P-36 軽油相当の炭化水素を大量生産可能なシアノバクテリアの創出

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Abstract

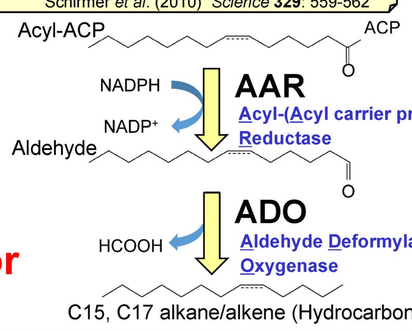
シアノバクテリアは、アシルACP還元酵素（AAR）とアルデヒド脱ホルミル化オキシゲナーゼ（ADO）を用い、光合成によって軽油相当の炭化水素を合成できることから、地球温暖化の防止に有効な再生可能バイオエネルギーの生産源として注目されている。しかし、両酵素の活性は低いため、高活性化が必要である。そこで本研究ではAARとADOの高活性型変異体を創出し、軽油相当の炭化水素を大量生産可能なシアノバクテリアの創出を目指した。まず、様々なシアノバクテリアに由来するAARを比較し、最も高活性なAARを同定した。次に100種類以上のAAR変異体を作製して、さらに高活性化させた。同様に、様々なシアノバクテリア由来ADOの中から高活性なADOを見出し、約40種類の変異体を作製して、高活性化に重要な部位を同定した。また、AARとADOの結合部位も解明した。最後に、これらの高活性型のAARとADOを多様な組み合わせで導入したシアノバクテリア*Synechocystis* sp. PCC 6803の変異株を24種類作製した結果、炭化水素合成量が野生株よりも5倍以上向上したシアノバクテリアの創出に成功した。

① Enzymes for alkane biosynthesis: AAR & ADO

Schirmer *et al.* (2010) *Science* **329**: 559-562

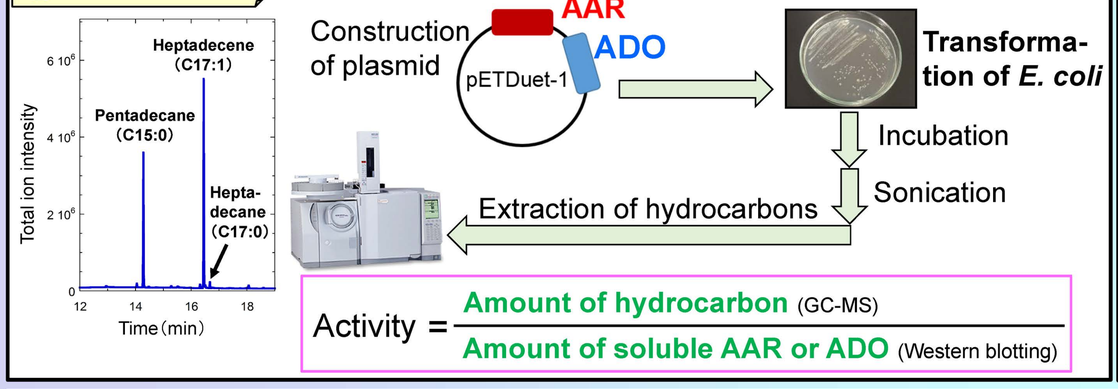
- AAR reduces acyl-ACPs to aldehydes, while ADO converts aldehydes into alkanes or alkenes corresponding to diesel fuels.
- *E. coli* co-expressing AAR & ADO can synthesize hydrocarbons.

AAR & ADO are essential for alkane biosynthesis.



② Methods

- Plasmid having AAR & ADO genes was introduced in *E. coli*.

