Characterization of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase hybrid complex from *Corynebacterium glutamicum*

Saori KOSONO

Graduate School of Agricultural and Life Science; Collaborative Research Institute for Innovative Microbiology, The University of Tokyo

Research objective

Pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase (ODH) are critical enzymes in central carbon metabolism. In *Corynebacterium glutamicum*, which is utilized for industrial production of amino acids, including L-glutamate, an unusual hybrid complex consisting of *Cg*E1p (thiamine diphosphate-dependent pyruvate dehydrogenase, AceE), *Cg*E2 (dihydrolipoamide acetyltransferase, AceF), *Cg*E3 (dihydrolipoamide dehydrogenase, Lpd) along with *Cg*E1o (thiamine diphosphate-dependent 2-oxoglutarate dehydrogenase, OdhA) has been suggested. In this study, the molecular architecture and function of the PDH-ODH hybrid complex from *C. glutamicum* was evaluated to elucidate a novel mechanism for a balanced control of PDH and ODH activities. The potential impact of lysine acetylation on the regulation of the PDH-ODH hybrid complex was also studied.

Methods

In vitro reconstitution of PDH and ODH was performed using recombinant proteins *Cg*E1p, *Cg*E1o, *Cg*E2, and *Cg*E3, which were produced in *E. coli.* The CgE2-*Cg*E3 subcomplex was copurified using N-terminal His-tagged *Cg*E3. Gel filtration chromatography and pull-down assays were used to determine the assembly and subunit interactions of the complex. Ultracentrifugation analysis was performed using *C. glutamicum* lysates to detect PDH and ODH complexes in vivo. K391 variant strains of *C. glutamicum* were constructed using a two-step homologous recombination strategy.

Results

1. **In vitro reconstitution and characterization of the PDH-ODH hybrid complex[1)]**

PDH and ODH activities were successfully reconstituted in vitro using *recombinant E. coli* proteins *Cg*E1p, *Cg*E1o, *Cg*E2, and *Cg*E3. *Cg*E2 formed a stable complex with *Cg*E3 (CgE2–*Cg*E3 subcomplex) in vitro, which was copurified using N-terminal His-tagged *Cg*E3. The molecular mass of the *Cg*E2–*Cg*E3 subcomplex was estimated to be 780 kDa by gel filtration chromatography with molecular size standards, which suggested six copies of *Cg*E2 and eight copies of *Cg*E3, giving two *Cg*E2 trimers and four *Cg*E3 dimers (**Figure 1**). Six copies of

*Cg*E2 in the core are rather compact compared to *E. coli* PDH and ODH that have twenty-four copies of E2. The mixture sample containing *Cg*E1o and *Cg*E2–*Cg*E3 provided a new peak fraction with a higher molecular mass than the individual *Cg*E1o and *Cg*E2–*Cg*E3 and ODH activity. This indicated a reconstituted ODH complex, the molecular mass of which was estimated to be approximately 1 MDa. Meanwhile, the mixture sample containing *Cg*E1p and *Cg*E2–*Cg*E3 exhibited PDH activity, but it was lost after gel filtration chromatography. This indicated that the association of *Cg*E1p with *Cg*E2–*Cg*E3 was too weak to be retained during gel filtration, and thus *Cg*PDH was a fragile complex.

Given that *Cg*E1p and *Cg*E1o subunits are associated with *Cg*E2–*Cg*E3 in a complex, *Cg*E1p (or *Cg*E1o) may compete with *Cg*E1o (or *Cg*E1p) for binding to *Cg*E2–*Cg*E3, resulting in competitive inhibition of ODH and PDH via *Cg*E1p and *Cg*E1o, respectively. As expected, *Cg*E1p- and *Cg*E1o-dependent inhibition of ODH and PDH activities, respectively, were observed. The inhibitory effect of *Cg*E1o on PDH activity was stronger than that of *Cg*E1p on ODH, which was consistent with the results obtained by gel filtration chromatography analysis. These results suggest that *Cg*E1p and *Cg*E1o associate with *Cg*E2-*Cg*E3 with different affinities, and that the change in the ratio of *Cg*E1p and *Cg*E1o associated with the complex may be involved in the balanced control of PDH and ODH activities.

