003056074.1	212	35.33	97	16.17	42	7.00	249	41.50	0.001 3	No
XP_002507519.1	209	36.35	97	16.87	44	7.65	225	39.13	0.023 5	No
XP_003083406.1	203	36.31	96	17.17	41	7.33	219	39.18	0.000 8	No
KAF6254198.1	208	34.55	103	17.11	41	6.81	250	41.53	0.001 9	No
PRW59596.1	220	35.26	91	14.58	38	6.09	275	44.07	0.002 6	No
KAI8462587.1	221	36.96	100	16.72	43	7.19	234	39.13	0.001 9	No
GBF95149.1	227	37.77	99	16.47	41	6.82	234	38.94	0.002 5	No
XP_011399073.1	207	36.64	100	17.70	39	6.90	219	38.76	0.000 7	No
EWM21792.1	366	34.24	194	18.15	97	9.07	412	38.54	0.000 9	No
QKY14898.1	201	36.09	93	16.70	42	7.54	221	39.68	0.001 8	No
Cyanobacteria										
MDJ0573558.1	202	37.20	97	17.86	36	6.63	208	38.31	0.016 2	No
MDJ0518457.1	206	37.87	97	17.83	38	6.99	203	37.32	0.009 3	No
MDJ0621091.1	197	36.21	94	17.28	41	7.54	212	38.97	0.010 4	No
PZV16368.1	200	36.76	87	15.99	37	6.80	220	40.44	0.012 2	No
PZO44876.1	203	37.32	86	15.81	39	7.17	216	39.71	0.012 2	No
PZU97200.1	211	38.86	85	15.65	36	6.63	211	38.86	0.013 1	No
HIK31139.1	196	36.10	101	18.60	35	6.45	211	38.86	0.010 5	No
HIK46815.1	204	37.50	94	17.28	37	6.80	209	38.42	0.012 2	No
MBW4461093.1	197	36.21	99	18.20	35	6.43	213	39.15	0.012 5	No
MDT9339139.1	196	36.03	97	17.83	33	6.07	218	40.07	0.005 0	No
MDE5092974.1	194	35.66	98	18.01	36	6.62	216	39.71	0.005 0	No
NJP19473.1	198	36.46	97	17.86	36	6.63	212	39.04	0.011 4	No
NJM98782.1	197	36.28	102	18.78	37	6.81	207	38.12	0.011 3	No
MCH2048694.1	202	37.13	97	17.83	36	6.62	209	38.42	0.005 0	No
MBS9770748.1	200	36.76	100	18.38	38	6.99	206	37.87	0.005 0	No
MCA6612058.1	195	35.85	99	18.20	41	7.54	209	38.42	0.024 8	No
BAY99341.1	197	36.08	100	18.32	39	7.14	210	38.46	0.003 6	No
KPQ33795.1	199	36.65	98	18.05	37	6.81	209	38.49	0.013 6	No
Rhodophyta										
KAA8499052.1	204	35.17	96	16.55	42	7.24	238	41.03	0.003 5	No
KAI0560001.1	204	34.93	99	16.95	37	6.34	244	41.78	0.003 4	No
XP_005717288.1	201	34.42	97	16.61	43	7.36	243	41.61	0.001 3	No
Diatoms										
GAX13287.1	374	35.32	175	16.53	85	8.03	425	40.13	0.001 0	No
GAX25814.1	378	35.69	175	16.53	95	8.97	411	38.81	0.001 0	No
XP_002185375.1	356	33.68	180	17.03	93	8.80	428	40.49	0.002 1	No
GFH52288.1	371	35.07	184	17.39	81	7.66	422	39.89	0.001 7	No
Phaeophyta										
AIQ80989.1	353	32.96	190	17.74	102	9.52	426	39.78	0.000 5	No

