High-throughput production of selenomethionine-labeled proteins in 2-liter PET bottles using an auto-inducing medium

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The production of protein labeled with selenomethionine (SeMet) has become a standard approach for determining experimental phases and structures by X-ray crystallography. CESG has implemented a high-throughput protocol for the routine production of SeMet-labeled proteins that provides high yield cell growth, maximizes expression and solubility, and gives a high percentage incorporation of SeMet. The protocol uses a 2-liter PET beverage bottle as the growth vessel and the Met auxotroph and codon-adapted strain *Escherichia coli* B834 pLacIRare as the expression host. An initial expression of the unlabeled protein in a medium containing tryptone and induced using 1mM IPTG has been used to assess target-dependent properties such as cell mass yield, protein expression level, solubility, purity after semi-automated purification, proteolysis from protein tags upon treatment with tobacco etch virus protease, stability of the cleaved protein, and identification of crystallization conditions. Cumulative favorable results from these successive pipeline steps triggers production of the SeMet-labeled protein, which is undertaken in a supplemented minimal medium containing SeMet, all other amino acids except Met, vitamins, trace metals, and the auto-inducing mixture of glucose and lactose. The complete cell growth process from inoculation of the PET bottles to cell harvest takes ~24 h, with protein expression commencing in the auto-inducing medium after ~9 h. The cell mass yields are target-dependent, with an average 7.2 ± 1.5 g of wet cells obtained per liter of culture medium (n = 29 different protein targets). Evaluation of the CESG database shows a strong correlation between the yield and purity results obtained in either the unlabeled or the SeMet-labeled proteins. ESI mass spectral analyses of SeMet-labeled proteins shows 90 ± 5% substitution of SeMet for Met. Recent examples of crystal structures solved from proteins expressed using this protocol will be presented. (This research funded by NIGMS GM645980).