Immune activating factor from fermented foods: Chemical synthesis and function of acetic acid bacterial lipid A

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Abstract

Lipid A, a membrane component of Gram-negative bacteria, is known as a representative innate immune stimulator, and we have developed low-inflammatory lipid A as an adjuvant. In this study, we focused on Kurozu, a traditional Japanese health food (black vinegar), which has been empirically reported to improve host immune functions and allergic symptoms. In this study, lipid A from *Acetobacter pasteurianus*, an acetic acid bacteria used in the fermentation process of Kurozu, was expected to be a safe and acid-resistant adjuvant candidate whose safety for is assured by food experience. *A. pasteurianus* lipid A, which consists of a unique tetrasaccharide backbone containing mannose and glucuronic acid, was synthesized by a systematic synthetic strategy involving challenging stereoselective 1,1′-α,α-glycosylation. Structure-activity relationship studies revealed that the glucuronic acid moiety is important for immune function and acid resistance.

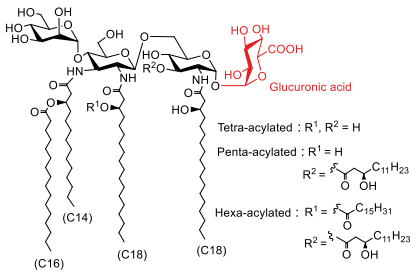
Background & Results

Lipopolysaccharide (LPS), an extracellular membrane component of Gram-negative bacteria, is one of the representative immunostimulators, and its active center is glycolipid lipid A. Canonical *Escherichia coli* lipid A exhibits potent immunostimulatory activity but also lethal toxicity, thus making it difficult to develop as an adjuvant. We have found that lipid A from *Alcaligenes faecalis*, which is resident in host Peyer's patches, an intestinal mucosal immune regulatory tissue, can effectively activate both mucosal and systemic immunity without inducing toxicity, and have developed it as a promising mucosal vaccine adjuvant. However, since *A. faecalis* lipid has a glycosyl phosphate structure that is unstable under acidic conditions, it is expected to be degraded in gastric acid when administered orally. Of course, enzyme resistance etc. would be necessary, but first we aimed to improve the acid resistance of lipid A. Focusing on acetic acid bacteria *A. pasteurianus* lipid A contained in fermented Kurozu, whose safety is anticipated by food experience and immune activation by oral intake has also been suggested, we systematically synthesized *A. pasteurianus* lipid A to identify the active center of *A. pasteurianus* LPS and evaluated their functions.

The trehalosamine skeleton containing the challenging 1,1'-α,α-glycosidic linkage was efficiently constructed by glycosylation using borinic acid as a catalyst, referring to the trehalose skeleton construction by Takemoto et al. (*Angew. Chem. Int. Ed.* 2020). A common tetrasaccharide intermediate was constructed by [2+2] glycosylation, followed by long-chain fatty acid condensation and deprotection, and the first chemical synthesis of *A. pasteurianus* lipid A was achieved. Hexa-acylated type showed the strongest immunostimulatory activity, identifying the active center structure of *A. pasteurianus* LPS. Furthermore, structure-activity relationship studies revealed that the glucuronic acid moiety is important for both immune function and acid resistance, providing a guideline for acid-resistant lipid A molecular design.

Significance of the research and Future perspective

Since antigens alone do not effectively confer immunity, the addition of adjuvants is necessary to increase the efficacy of vaccines. However, it is not easy to develop adjuvants that are both effective and safe. Acetic acid bacteria lipid A is expected to be a seed for adjuvants having both safety, chemical stability, and immunostimulatory properties.



A. pasteurianus Lipid A

Figure 1

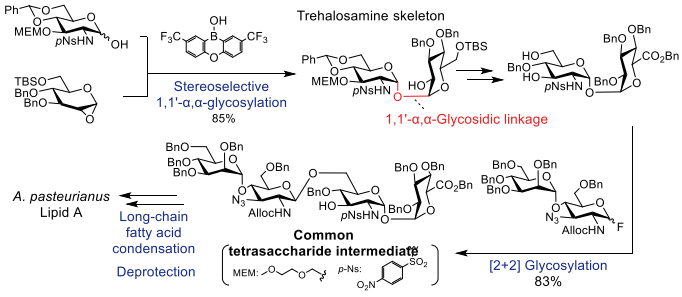


Figure 2

Patent	Japanese Patent No. 7092308
Treatise	Shimoyama, Atsushi; Fukase, Koichi et al. Chemical synthesis of *Acetobacter pasteurianus* lipid A with a unique tetrasaccharide backbone and evaluation of its immunological functions. Angew. Chem. Int. Ed. 2024, 63(24), e202402922. doi: 10.1002/anie.202402922 Shimoyama, Atsushi; Kunisawa, Jun; Fukase, Koichi et al. Lipopolysaccharide from gut-associated lymphoid-tissue-resident *Alcaligenes faecalis*: Complete structure determination and chemical synthesis of its lipid A. Angew. Chem. Int. Ed. 2021, 60(18), 10023-10031. doi: 10.1002/anie.202012374
URL	
Keyword	adjuvant, vaccine, acetic acid bacteria, immunity, glycosylation