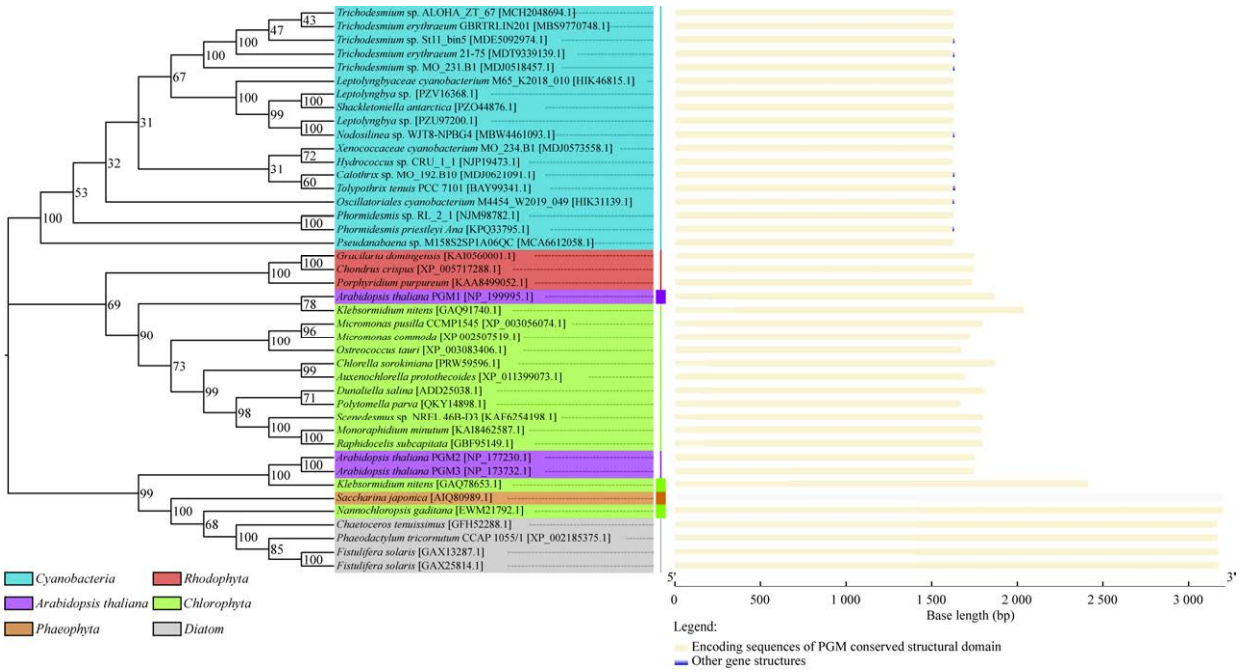

图 1　微藻 PGM 蛋白系统发育树和蛋白质保守结构域编码序列分析

Figure 1　Microalgae PGM protein system phylogenetic tree and conserved domain coding sequence analysis. Phylogenetic tree was constructed using the neighbour-joining algorithm in MEGA 11 with 1 000 bootstrap replicates, and the number on the branch represents bootstrap calculated after 1 000 repetitions. The accession number of the corresponding gene was shown in parentheses. The conservative structural domain model was constructed based on the predictions from the NCBI Conserved Domain Search (Table 3); Search against database: CDD v3.21-62456 PSSMs.

2　微藻葡萄糖磷酸变位酶的功能

光合生物 PGM 参与的代谢途径如图 2 所示，PGM 是光合生物合成碳水化合物途径中重要的调节酶，催化葡萄糖-6-磷酸与葡萄糖-1-磷酸之间的可逆转化，被认为能够调控磷酸己糖之间的相互平衡，形成的葡萄糖-6-磷酸产物分别被戊糖磷酸途径和糖酵解途径进一步代谢[10]。

在植物中，cPGM 对蔗糖代谢、细胞壁合成、植株生长发育等方面起着至关重要的作用[12,63]。当叶绿体无光合作用驱动的碳输出时，拟南芥叶片蔗糖的形成取决于 cPGM 的活性[63-64]。cPGM 催化生成的葡萄糖-1-磷酸是 UDP-葡萄糖焦磷酸化酶的底物 UDP-葡萄糖是细胞壁合成的主要原料[65]。Uematsu 等[11]通过植物过表达载体 pIG121 将拟南芥 cPGM 基因利用农杆菌转入烟草(*Nicotiana tabacum* cv. Xanthi)植株，cPGM 过表达品系 cPGM 活性比野生型植物高 2.1−3.4 倍，叶片淀粉含量下降至野生型的 60%−70%，但蔗糖含量未见明显差异。作者认为 cPGM 活性升高可能会刺激光合碳流向蔗糖，而蔗糖浓度的增加可能会提高蔗糖转运蛋白活性，进而使合成的蔗糖从叶片迅速转运到分生组织[11]。研究结果表明，马铃薯(*Solanum tuberosum* cv. Desiree)降低 cPGM 的活性会明显影响光合作用的效率，转化植株的气体交换率与野生型对比降低了约 30%，CO_2 同化率明显降低，最终导致其转化植株淀粉含量显著低于野生型，蔗糖转运能力也显著下降[66]。在拟南芥植株发育过程中，缺失 cPGM 会严重影响植物的代谢并降低植物的适应性，甚至导致植物死亡[10]。

表 3 微藻 PGM 蛋白保守结构域预测

Table 3 Conserved domain prediction of microalgae PGM proteins

GenBank ID	PSSM-ID	Starting position of conservative structural domain	End position of conservative structural domain	*E*-value	Bit score	Short name	Superfamily
Chlorophyta							
ADD25038.1	177 942	47	604	0	1 047.32	PLN02307	cl38939
GAQ78653.1	177 942	222	806	0	1 059.65	PLN02307	cl38939
	469 662	56	211	4.47E−18	82.73	DNA_BRE_C superfamily	cl00213
GAQ91740.1	177 942	117	681	0	1 134.37	PLN02307	cl38939
XP_003056074.1	177 942	33	600	0	1 130.14	PLN02307	cl38939
XP_002507519.1	177 942	8	575	0	1 157.49	PLN02307	cl38939
XP_003083406.1	177 942	1	559	0	1 126.67	PLN02307	cl38939
KAF6254198.1	177 942	37	602	0	1 062.34	PLN02307	cl38939
PRW59596.1	177 942	62	624	0	1 034.61	PLN02307	cl38939
KAI8462587.1	177 942	39	598	0	1 018.43	PLN02307	cl38939
GBF95149.1	177 942	53	601	0	1 008.03	PLN02307	cl38939
XP_011399073.1	177 942	2	565	0	991.08	PLN02307	cl38939
EWM21792.1	476 822	471	1 069	0	960.65	Phosphohexomutase	cl38939
	460 300	28	442	7.12E−148	447.73	superfamily UDPGP	cl46593
QKY14898.1	177 942	1	557	0	1 054.64	PLN02307	cl38939
Cyanobacteria							
MDJ0573558.1	100 087	4	543	0	1 026.78	PGM1	cl38939
MDJ0518457.1	100 087	4	544	0	1 037.18	PGM1	cl38939
MDJ0621091.1	100 087	4	544	0	1 042.57	PGM1	cl38939
PZV16368.1	100 087	4	544	0	1 033.71	PGM1	cl38939
PZO44876.1	100 087	4	544	0	1 032.94	PGM1	cl38939
PZU97200.1	100 087	4	543	0	1 030.25	PGM1	cl38939
HIK31139.1	100 087	4	543	0	1 037.18	PGM1	cl38939
HIK46815.1	100 087	4	544	0	1 016.38	PGM1	cl38939
MBW4461093.1	100 087	4	544	0	1 025.62	PGM1	cl38939
MDT9339139.1	100 087	4	544	0	1 023.31	PGM1	cl38939
MDE5092974.1	100 087	4	544	0	1 021.39	PGM1	cl38939
NJP19473.1	100 087	4	543	0	1 032.56	PGM1	cl38939
NJM98782.1	100 087	4	543	0	1 024.08	PGM1	cl38939
MCH2048694.1	100 087	4	544	0	1 021.77	PGM1	cl38939
MBS9770748.1	100 087	4	544	0	1 017.15	PGM1	cl38939
MCA6612058.1	100 087	4	544	0	1 014.84	PGM1	cl38939
BAY99341.1	100 087	6	546	0	1 022.16	PGM1	cl38939
KPQ33795.1	100 087	4	543	0	1 036.41	PGM1	cl38939
Rhodophyta							
KAA8499052.1	100 087	7	580	0	966.69	PGM1	cl38939
KAI0560001.1	476 822	3	584	0	947.94	Phosphohexomutase superfamily	cl38939
XP_005717288.1	476 822	8	584	0	928.17	Phosphohexomutase superfamily	cl38939