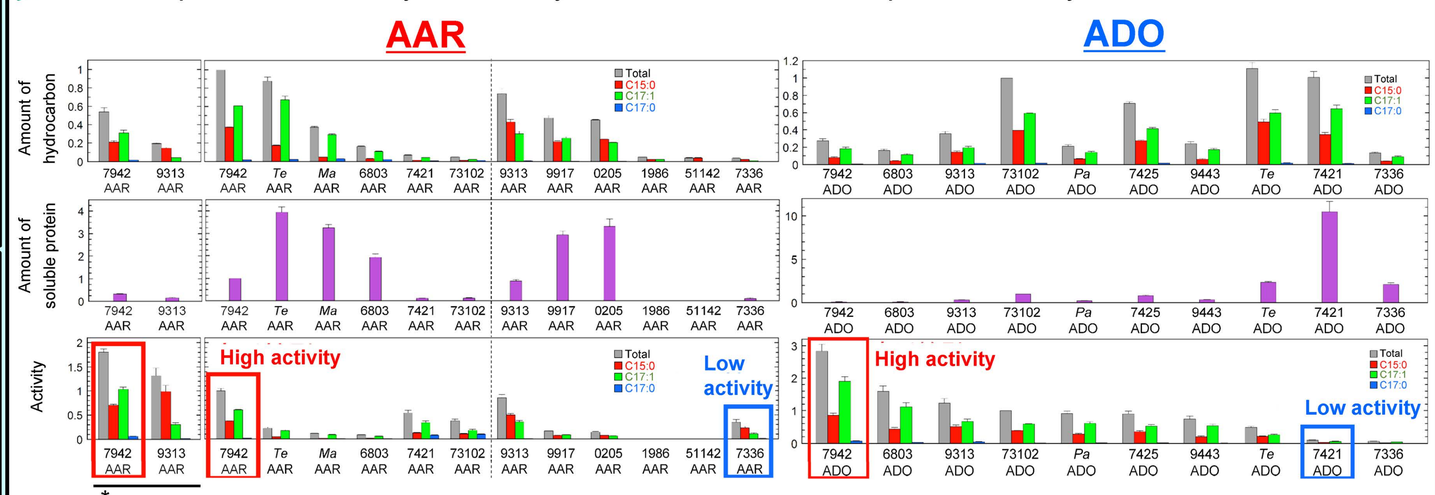


$$\text{Activity} = \frac{\text{Amount of hydrocarbon (GC-MS)}}{\text{Amount of soluble AAR or ADO (Western blotting)}}$$

③ To select highly active AAR & ADO from various cyanobacteria

Kudo H *et al.* (2016) *Biotechnology for Biofuels*, 9:234　　Kudo H *et al.* (2019) *Biotechnology for Biofuels*, 12:89

- We compared the solubility and activity of AARs & ADOs from representative cyanobacteria.



*The results were obtained when the expression level of AAR was decreased by introducing a mutation into the T7 promoter, to prevent the ADO reaction from being the rate-limiting step.

- The amount of soluble protein and activity are different among AARs & ADOs.
- **7942AAR and 7942ADO have the highest activity.**

④ Purpose

To improve the AAR & ADO activities for biofuel production,

it is important to identify the residues essential for improving the activities of AAR & ADO.

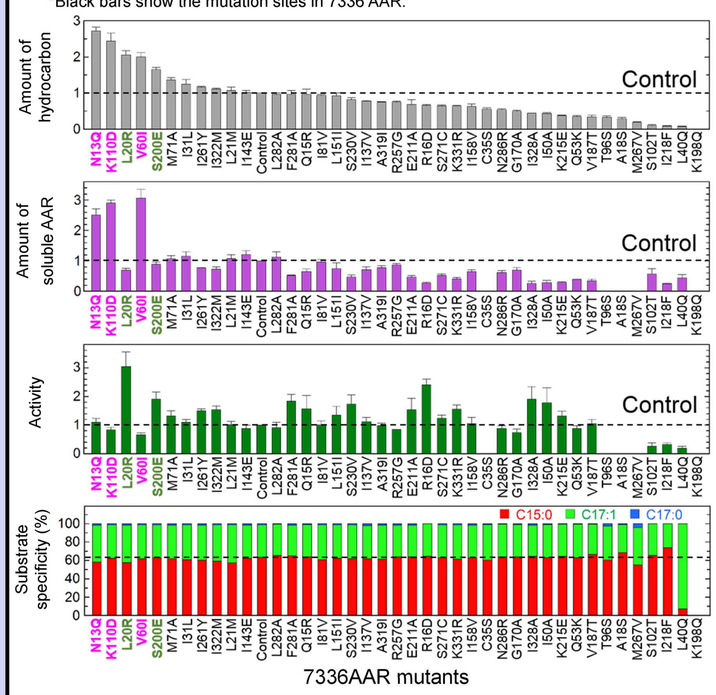
We introduced single amino acid substitutions into AAR & ADO with low activity (7336AAR and 7421ADO), to make these sequences close to those of the highly active AAR & ADO (7942AAR and 7942ADO), respectively.

