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Search for Salt-Enhancement Materials from Natural Resources by Using GABA-Synthesizing Enzyme, Development of Low-Salt Content Foods, and Study of Salt Signal Transduction Mechanism

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Summary

Low salt diet is desired for those people concerning high blood pressure, diabetes, kidney disease, etc. Those having a risk toward high blood pressure and diabetes, called life style related disease, are also targeted for low salt diet. For elderly people especially, low salt diet tends to decrease quality of taste due to the salt effect on Umami. We have been interested on the effect of salt on Umami how salt information interacts with Umami on the taste cells. Recently, we found that GABA producing enzyme, GAD67, is expressed in type III taste cells where expression of $GABA_A$ receptor was also shown. Since $GABA_A$ receptor is known as GABA-gated chloride ion channel, the results suggested a possibility that GABA may play some role in salt signal pathway. We went through extensive human taste tests and found that some spice extracts enhanced salt taste with maintaining Umami. Hence, in this project, following themes were proposed. 1) Screening salt enhancer from natural products by extracting effective components. 2) Preparing low salt recipe that contains salt enhancer from natural products. Low salt bread and other foods are compared with foods prepared with normal recipe by human taste tests. 3) Appling Surface Plasmon Resonance method, salt signal transaction pathway is investigated.

Spice extracts were incubated with GAD67 enzyme and substrate, L-glutamate, where GABA produced was quantitatively measured. At the same time, those spice extracts were mixed with standard salt water where panelists examined taste if saltiness be enhanced or reduced. Both enzyme activity change and saltiness change were converted to numbers, hence, correlation plot was drawn. We observed from these *in vitro* experiments there is a rule that those increased GAD activities can be salt enhancers. Screening was carried out to make a library for spices that enhance salty tastes.

Meanwhile, we prepared low-salt recipe for bread, dressing, and soup. Specific aim was to maintain saltiness and umami taste by adding spice extracts, since lowering the salt contents reduces umami taste. Some of spice extracts indeed showed salt enhancing effect and maintain umami taste, but those spice extracts were not necessarily the same spice extracts activated GAD67. The reasons are not clear at the moment, but it is projected that actual food materials contain multiple materials, including many free amino acids, that affected the taste. We shall continue to investigate this point.

About the signal transaction for salt, protein complex formation was investigated. Peptides having hydrophobic region found in N-terminal region of GAD65 and 67 were synthesized and attached to Biacore sensor chips. Interaction with brain extracts, there was a protein fraction positively interacted with GAD67peptide#1. After tryptic digestion and LC-MS analysis, we found this protein was GAPDH. GAD67 native protein was also found to interact specifically with GAPDH; thus, it is probable that GAD67 and GAPDH forms a protein-protein complex. It has been known that GAPDH interacts with alpha1 subunit of chloride ion channel, probably phosphorylates Ser/The residue with its kinase activity. We also found LDH to interact with both GAD67 and GAPDH. Physiological meaning of the complex formation between these three proteins are still not clear. We strongly believe they play important roles in salt signal pathway.