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Using N_2 as a final electron receptor in yeast to produce NH_3 in bioreactors

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Abstract

This research proposal aims to replacement of N_2 molecule (instead of O_2) as a final electron receptor in micro-organisms so that they can produce NH_3, which is important industrially, instead of H_2O. This idea is based on the hypothesis that replacement of the central atom in gas-carrying compounds in living organisms such as chlorophyll, heme and hemocyanin would replace the fixed gas. However, NH_3 is an expensive product because it is produced using Haber-Bosch reaction technique which requires high temperature and pressure. Therefore, it is predictable here to produce NH_3 so simply in bioreactors which is a great economic benefit.

Keywords

Nitrogen fixation; chlorophyll; heme; ammonia, ferrochelatase and adaptive mutation.

Objectives

NH_3 (ammonia or azane gas) is an important product; it has a number of vital uses such as fertilizers, cleaners, fermentation industry and antimicrobial agent in food products. NH_3 is currently produced using Haber-Bosch reaction technique which requires high temperature (400-500°C) and pressure (150-250 bar) that is why it is so expensive, so the production of ammonia gas in our proposed way would be a great economic benefit.

Impact

1. Production of NH3 in bioreactors (low-cost), instead of using Haber-Bosch reaction technique (high-cost).
2. Great scientific achievement: If our purpose succeeded, our new yeast strain, would be the first of its type ever; we would have an organism that "breath" N_2, instead of O_2 for the first time on Earth.

3. Our new yeast strain would start new scientific horizons in terms of evolution, microbiology, adaptive mutations and life itself.

Introduction/Background

Photosynthesis and respiration are two vital processes that are the prerequisites for all life on Earth. The two pigments responsible for these processes are chlorophyll and heme respectively. The so-similarity between chlorophyll and heme has been a mysterious subject of research by some researches for a long (Vernon and Seely 1966); they are identical in structure except for the central atom: magnesium in chlorophyll and iron in heme (Bryson and Vogel 1965). Chlorophyll has been proven to have the ability to turn into hemoglobin (Hughes and Lanter 1936, Kohler et al. 1939).

O_2 is fixed by living organisms to be used as a final electron receptor, to be turned into H_2O. If we managed to replace N_2 by O_2 in yeast (because yeast contains heme) and green bacteria, we are going to get NH_3, and that is a real economic benefit. NH_3 (ammonia or azane gas) is an important product; it has a number of vital uses such as fertilizers, cleaners, fermentation industry and antimicrobial agent in food products. NH_3 is currently produced using Haber-Bosch reaction technique which requires high temperature (400-500°C) and pressure (150-250 bar) that is why it is so expensive, so the production of ammonia gas in our proposed way would be a great economic benefit.

Heme is the compound that forms, with some proteins, hemoglobin; the compound that exists in red blood cells in most animals. It has the ability to deliver O_2 and CO_2. Its chemical structure is $C_{55}H_{72}N_4Fe$. Hemocyanin is the compound that delivers O_2 and CO_2 in mollusk and arthropod species the same way hemin does, but less effectively. Its chemical structure is $C_{55}H_{72}N_4Cu$. Chlorophyll is the compound that fixes O_2 and CO_2 in plants. It also absorbs sun light's energy necessary for photosynthesis. Its chemical structure is $C_{55}H_{72}N_4Mg$. Hemin has a semi- similarity with other compounds such as cobalamin (vitamin B_{12}) which has Co atom as a central atom in its structure.

Research Approach and Methodology

Accurately, gases do not bind heme, as a whole, they bind only the central atom (iron) within it. This makes a sense that if we managed to change the central atom in heme, the fixed gas would be changed. When we have a breath in our lungs, only oxygen binds hemoglobin, not because oxygen is more suitable than other gases, but because of the central atom in heme, iron, which interacts with oxygen naturally.

Iron exists naturally in the form of oxides i.e. FeO, Fe_2O_3, Fe_3O_4. Copper, in hemocyanin, exists in nature in the form of oxides too i.e. Cu_2O CuO, CuO_2, Cu_2O_3. Magnesium, in chlorophyll interacts with oxygen and carbon dioxide easily too. Therefore, our hypothesis is "Changing the central atom in hemoglobin, hemocyanin and chlorophyll, changes the fixed gas by them".

While some researchers managed to replace the central atom in chlorophyll with copper and zinc *in vitro* (Petrovic et al. 2006), no attempts have been made to replace the central atom either in chlorophyll nor in heme in living organism for two reasons, to the author's point of view. They are:

1. The absence of purpose, which is available in this proposal; bio-production of ammonia gas.
2. Technical complexity, which may seem to be impossible at first sight for two reasons. Firstly, hemin and chlorophyll are synthesized through a set of bioreactions, so if you need to do a specific modification in heme, for instance, you will need to make a number of "specific" genetic alterations or mutations in yeast, for instance, which sounds to be impossible. Secondly, if you replaced the central atom in heme with Ca^{2+} *in vitro*, obtained Ca-Heme and managed to replace it with the heme of yeast (using microinjection technique), your new trait wouldn't be inherited to next generations because of ferrochelatase enzyme which is selective to Fe ions (Ogun et al. 2023).