⑤ Mutational analysis of 7336AAR (40 mutants)

Kudo H *et al.* (2019) *Biotechnology for Biofuels*, 12:291

- S298A of 7336AAR improved the hydrocarbon yield more than 6-fold compared to WT.
- We constructed and analyzed 40 mutants of 7336AAR S298A (Control) with an additional amino acid substitution.

*Black bars show the mutation sites in 7336 AAR.



7336AAR mutants

*The 7336AAR S298A mutants were coexpressed with 73102ADO in *E.coli*.

- 5 mutations remarkably increased the amount of hydrocarbon.

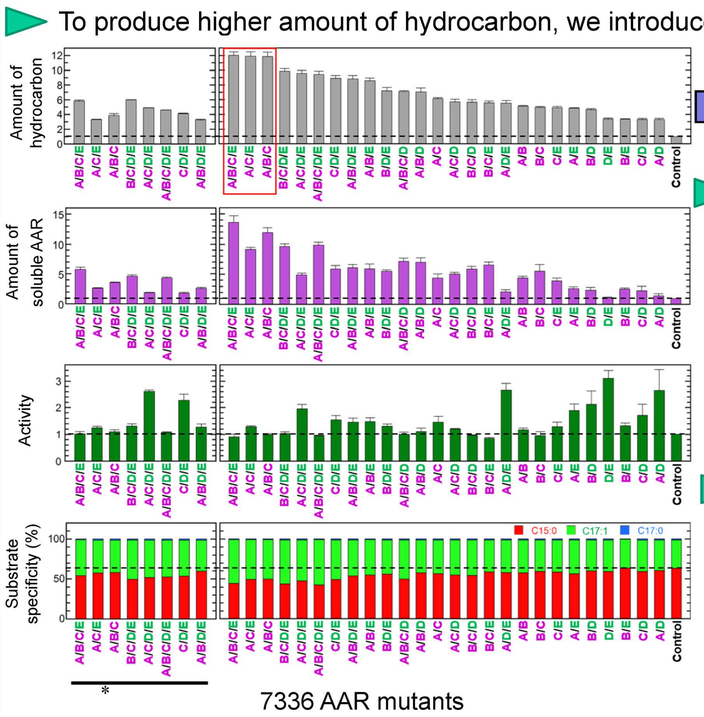
Mutation	Amount of soluble AAR	Activity
N13Q	↑	-
V60I	↑	-
K110D	↑	-
L20R	-	↑
S200E	-	↑

We identified the residues essential for improving the activity of AAR and amount of soluble AAR.

⑥ Multiple mutations of 7336AAR (26 mutants)

Kudo H *et al.* (2019) *Biotechnology for Biofuels*, 12:291

- To produce higher amount of hydrocarbon, we introduced multiple mutations into 7336AAR.



7336 AAR mutants

*The left panel shows the results obtained when the expression level of AAR was decreased by introducing the mutation into T7 promoter, to prevent the ADO reaction from being the rate-limiting step.