(待续)

(续表 3)

GenBank ID	PSSM-ID	Starting position of conservative structural domain	End position of conservative structural domain	*E*-value	Bit score	Short name	Superfamily
Diatoms							
GAX13287.1	177 942	464	1 059	0	1 008.41	PLN02307	cl38939
	460 300	21	429	5.48E−144	437.33	UDPGP	cl46593
GAX25814.1	177 942	464	1 059	0	999.94	PLN02307	cl38939
	460 300	21	429	3.42E−143	435.02	UDPGP	cl46593
XP_002185375.1	177 942	462	1 057	0	1 051.17	PLN02307	cl38939
	132 998	65	373	2.21E−143	431.29	UGPase_euk	cl11394
GFH52288.1	177 942	469	1 058	0	1 002.25	PLN02307	cl38939
	460 300	21	428	2.49E−146	443.49	UDPGP	cl46593
Phaeophyta							
AIQ80989.1	177 942	475	1 071	0	981.07	PLN02307	cl38939
	460 300	33	439	1.07E−147	447.35	UDPGP	cl46593

Data source: NCBI Conserved Domain Database. PSSM-ID: The unique identifier for a domain model's position-specific scoring matrix (PSSM); *E*-value: The expect value, or *E*-value, indicates the statistical significance of the hit as the likelihood the hit was found by chance, the lower the *E*-value, the higher the credibility; Bit score: The value S' is derived from the raw alignment score S in which the statistical properties of the scoring system used have been taken into account, a higher bit score indicates greater reliability; Short name: The short name of a conserved domain, which concisely defines the domain; Superfamily: Populated only for domain models that are specific or non-specific hits, and it lists the accession number of the superfamily to which the domain model belongs.

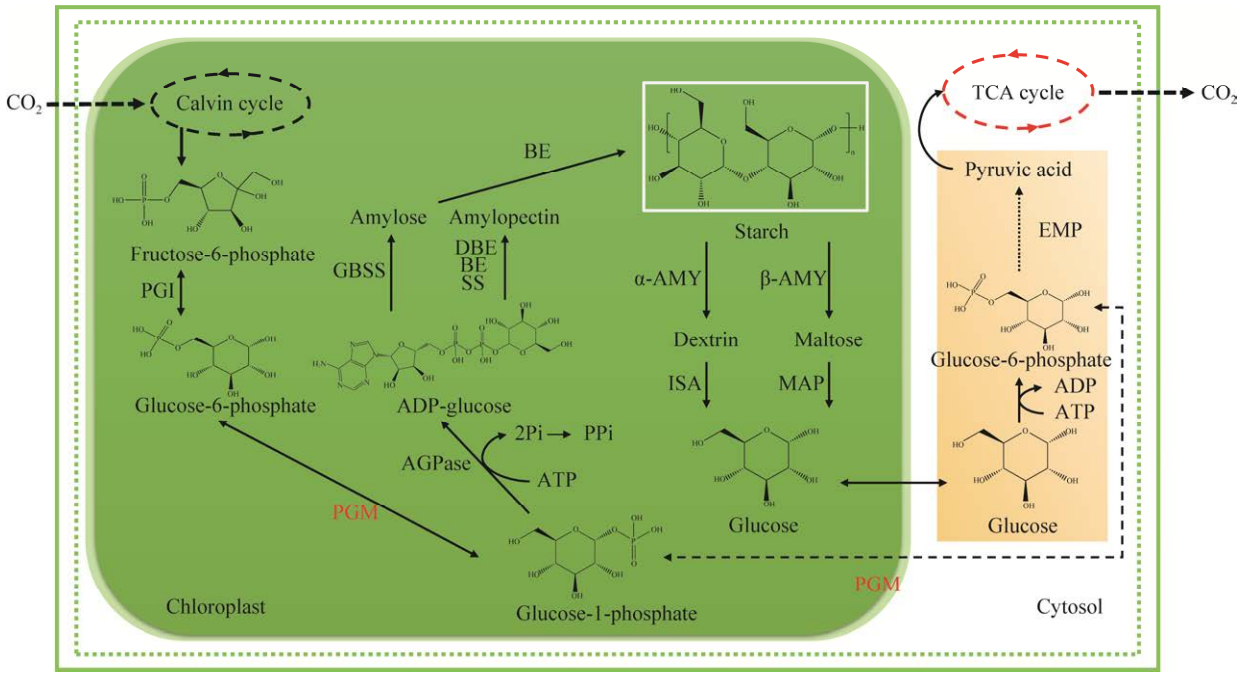

图 2　PGM 参与的淀粉合成与降解途径

Figure 2　The starch biosynthesis and degradation pathways by PGM. Chloroplast, PGI: Glucose-6-phosphate isomerase; PGM: Phosphoglucomutase; AGPase: ADP-glucose pyrophosphorylase; GBSS: Granule bound starch synthase; SS: Starch synthases; BE: Branching enzyme; DBE: Starch debranching enzyme; AMY: Amylase; ISA: Isomaltase; MAP: Maltose phosphorylase. Cytosol, EMP: Embden-Meyerhof-Parnas Pathway; TCA Cycle: Tricarboxylic acid cycle.