Ferrochelatase (or protoporphyrin ferrochelatase) is an enzyme which is encoded by the FECH gene in humans (Anonymous In Press). It catalyses the terminal (8^{th}) step in the biosynthesis of heme, turning protoporphyrin IX into heme B. The interesting fact (to this proposal) is that ferrochelatase can also insert other divalent metal ions into protoporphyrin. Some ions such as Zn^{2+}, Ni^{2+} and Co^{2+} form other metalloporphyrins (Medlock 2009). Zinc protoporphyrin (ZPP) is a compound found in red blood cells when heme production is inhibited by lead and/or by lack of iron (Labbe et al. 1999). Substrate inhibition happens in the order $Cu^{2+} > Zn^{2+} > Co^{2+} > Ni^{2+}$ and can be alleviated by supplementation of metal complexing agents such as β-mercaptoethanol or imidazole to the reaction buffer (Hunter et al. 2008). Magnesium-chelatase is a three-component enzyme that catalyses the insertion of Mg^{2+} into protoporphyrin IX. This is the first unique step in the synthesis of chlorophyll and bacteriochlorophyll (Willows 2003, Bollivar 2006).

Although it is possible to replace the central atom in heme and chlorophyll, it looks greatly difficult to make specific genetic manipulations to reach the target of our proposal. Therefore, our goal can be possible if we follow an adaptive mutation technique. Adaptive mutation states that rather than mutations and evolution being random, they are responsive to certain stresses. In other words, the mutations which happen are more beneficial and specific to the given stress, neither random nor a response to anything in particular. The term stress refers to any alteration in the environment, such as temperature, nutrients, population size, etc. Tests with microorganisms have found that for adaptive mutation, more of the mutations remarked after a given stress were found to be more efficient at dealing with the stress than chance alone would suggest is possible (Foster 1993, Sniegowski and Lenski 1995). Adaptive mutation is a very controversial topic so there have been many experiments to prove or discredit the theory. Three key examinations are the SOS response (McKenzie 2000), responses to starvation in Escherichia coli (Foster 2000) and testing for revertants of a tryptophan auxotroph of Saccharomyces cerevisiae (Foster 2000).

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Proposed experiment

Model of research:

- The micro-organism(s) that we are going to use in our experiment must have two properties:

1. Obligate aerobic: Because if a facultative aerobic micro-organism was used, it is highly probable to simply turn into anaerobic pathway when aerobic conditions are not suitable to it.
2. Contains heme or bacteriochlorophyll.

- The advantages of using a yeast strain over using a bacterium strain as a model of research are:

1. Ferrochelatase adds ferrous ion as a terminal step in heme biosynthesis, while magnesium chelatase adds magnesium ion as a first step in bacteriochlorophyll synthesis; therefore, if any modification was made in magnesium chelatase enzyme, it may be required to be followed by further modifications in the enzymes that synthesize bacteriochlorophyll which is more difficult. It is much easier to modify the top of a pyramid than modifying its base.
2. The biosynthesis of heme looks simpler than the biosynthesis of bacteriochlorophyll. Besides, the structure of ferrochelatase is simple and its molecular weight is low relative to magnesium chelatase.

On the other hand, mutation rate in prokaryotes, in general, is much higher than mutation rate in eukaryotes which is a disadvantage of using a yeast strain over using a bacterium strain as a model of study in this proposal. Therefore, it is proposed here to use a yeast strain and a bacterium strain at the same time to avoid the need to use any mutants that may induce unpredictable, undesired mutations. *Torulopsis psychrophile*, *T. austromarina*, *L. gelidum*, and *L. nivalis* are examples for obligate aerobic yeasts (Anonymous 2023), so they are candidates to be models of study.

Fe^{2+}, Cu^{2+} and Co^{2+}, are naturally selected by ferrochelatase, all lie in the fourth group of the periodic table. They are all bivalent, and their electronegativity ranges from 1.83 to 1.90. Besides, they are all reactive to O_2. Therefore, if we are to replace ferrous ions at the hemin of yeast, we should focus on an element that is:

1. Bivalent.
2. Have in-range or close electronegativity.
3. Reactive to N_2.

As previously mentioned, Zn^{2+} is naturally selected by ferrochelatase (causing a disease in humans). Its electronegativity is 1.65, and it is reactive to N_2 forming Zn_3N_2. Thus, Zn^{2+} looks to be perfect to be our first candidate to be replaced by ferrous ions in hemin. The electronegativity of Ni^{2+} is 1.91. it is reactive to N_2 (forming NiN), so it likes to be a good fit

to be our second candidate. At the third place, Mn^{2+} comes. Its electronegativity equals 1.55. It is also reactive to N_2 forming Mn_3N_5, Mn_3n_2 and MnN_2.

