【Grant-in-Aid for Scientific Research (S)】

Development of structure analysis method for femto to picogram scale metabolites

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Purpose and Background of the Research

●Outline of the Research

This study aims to develop the Micro-scale Crystalline Sponge method (MicroCS), a technique enabling structural analysis of compounds ranging from femto- to picogram quantities. In cutting-edge life science research, numerous compounds with significant physiological activity exist in extremely small quantities within living organisms, making it challenging to determine their structures. This study seeks to develop the MicroCS method, which will enable the structural analysis of ultra-trace substances, particularly metabolites, ranging from femto- to picogram quantities. This endeavor targets a paradigm shift in life science and drug discovery research.

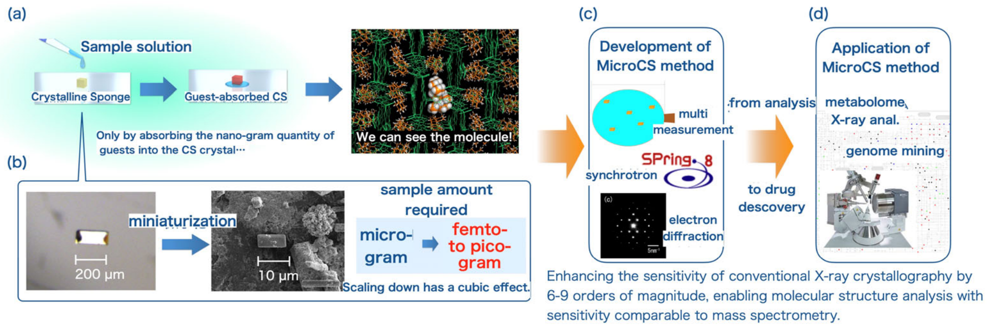

Figure 1. Overview: (a) The Crystal Sponge method (CS method) which does not require sample crystallization; (b) (c) Concept of this study: Development of MicroCS method, which improves the sensitivity of CS method by 6-9 orders of magnitude through miniaturization; (d) Application of MicroCS method.

●Background: As history proves, the emergence of new molecular structure analysis techniques has repeatedly led to breakthroughs in natural science research. Among the various methods of molecular structure analysis, the most reliable technique is single crystal X-ray crystallography, which directly provides information about the three-dimensional structure of molecules. However, X-ray crystallography has a significant measurement barrier that cannot be avoided: the requirement for "crystallization of the sample," which has long been a challenge for researchers.

In 2013, we introduced the X-ray structural analysis technique called "crystalline sponge method (CS method)," which does not require crystallization of the sample (M. Fujita et al., Nature 2013, 495, 461). By utilizing the periodically arranged space in porous metal complexes as a template, it becomes possible to create a periodic arrangement of absorbed organic molecules afterwards. Applying this principle to single crystal X-ray structural analysis led to the CS method (Figure 1a). The CS method has expanded its application as a versatile molecular structure analysis technique not only in academic fields such as organic chemistry, natural product chemistry, and biochemistry but also in industrial sectors including pharmaceuticals, foods, and fragrances.

●Research Objective: The crystalline sponge (CS) method eliminates the need for the conventional single crystal preparation step, which typically requires milligram quantities of samples, allowing the required sample amount for measurement to be reduced to the microgram order, approaching the detection limit of the instrument. In this study, we aim to develop a MicroCS method capable of analyzing molecular structures in the femto- to pico-gram range by leveraging the use of extremely small CS crystals (~10 μm) and high-sensitivity electromagnetic diffraction methods (such as synchrotron X-ray multi-measurement or electron diffraction) (Figure 1b,c). This will substantially improve the measurement sensitivity of conventional X-ray structural analysis by 6-9 orders of magnitude, enabling the establishment of a groundbreaking molecular structural analysis technique that can analyze the three-dimensional structures of molecules with sensitivity comparable to mass spectrometry. Furthermore, we aim to demonstrate that the MicroCS method serves as a powerful booster for various research fields (especially drug discovery research) facing bottlenecks in the structural analysis of minute unknown compounds.

Expected Research Achievements

●Development of Microcrystalline Sponge Method (MicroCS Method): By downsizing the crystalline sponge (CS) crystals used in the CS method, leveraging the cubic volume effect where the scale-down effect works cubically, we aim to reduce the weight of samples required for analysis to the femto to picogram range. We believe that the MicroCS method in the femto to picogram range can be realized by reducing the technical barriers associated with handling tiny crystals, such as capillary manipulation and the use of pipettors and 96-well plates.

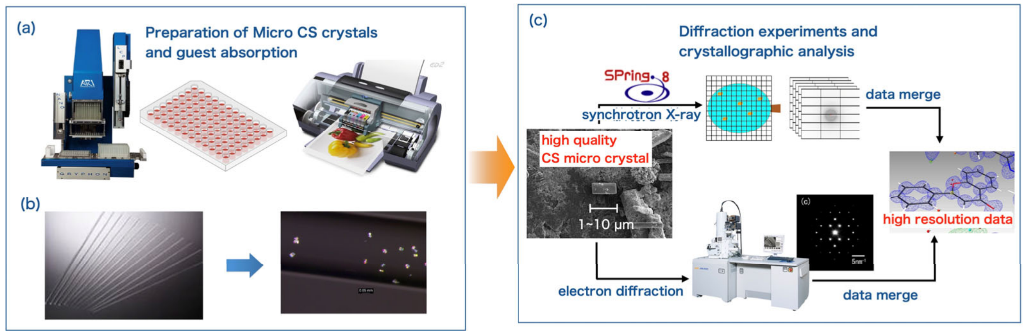

Figure 2. **MicroCS Method:** (a) Reduction of crystalline sponge (CS) crystal size using devices and equipment such as pipettors, 96-well plates, and inkjet printers, and (b) miniaturization of the synthesis, solvent exchange, and guest absorption processes of microcrystalline CS utilizing capillary spaces. (c) Utilization of diffraction experiments using synchrotron X-rays or electron beams, as well as analysis techniques such as data merging, to achieve high-resolution analytical results.

●Structural Analysis of Trace Physiologically Active Compounds : At the forefront of life science research, the structural analysis of trace unknown compounds often becomes a limiting factor, hindering their exploration. Focusing on such cutting-edge fields as genome information exploration, metabolome analysis, and soil microbiology research, we actively engage in collaborative research with researchers in these fields to elucidate the structures of crucially active compounds that act in trace amounts.

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