pPGM 是卡尔文循环途径的关键酶。当植物细胞处于异养状态时，葡萄糖-6-磷酸从细胞质转运到质体，这种反应需要由 pPGM 介导胞内葡萄糖-6-磷酸到葡萄-1-磷酸的转化，葡萄糖-1-磷酸进入叶绿体基质后，可以通过各种淀粉合酶同工酶的介导，进入淀粉生物合成的途径，也可以直接作为葡聚糖延伸的葡萄糖基供体[12]。在产淀粉植物豌豆(*Pisum sativum* L.)中，质体内 pPGM 为淀粉合成提供底物葡萄糖-1-磷酸，而缺失 pPGM 活性会降低细胞内淀粉产量或导致无淀粉产生[67]。通过突变拟南芥 pPGM，会限制葡萄糖-6-磷酸转化为葡萄糖-1-磷酸，进而抑制 ADP-葡萄糖合成，使突变体淀粉含量降低[68]。同样的是，通过 CRISPR-Cas9 技术分别突变白杨树(*Populus tremula*×*tremuloides*) 2 个 pPGM (PGM1、PGM2)基因，2 个品系完全展开的叶片、韧皮部组织和根尖中都缺乏淀粉[69]。此外，研究表明在异养状态下，植物组织 pPGM 调节淀粉合成和碳水化合物氧化代谢的碳分配中起着关键作用。例如，研究人员构建了一株拟南芥 pPGM1 功能缺失突变体，在早期种子发育过程中，由于 PGM 功能的缺失无法合成淀粉，突变体种子的碳存储较少，难以维持脂质合成；通过气相色谱分析种子总脂质提取物中的脂肪酸甲酯，结果显示，在同一培养条件下，pPGM1 突变体的种子油脂含量减少了 40%[70]。另外，有研究指出蓝藻 PGM 对糖原和中心碳代谢物的调节起着重要的作用，缺乏 PGM 的蓝藻会降低对强光或缺氮等压力的适应度；当磷酸甘露糖酶/葡萄糖磷酸变位酶复合酶(phosphomannomutase/phosphoglucomutase, PMM/PGM)过表达时，蓝藻对这些应激的反应会降低[71]。在微藻糖代谢途径中，PGM 似乎不只限于参与淀粉合成，还间接参与其他的糖代谢，一些藻类甘露糖-1-磷酸鸟苷酸转移酶(mannose-1-phosphate guanylyltransferase, MPG)能催化葡萄糖-1-磷酸或甘露糖-1-磷酸合成海藻酸[72]，再通过尿苷二磷酸葡萄糖焦磷酸化酶(UDP-glucose pyrophosphorylase, UDPase)或 GDP 甘露糖焦磷酸化酶(GDP-mannose pyrophosphorylase, GMP)催化合成卡拉胶[73]或琼脂[74]。

近年来，有研究人员在对微藻进行光自养培养和异养发酵时发现，微藻不仅能光合自养通过卡尔文循环获得多糖合成所需的物质，还能够通过从环境中吸收单糖合成淀粉[6,75-77]。Koo 等[78]通过 γ 辐射获得高产淀粉莱茵衣藻(*Chlamydomonas reinhardtii*)突变体，在所有突变体中，PGM 过表达的莱茵衣藻细胞内葡萄糖- 6-磷酸浓度低且淀粉含量高，这表明过表达 PGM 有利于藻细胞内葡萄糖-6-磷酸向葡萄糖- 1-磷酸的转化，促进淀粉合成。有研究表明 PGM 的过表达可显著提高三角褐指藻(*Phaeodactylum tricornutum*)金藻昆布多糖含量并降低其脂质含量，过表达硅藻 PGM，可能导致其多糖合成关键基因的表达水平显著增加，而脂质合成关键酶转录丰度显著降低，差异转录表明 PGM 协调了硅藻中金藻多糖和脂质生物合成途径[79]。这些结果可推断，PGM 可能是碳水化合物和脂质代谢之间碳分流过程的关键调控节点。

在植物细胞中，PGM 分为质体定位的 pPGM 和胞质定位的 cPGM 2 个亚型[14,80]，cPGM 主要参与光合作用、呼吸作用、蔗糖代谢和细胞壁合成[10,25,81-83]，pPGM 主要确保淀粉合成过程中葡萄糖-1-磷酸的供应[11]。然而，真核微藻 PGM 的分类及潜在生物学功能仍不明确。真核微藻作为低等光合生物，其 PGM 可能同时拥有植物 cPGM 和 pPGM 的功能，或是具有 PMM/PGM 复合蛋白催化相关反应的特性[71-72]。通过基因编辑技术和组学分析进一步发掘真核微藻 PGM 的生理功能，可为调控微藻合成高值目标物质提供理论依据。