It was previously considered that a *Cg*E1o dimer associates with *Cg*E2–*Cg*E3, as reported for other ODHs; however, it was found that *Cg*E1o existed mainly as a hexamer in solution. *Cg*E1p existed as a dimer in solution, but it migrated to higher molecular mass fractions independent of *Cg*E2 in ultracentrifugation analyses using lysates, suggesting that *Cg*E1p may interact with unknown protein(s) other than *Cg*E2.

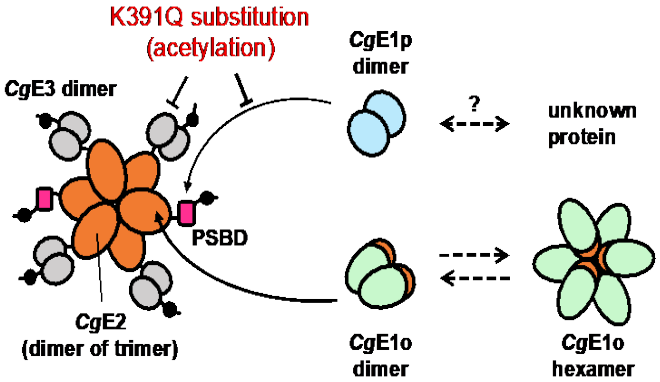

Figure 1 A schematic model of the PDH-ODH hybrid complex of *C. glutamicum*.

2. Characterization of a lysine acetylation site in the peripheral subunit-binding domain (PSBD) of the *Cg*E2 subunit[2)]

Protein lysine acetylation is emerging as a mechanism that controls the activity of metabolic enzymes. Lys391 in the PSBD of *Cg*E2 was found to be acetylated by our acetylome analysis. Interestingly, the *Cg*E2–K391R variant strain showed increased L-glutamate production compared to the wild-type and acetylation-mimic K391Q variant strains. This result suggests that acetylation of Lys391 affects L-glutamate production.

To understand the reason for the increased L-glutamate production in the K391R strain, the effect of K391 substitutions on the assembly and activity of PDH and ODH was analyzed. The acetylation-mimic K391Q substitution, but not the K391R, severely reduced the interaction of *Cg*E2 with *Cg*E1p and *Cg*E3, but did not affect the interaction with *Cg*E1o. This indicated that Lys391 is a critical residue in the assembly of *Cg*E1p and *Cg*E3 into the complex, suggesting that its acetylation affects the assembly of PDH and ODH (**Figure 1**).

Both K391R and K391Q substitutions also affected enzymatic activities, especially PDH. Kinetic analyses of PDH and ODH using *C. glutamicum* lysates showed that the apparent V_{max} value of PDH decreased with the K391R variant and further decreased with the K391Q variant. Interestingly, the apparent K_m values for pyruvate and 2-oxoglutarate decreased in both K391R and K391Q variants. The calculated PDH activity of the wild-type, K391R, and K391Q variants toward 1 mM pyruvate were estimated using the obtained apparent kinetic parameters and the Michaelis–Menten equation. The activity was higher in K391R than in wild-type and K391Q. Pyruvate production was lower in K391R than in wild-type and K391Q.

Collectively, these results suggest that the K391R variant may efficiently convert pyruvate to acetyl-CoA to increase the carbon flux into the citric acid cycle, which may be the reason for the increased glutamate production in the K391R strain.

Conclusion

This study revealed several unique features of PDH and ODH hybrid complexes from *C. glutamicum*. Since the hybrid complex is compact and composed of a smaller number of subunits compared to *E. coli* PDH and ODH, the change in the subunit stoichiometry may easily affect the balance between PDH and ODH activities, which critically influences the central carbon flux. It has also been suggested that lysine acetylation has an impact on PDH and ODH function and L-glutamate production.

References

1) Kinugawa H., Kondo N., Komine-Abe A., Tomita T., Nishiyama M., Kosono S. (2020) In vitro reconstitution and characterization of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase hybrid complex from *Corynebacterium glutamicum*. *MicrobiologyOpen* **9**:

e1113.

2) Komine-Abe A., Kondo N., Kubo S., Kawasaki H., Nishiyama M., Kosono S. (2021) Characterization of lysine acetylation in the peripheral subunit-binding domain of the E2 subunit of the pyruvate dehydrogenase-2-oxoglutarate dehydrogenase hybrid complex from *Corynebacterium glutamicum*. *Biosci. Biotechnol. Biochem*. **85**:874-881.