Combination of 5 mutations:
N13Q (A), V60I (B), K110D (C), L20R (D), S200E (E)

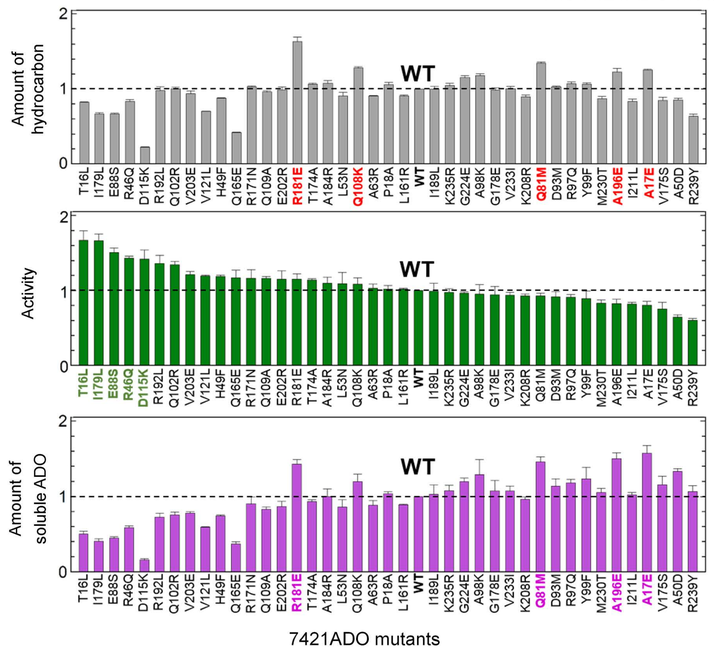
- A/B/C/E, A/C/E, and A/B/C showed **the highest yields of hydrocarbon:**
  ~70-fold higher than WT and ~1.5-fold higher than 7942AAR.
- Multiple mutations improved both the activity of AAR and amount of soluble AAR.

⑦ Mutational analysis of 7421ADO (40 mutants)

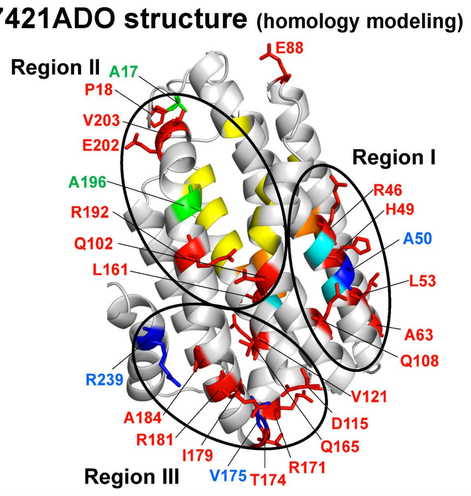
Kudo H *et al.* (2019) *Biotechnology for Biofuels*, 12:89

- We constructed and analyzed 40 single mutants of 7421ADO.

*The 7421ADO mutants were coexpressed with 7942AAR in *E.coli*.



7421ADO structure (homology modeling)



Fe-binding site (Active center: Orange)
Substrate-binding site (Yellow)
Proton channel (Lightblue)

Mutation sites

Activity up↑ (Red)　Activity down↓ (Blue)
Amount of soluble ADO up↑ (Lightgreen)

- Both the activity of ADO and amount of soluble ADO were increased by introducing the single mutations.

We identified the residues essential for improving the activity of ADO and amount of soluble ADO.

- The mutation sites of 19 mutants that improved the activity can be categorized into three regions.

⑧ Conclusions

Enhancement of both the activity and amount of soluble protein of AAR & ADO by multiple mutations is effective in producing higher amount of hydrocarbon.

We succeeded in identifying the residues essential for improving the activities of AAR & ADO and the amounts of soluble AAR & ADO.

⑨ References

Arai, M., Hayashi, Y., Kudo, H. (2018) *Adv. Exp. Med. Biol*. 1080, 119-154.

Kudo, H., Hayashi, Y., & Arai, M. (2019) *Biotechnology for Biofuels* 12, 89.

Kudo, H., Hayashi, Y., & Arai, M. (2019) *Biotechnology for Biofuels* 12, 291.

Chang, M., Shimba, K., Hayashi, Y., & Arai, M. (2020) *Biosci. Biotech. Biochem*. 84(2), 228-237.