3 微藻葡萄糖磷酸变位酶的活性调控

3.1 氨基酸基序与 PGM 活性的关系

PGM 是糖代谢的关键调节点，翻译后修饰是控制其活性的重要机制。微藻 PGM 被鉴定为具有 2 个丝氨酸磷酸化位点——Ser^{47} 和 Ser^{152}，其中 Ser^{47} 位于酶的表面，处于一个被称为“闩锁”的环中；Ser^{152} 对应于催化活性丝氨酸残基，负责糖分子与酶之间的磷酸转移，通过对 Ser^{47} 和 Ser^{152} 的磷酸化和去磷酸化能调节藻类 PGM 活性[84-85]。研究表明，PGM 催化的反应需要葡萄糖-1,6-二磷酸作为中间体实现磷酸基团在底物分子上的转移，葡萄糖-1,6-二磷酸的磷酸基团在酶分子活性位点被重新分配，1′或 6′位置的磷酸基团转移到催化活性丝氨酸残基，从而实现葡萄糖-1-磷酸和葡萄糖-6-磷酸的相互转化，由于只有一个磷酸基团发生解离，因此酶活性位点在反应中持续保持磷酸化(图 3)[84,86]。Doello 等[85]证实了，在氮饥饿时，蓝藻 PGM 残基 (Ser^{47}) 被磷酸化后其活性显著下降。Periappuram 等[70]从拟南芥中鉴定了一种 PGM 酶的编码序列(coding sequence, CDS) (GeneBank 登录号：AJ242601)，将蛋白命名为 At-PGMp，氨基酸序列分析发现 At-PGMp 最保守的区域有催化反应中心(Ser-Ala-Ser^{181}-His-Asn)、金属结合位点(Asp-Gly-Asp-Gly-Asp)和葡萄糖环结合位点(Cys-Gly-Glu-Glu-Ser-Phe)，PGM 蛋白氨基酸序列中 2 个 Asp 残基对协调辅因子 Mg^{2+} 是必需的。在褐藻 *Saccharina japonica* 中鉴定的 PMM/PGM 复合蛋白保守基序显示，PMM/PGM 蛋白由帽结构域和核心结构域组成，帽结构域(Arg^{125}、Arg^{136} 和 Arg^{143})氨基酸残基会与核心结构域(Arg^{23} 和 Lys^{53})氨基酸残基形成静电斥力，使这 2 个结构域向底物开放；当带负电荷的底物与帽结构结合时，结构域会闭合形成催化反应所需的溶剂排斥环境，底物结合到帽结构域(Arg^{136}、Arg^{143} 和 Ser^{181})形成磷酸盐结合位点；Asp^{183}、Arg^{125} 和 Arg^{23} 结合甘露糖-1-磷酸或葡萄糖-1-磷酸，随后被推向活性位点[72]。

3.2 金属离子对 PGM 的活性调控

在植物中，金属离子是 PGM 活性调节的重要元素(表 4)。Mg^{2+} 是维持 PGM 活性所必需的[73,87,91]。Be^{2+} 离子对葡萄糖磷酸变位酶有较强的抑制作用，在 10 μmol/L $BeCl_2$ 存在的情况下，Be^{2+} 导致 PGM 快速失活；在添加 Mg^{2+} 离子

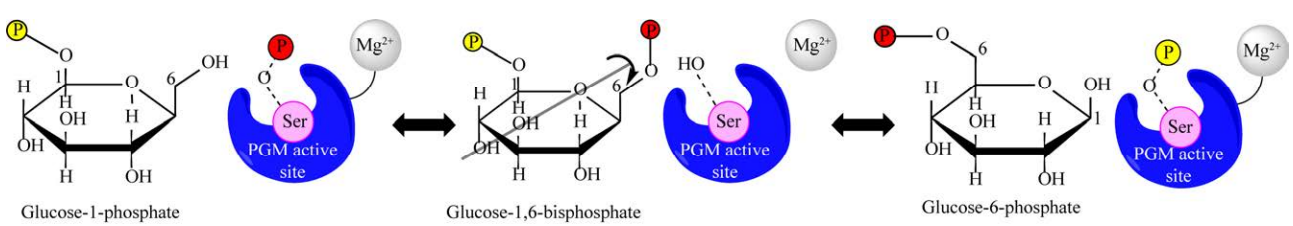

图 3 PGM 催化的反应[84,86]

Figure 3 The reaction catalyzed by PGM[84,86]. The reversible conversion of glucose-1-phosphate and glucose-6-phosphate was catalyzed by phosphoglucomutase (PGM). Glucose-1,6-bisphosphate was an intermediate in this reaction. The initial step performed the transfer of a phosphate group (represented in red) from the serine residue at the active site of PGM. The intermediate was rotated at the active site with 180°, then the phosphate group (depicted in yellow) from the substrate was returned to the PGM active site for restoring the enzyme towards its active state. Ultimately, glucose-1-phosphate was converted into glucose-6-phosphate. This reaction is a reversible reaction.

和 EDTA 后，酶活性被修复，并且恢复了约20%的酶活力，这可能是因为 EDTA 从酶上解离了抑制性金属离子[87]。从菠菜分离得到的 2 种 PGM 酶活性均表现出对 $MgCl_2$ 的高度依赖，当反应体系 EDTA 的浓度超过 Mg^{2+} (10 mmol/L) 达到 30 mmol/L，PGM 活性被完全抑制；另外，在咪唑缓冲体系中，当 EDTA 浓度为 3 mmol/L 低于 Mg^{2+}时，PGM 酶的活性会增加，这表明 Mg^{2+} 是维持 PGM 酶活性所必需的，低浓度 EDTA 可将酶结合位点解离抑制性盐离子，使 Mg^{2+}更好地与酶结合位点作用；而高浓度 EDTA 会抑制 Mg^{2+}与酶活性位点结合而导致 PGM 的失活[91]。