Proposed experiment steps:

- In a lab fermenter, *Torulopsis psychrophile*, for instance, should be grown on a suitable medium with the replacement of ferrous with zinc under normal aerobic conditions. It is predictable here that Zn-heme will be synthesized in the yeast cells.
- pH value of the medium may be needed to be modified several times because to G. A. Hunter (August 29, 2008), ferrochelatase selectivity is pH-dependent.
- N_2 gas should be replaced by the same volume of O_2 gas gradually as a trial to induce an adaptive mutation.
- N_2 is less reactive than O_2 (bond energy of oxygen equals 119 k.cal/mole, but equals 226 k.cal/mole for nitrogen), so temperature may be needed to be increased. As a result, the yeast strain to be chosen as a model of research should be thermophilic or superthermophilic.
- Test the aerobic environment of the bioreactor for ammonia gas periodically. If found, we have reached our goal.
- Mutants may be needed to be used.

Ethics and security

It is recommended to keep our (to be) new yeast train "safe" in laboratory, and not to be used for commercial purposes before carefullly studying the risks and/or advantages of its spread in nature.

Conflicts of interest

The authors have declared that no competing interests exist.

References

- Anonymous (2023) The Yeasts Trust Database. URL: https://theyeasts.org/homepage-yeasts
- Anonymous (In Press) P22830 · HEMH_HUMAN. UniProtKB. URL: https://www.uniprot.org/uniprotkb/P22830/entry
- Bollivar DW (2006) Recent advances in chlorophyll biosynthesis. Photosynthesis Research[In English]. URL: https://link.springer.com/article/10.1007/s11120-006-9076-6
- Bryson V, Vogel HJ (Eds) (1965) Evolving Genes and Proteins. Elsevier [In English]. URL: https://www.sciencedirect.com/book/9781483227344/evolving-genes-and-proteins [ISBN 978-1-4832-2734-4]
- Foster PL (1993) Adaptive mutation: the uses of adversity. Annual Review of Microbiology[In English]. URL: https://www.annualreviews.org/content/journals/10.1146/annurev.mi.47.100193.002343

- Foster PL (2000) Adaptive mutation: implications for evolution. Bio Essays[In English]. URL: https://onlinelibrary.wiley.com/doi/10.1002/1521-1878(200012)22:12%3C1067::AID-BIES4%3E3.0.CO;2-Q
- Hughes JH, Lanter AL (1936) Chlorophyll and haemoglobin regeneration after haemorrage. The Journal of Physiology[In English]. URL: https://physoc.onlinelibrary.wiley.com/doi/10.1113/jphysiol.1936.sp003374
- Hunter GA, Sampson MP, Ferreira GC (2008) Metal Ion Substrate Inhibition of Ferrochelatase. Journal of biological chemistry[In English]. URL: https://www.jbc.org/article/S0021-9258(19)49280-6/fulltext
- Kohler GO, Elvehjem CA, Hart EB (1939) The relation of pyrrole-containing pigments to hemoglobin synthesis. Journal of biological chemistry[In English]. URL: https://www.sciencedirect.com/science/article/pii/S0021925818737058?ref=pdf_download&fr=RR-2&rr=868ca64729d10db9
- Labbe RF, Verman HJ, Stevenson DK (1999) Zinc protoporphyrin: A metabolite with a mission. *Clinical Chemistry*[In English]. URL: https://academic.oup.com/clinchem/article/45/12/2060/5643290
- McKenzie GJ, et al. (2000) The SOS response regulates adaptive mutation. PNAS[In English]. URL: https://www.pnas.org/doi/full/10.1073/pnas.120161797
- Medlock AE, et al. (2009) Product Release Rather Than Chelation Determines Metal Specificity for Ferrochelatase. Journal of Molecular Biology[In English]. URL: https://www.sciencedirect.com/science/article/abs/pii/S0022283609010389?via%3Dihub
- Ogun AS, Joy NV, Valentine M (2023) Biochemistry, Heme Synthesis. https://www.ncbi.nlm.nih.gov/books/NBK537329/. Accessed on: 2020-7-10.
- Petrovic J, Nicolic G, Markovic D (2006) In vitro complexes of copper and zinc with chlorophyll. Journal of Serbian Chemical society URL: https://doiserbia.nb.rs/Article.aspx?id=0352-51390605501P
- Sniegowski PD, Lenski RE (1995) MUTATION AND ADAPTATION: The Directed Mutation Controversy in Evolutionary Perspective. Annual Review of Ecology and Systematics[In English]. URL: https://www.annualreviews.org/content/journals/10.1146/annurev.es.26.110195.003005
- Vernon LP, Seely VR (Eds) (1966) The Chlorophylls. 1st ed.. Elsevier [In English]. URL: https://shop.elsevier.com/books/the-chlorophylls/vernon/978-1-4832-3289-8 [ISBN 9781483267722]
- Willows RD (2003) Biosynthesis of chlorophylls from protoporphyrin IX. Natural Product Reports[In English]. URL: https://pubs.rsc.org/en/content/articlelanding/2003/np/b110549n