60/125

Poster 9

Cost-effective Isotope-labeling Technique Developed for $^{15}N/^{13}C$-labeled Novel Human Protein Tyrosine Kinas6(PTK6)

Eunmi Hong, Joon Shin, Min-hyung Kim, Seung-taek Lee and Weontae Lee
Dept. of Biochemistry, College of Science, Yonsei University, Seoul, 120-740, Korea

Non-receptor protein tyrosine kinases(PTKs) plays an essential role in important intracellular functions such as cell proliferation and differentiation by signal transduction from cell surface receptors to their intracellular targets. Protein tyrosine kinases 6(PTK6),a member of the family of non-receptor protein kinase,was first identified by reverse transcriptation-PCR of normal human melanocyte mRNAs and suggested to be related in the development and differentiation of certain cancer and epithelial cells. PTK6 consists of a src homology 2(SH2), a src homology 3(SH3) and catalytic tyrosine kinase domains. Human PTK6 SH2 domain was cloned, overexpressed and purified to characterize biochemical and structural properties. We cultured the cells initially in M9 minimal medium with ^{12}C-D_6-glucose and then provided isotopically labeled ^{13}C-D_6-glucose only after the induction for cost-effective purification of double labeled protein in the ^{15}N, ^{13}C source (the yield of labeled protein >95%). We observed the decrement of ^{12}C-D_6-glucose and the turbidity of the M9 minimal medium to determine the changing point of ^{12}C-D_6-glucose to ^{13}C-D_6-glucose. A limited amount of ^{12}C-D_6-glucose is indispensable in the early stage of culture but it should be removed from the M9 minimal medium for the uniform labeling. We harvested the cells and removed the ^{12}C-D_6-glucose when the value of OD_{600} reached 6-7. As a result, we successfully removed all ^{12}C-D_6-glucose in the M9 cultural medium before the protein(SH2) synthesis is initiated. The labeling of $^{15}N/^{13}C$ was finally confirmed by both ^{15}N-H HSQC and triple-resonance 3D experiments. In conclusion, this result will help us to save expensive isotopes with same isotope-labeling efficiency.