褐藻 *Saccharina japonica* 的 PMM/PGM 表征发现，5 mmol/L 金属化合物[如 $MgCl_2$ (Mg^{2+})]会显著提高 PGM 的活性，相较于未处理增加了约 21 倍，即由 0.22 U/mg 增加到 4.63 U/mg；$MnCl_2$ (Mn^{2+})对 PGM 活性也有一定的增强作用，PGM 活性可提高到 0.80 U/mg[72]。Jackson 等[88]研究了在不同温度、pH 和 5 mmol/L 不同金属离子 ($MgCl_2$、LiCl、$CoCl_2$、$MnCl_2$、$CaCl_2$、$NiCl_2$ 和 $ZnCl_2$)对褐藻 *Laminaria digitata* PGM 活性的影响，与对照相比，Mg^{2+}、Mn^{2+}和 Zn^{2+}对 PGM 的活性有显著促进作用，分别提高了 10 倍、6 倍和 6 倍；Ca^{2+}、Co^{2+}、Ni^{2+}和 Li^{2+}对 PGM 活性有一定的抑制作用；PGM 在 35−45 ℃温度区间内保持高活性，而 65 ℃以上则失活；PGM 在 pH 8.0 时表现最大活性。正钒酸钠(sodium orthovanadate)是一种常用的磷酸酯酶抑制剂，研究表明 PGM 活性会被葡萄糖-1-磷酸和无机钒酸盐形成的复合物所抑制[92]。

一般认为，重金属对酶活性有一定的抑制作用。Devi 等[89]研究探讨了外源铅(Pb)对豌豆幼苗生长、蔗糖代谢、淀粉降解、磷酸戊糖途径、糖酵解等途径重要酶活性的影响，发现在施加 0.5 mmol/L Pb 的胁迫条件下，豌豆 PGM 的活性在嫩茎和叶片中分别下调 30%和 24%，铅可能通过影响组织的碳水化合物状态，干扰生物体的吸水能力，从而抑制生物体的生长。

3.3 有机化合物对 PGM 活性的影响

一些有机物也被证明可调节PGM活性(表4)。分散兰 56 (disperse blue 56)是一种基于蒽醌的染料，在水溶液中会形成有机分子聚集体，是PGM的非竞争性抑制剂，在体外显示出强烈的抑制作用，能够在酶表面富集以抑制酶活性[93]。Chen 等[74]发现在 5 μmol/L 分散兰 56 浓度下，红藻 *Gracilariopsis lemaneiformis* PGM 酶活性和琼脂含量下降了 40%。

在微藻生长过程中，淀粉合成途径中间产物对 PGM 活性也有影响。在含有 2.4 mmol/L 果糖-1,6-二磷酸的情况下，拟南芥 PGM 活性下降了 50%[70]。此外，在分别含有 1 mmol/L 的代谢物 D-果糖-1-磷酸、D-果糖-6-磷酸、D-甘油-2-磷酸、果糖-1,6-二磷酸、甘油-2,3-二磷酸、UDP-葡萄糖、AMP、ADP 或 ATP 的体系检测酶活性，与未添加抑制剂相比，2 种二磷酸盐抑制了 PGM 56%−60%的酶活性，而其他物质抑制了 PGM 5%−13%的酶活性[87]。这种由于多糖代谢途径中间产物而影响 PGM 活性的情况在莱茵衣藻中也有发现，葡萄糖-6-磷酸含量升高正反馈调节 PGM 表达，促进了淀粉的过度积累[78]。研究表明，甲硫氨酸会降低 PGM 转录水平，相比对照组减少了约 53%，巯基保护试剂能提高酶制剂的稳定性，但相关的机理尚不明确[73]。

24-表油菜素内酯(24-epibrassinolide, 24-epiBL)是一类人工合成的油菜素内酯类似物，对植物种子萌发、脂质过氧化、脯氨酸含量等均有调节作用[94]。汤小彬等[90]研究不同浓度 24-epiBL 和 3 种温度对红藻(*Gracilariopsis lemaneiformis*) PGM 表达量的影响，结果表明，在 15 ℃时，0.5 mg/L 24-epiBL 的 PGM 表

表 4　不同化合物对 PGM 活性的影响

Table 4　Effects of different compounds on PGM activity

Chemical compound	Concentration	Effects of PGM activity	References
Fructose-1,6-bisphosphate	2.4 mmol/L	Activity reduced by 50%	[70]
Magnesium chloride ($MgCl_2$)	5.0 mmol/L	Enhanced activity by 21 times	[72]
Manganese(II) chloride ($MnCl_2$)		Enhanced activity by 4 times	
Methionine	10.0 mmol/L	Reduce transcription levels	[73]
Disperse blue 56	5.0 μmol/L	Activity reduced by 40%	[74]
Ethylene diamine tetraacetic acid (EDTA)	0.2 mmol/L	Improve activity when exist together	[87]
Magnesium chloride ($MgCl_2$)	1.0−10.0 mmol/L		
Fructose-1,6-bisphosphate	1.0 mmol/L	Activity reduced by 56%−60%	
Fructose-1-phosphate		Activity reduced by 5%−13%	
Fructose-6-phosphate			
2-phosphoglycerate			
UDP-glucose			
Adenosine monophosphate (AMP)			
Adenosine diphosphate (ADP)			
Adenosine triphosphate (ATP)			
Manganese(II) chloride ($MnCl_2$)	5.0 mmol/L	Enhanced activity by 6 times	[88]
Zinc chloride ($ZnCl_2$)			
Sodium chloride (NaCl)		Inhibition as concentration increases	
Calcium chloride ($CaCl_2$)		Inhibition of activity	
Cobalt(II) chloride ($CoCl_2$)			
Nickel chloride ($NiCl_2$)			
Lithium chloride ($LiCl_2$)			
Lead (Pb)	0.5 mmol/L	Activity reduced by 24%−30%	[89]
24-epibrassinolide (24-epiBL)	1.0 mg/L	Enhanced activity by 2.38 times at 25 ℃	[90]

达量最高，为对照组的 1.72 倍；在 25 ℃和 31 ℃时，1.0 mg/L 24-epiBL处理组的PGM表达量最高，分别为对照组的 2.38 倍和 1.51 倍，藻细胞的琼脂含量与 PGM 表达量呈正相关关系。

4　微藻 PGM 潜在影响高值化产物合成

微藻可合成蛋白质、多不饱和脂肪酸、色素等高附加值产物，已广泛应用于医药、食品、饲料、化妆品等领域。目前，微藻科技工作者一直致力于提高微藻目标物质合成的技术工艺，推动微藻生物技术和行业的发展。为此，本节将介绍现有微藻 PGM 对其高附加值产物合成的潜在影响，旨在为高效精准调控合成目标产物提供实践和理论依据。

4.1　蛋白质

在自养且高氮水平条件下，微藻细胞可合成高丰度的蛋白质。例如，螺旋藻(*Spirulina*)在适宜的生长环境下，蛋白质含量可达 60%−70%[95]。小球藻在光自养且氮源充足的条件下，其蛋白质含量可达 50%以上[96-97]。现有关于提高自养微藻蛋白质含量的研究工作，重心都集中在培养基优化，例如周有彩等[19]研究不同氮源对微藻细胞生长及蛋白质代谢的影响发现，以尿素氮为小球藻培养过程中的唯一氮源可使异养条件下蛋白质含量(<40%)提高至 52.3%，与光自养条件相当。然而，自养微藻合成蛋白质所需的碳骨

架主要来自其胞内淀粉降解形成的中间糖类代谢物(如丙酮酸、草酰乙酸等)[7,98]。然而，在自养微藻淀粉代谢方面，研究者主要关注核酮糖-1,5-二磷酸羧化酶/加氧酶(ribulose-1,5-bisphosphate carboxylase/oxygenase, Rubisco)、AGPase 等关键酶的调控[13,99]，关于明确微藻 PGM 是否调控蛋白质合成的研究报道还尚未发现。综上所述，微藻 PGM 是微藻淀粉合成的关键酶之一。因此，调控 PGM 活性是否影响自养微藻蛋白质合成值得更深入研究。

与自养培养不同，异养微藻可利用有机碳源进行发酵获得高蛋白含量的微藻生物质。以异养小球藻为例，其蛋白含量普遍较低(35%以下)，导致藻类商业化开发困难。近年来，有研究人员提出微藻对营养物质的过度补偿理论[96]，研究表明营养调控会影响异养小球藻淀粉代谢途径相关酶的基因表达，调控高值化产物合成所需的碳骨架流量[100-102]。Xiao 等[6]通过两阶段氮调控提高异养小球藻蛋白质合成的研究发现，小球藻 MBFJNU-17 在缺氮培养下淀粉含量可达 50.60%；随后进行富氮调控，此时微藻细胞开始吸收培养环境中的氮以合成蛋白质，当蛋白质含量达到峰值(55.60%)时，胞内淀粉含量会迅速下降到 20.61%，这表明在异养培养过程中，淀粉代谢是小球藻 MBFJNU-17 胞内生物质转化的关键点。此外，通过转录组分析氮调控前后的小球藻基因表达表明，在小球藻 MBFJNU-17 淀粉代谢途径中，AGPase、PGM、SS 等关键酶的基因在氮调控前后存在明显表达差异[6]。为此，着手开展包括 PGM 在内的关键酶活性调控，阐明这些关键酶如何整体上影响小球藻蛋白质合成的调控机制，可为异养小球藻蛋白质开发提供理论依据。

4.2 油脂

原核微藻的油脂合成代谢分布在细胞质，真核微藻的油脂合成代谢分布在叶绿体膜和内质网膜[103]。此外，由于不同微藻拥有自身独特的油脂代谢途径，使不同微藻合成的多不饱和脂肪酸种类及其丰度存在较大差异。例如，螺旋藻 *Spirulina platensis* 主要合成的多不饱和脂肪酸是 γ-亚麻酸[104]，小球藻主要合成亚油酸和 α-亚麻酸[105-106]，微拟球藻(*Nannochloropsis oceanica*)可合成二十碳五烯酸[107]。因此，开发微藻多不饱和脂肪酸也是微藻研究领域的热点方向。

目前，调控微藻油脂合成的研究工作集中在营养缺乏策略和分子生物学调控油脂代谢关键酶表达。为应对环境中营养缺乏带来的生长压迫，微藻会以糖类或脂质的形式储存能量[108-111]。研究表明在氮胁迫条件下，莱茵衣藻将淀粉转化为脂质，以三酰甘油酯(triacylglycerol, TAG)形式积累到细胞内[112]。藻类脂质合成受到中央碳代谢途径的转录调控，在缺氮时期，佐夫色绿藻(*Chromochloris zofingiensis*)的 PGM 转录水平上调，淀粉合成保持不变，但淀粉降解酶活性的提高使细胞内糖酵解途径的碳流主要分配到脂肪酸合成途径，促进油脂积累[110-111]。同样地，链带藻(*Desmodesmus* sp.)在氮胁迫条件下也表现出油脂含量的大幅增加(40%−50%)[113]。在硅藻 *Phaeodactylum tricornutum* 中，双功能 UGPase-PGM 融合转录本的敲除导致 TAG 积累急剧增加 45 倍[114]。微藻从环境中吸收 CO_2 的过程，Rubisco 起着关键作用，Wei 等[115]通过在微拟球藻(*Nannochloropsis oceanica*)中过表达藻自身的 Rubisco 以提高光合作用效率，使生长率提高了 32%，生物质积累提高约 50%，脂质产量提高约 41%。值得注意的是，调控微藻油脂合成的碳流量来自机体内的碳水化合物和蛋白质，在缺氮条件下微藻会降解多糖和蛋白质将碳重新分配到脂质中[110]。微藻从光合作用或糖酵解中获得的过量碳被重新分配到含碳化合物中，如

葡萄糖-6-磷酸、果糖-6-磷酸、磷酸烯醇丙酮酸，然后通过 γ-氨基丁酸途径、糖酵解和三羧酸循环转移到脂质代谢中，用于合成脂质[116]。对于自养微藻，提高自养微藻固碳效率促进细胞内碳水化合物的积累，再通过调控后有利于碳水化合物向油脂和/或多不饱和脂肪酸的合成[117-118]。正如 4.1 所述，促进自养微藻细胞内碳水化合物积累的机理研究多集中在 Rubisco、AGPase 等关键酶。目前，调控 PGM 活性对自养微藻油脂和多不饱和脂肪酸合成的响应特征，值得深入探究。

对于异养微藻，微藻合成油脂的碳骨架来自外源的有机碳源。在异养条件下微藻能够快速吸收葡萄糖，通过上调糖酵解和三羧酸循环加速葡萄糖的分解代谢，快速合成丙酮酸；同时，参与脂肪酸合成的大多数酶的上调和参与脂肪酸降解的酶的下调均有利于细胞内脂肪酸的合成[35]。近期，研究表明通过缺氮调控后，一些异养微藻(如小球藻、栅藻等)可将碳水化合物转化为淀粉[6,119]，这无疑不利于高附加值油脂的开发和应用。因此，若使糖酵解受阻，在代谢途径改变时，细胞可能寻找其他方式来产生能量和维持正常功能，这可能会促进脂质合成[74]。因此，揭示 PGM 等关键酶对调控异养微藻合成油脂和多不饱和脂肪酸的作用机制，可为高值化开发微藻多不饱和脂肪酸奠定理论基础。

4.3 色素

不同微藻种属具有不同的色素合成通路，赋予其合成不同种类色素的能力。雨生红球藻(*Haematococcus pluvialis*)可合成虾青素[120-122]，等鞭金藻(*Isochrysis*)合成岩藻黄素[123]，杜氏盐藻主要合成 β-胡萝卜素[121]。有些色素(如岩藻黄素)也是微藻重要捕光色素，对微藻光合作用具有重要的作用。代谢组学分析表明，在高碳源诱导条件下，微藻糖酵解、磷酸戊糖途径和三羧酸循环途径会增强，进而促进色素积累[124]。此外，藻类研究者尝试提高自养微藻细胞色素含量，一方面提高微藻的光合固碳性能，另一方面可获得含高浓度色素的微藻生物质资源。例如，在高密度培养微藻时，光无法均匀穿透培养基，导致光合作用受到抑制。为此，有研究人员提出，通过基因工程手段，降低莱茵衣藻叶绿素合成调控基因(*tla1*)的表达可减少细胞叶绿素含量，使更多的细胞参与到光合作用，提高整体光合效率[125]。微藻色素合成受生长条件和环境中营养物质丰度影响[126]，培养基中的磷、氮和硫等元素已被证明是色素合成所必需的，其中不同的氮源对微藻色素含量影响最大[127-128]。研究表明在培养基中额外添加葡萄糖和硝酸盐可提升念珠藻(*Nostoc commune*)中藻蓝蛋白含量[129]。Kovač 等[130]发现，提供稳定持续的光照和碳源会提高螺旋藻藻蓝蛋白(12 倍)和别藻蓝蛋白(16 倍)的含量。异养微藻合成目标产物的碳骨架来自培养基的有机碳[131]，通过调节培养基营养组成(尤其是碳源)能明显影响藻类色素的合成；然而，关于碳源代谢酶与色素合成的调控机制研究仍然较少。尽管有报道指出碳通量变化会调控藻类色素合成速率，但其中的相关机理尚不明确[131]。因此，探明 PGM 等淀粉合成关键酶对调控微藻色素合成相关机制，可为微藻合成高值化产物工业化提供理论基础。

大量研究表明，异养培养的微藻也能合成其自养模式下的色素种类，但是色素含量存在差异。例如，异养培养被应用于藻类叶黄素的生产，通过异养培养小球藻可获得 5.3 mg/g 的藻类生物质叶黄素[132]，而在光自养条件下，藻类叶黄素产量可达到约 7.5 mg/g[131]。光自养条件下螺旋藻可合成获得高含量的天然藻蓝蛋白[131,133]；除了光自养，螺旋藻还可利用葡萄糖进行异养生长，在异养培养条件下，藻蓝蛋白的含量约为 57 mg/g，大约是混合营养条件下的一半[131,134]。正如 4.2 所

述，异养微藻合成目标产物的碳骨架主要来自培养基的有机碳。在异养模式下，微藻通过碳代谢酶将从环境中获得的有机碳转化为淀粉等聚合物，为碳代谢反应提供中间糖代谢物[131]。PGM 是淀粉合成的关键酶，调控异养微藻 PGM，降低其淀粉合成，进而调节异养微藻的碳骨架流量，这是否可提高异养微藻目标色素的合成？跟踪现有的文献，这一研究尚处于空白的领域。

5 展望

目前，微藻多糖、蛋白、脂质等高附加值产物产量低限制了微藻工业的发展。微藻 PGM 作为淀粉合成途径中关键的限速酶，对其分子特性和活性调控的研究仍然较少，对 PGM 影响藻类高附加值产物的研究仍聚焦于淀粉、琼脂等生物多糖，对 PGM 间接调控蛋白、油脂、色素等其他产物的研究关注不足。随着生物信息学和基因工程技术的发展，我们有望深入发掘 PGM 催化高值化产物合成的分子机制、调控途径及在其微藻发酵工业上潜在的应用价值，进一步明确藻类淀粉作为碳源转化为高附加值产物合成途径，深入研究 PGM 与淀粉合成途径中其他关键酶的相互作用。从分子水平出发，通过转录组学、代谢组学、蛋白组学等组学和结构生物学等技术，揭示微藻淀粉代谢的动态调节机制，为提高微藻在不同培养环境中的适应性和调控目标产物合成提供理论支持。这不仅有助于解析微藻淀粉合成的分子机制，也为未来对藻类工程改造提供了更准确的靶点，为发展微藻固碳技术和高值化开发微藻提供新思路，助力我国“双碳”目标奠定理论基础。

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