

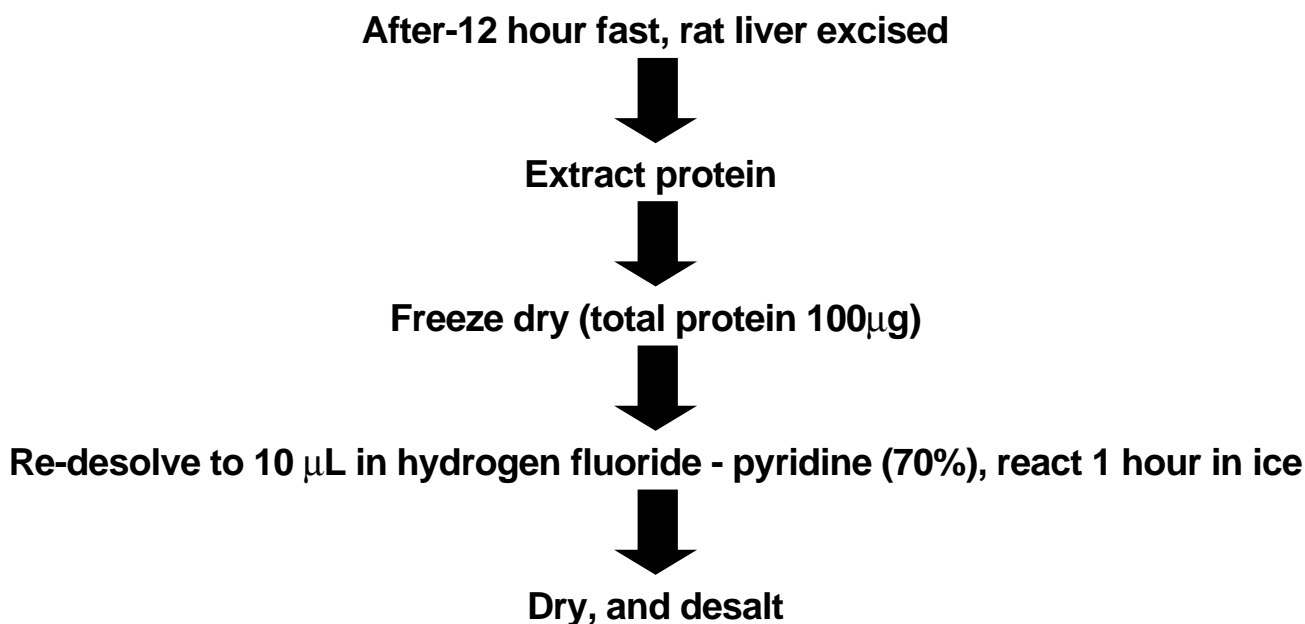
Detection of Phosphorylated Proteins by Chemical Dephosphorylation with Hydrogen Fluoride

The phosphorylation of proteins plays an important role in a variety of cellular functions, such as information transmission, growth, metabolism, multiplication, movement, differentiation, and cancerous change. The analysis of protein phosphorylation is an important component in ongoing efforts to describe such life phenomena.

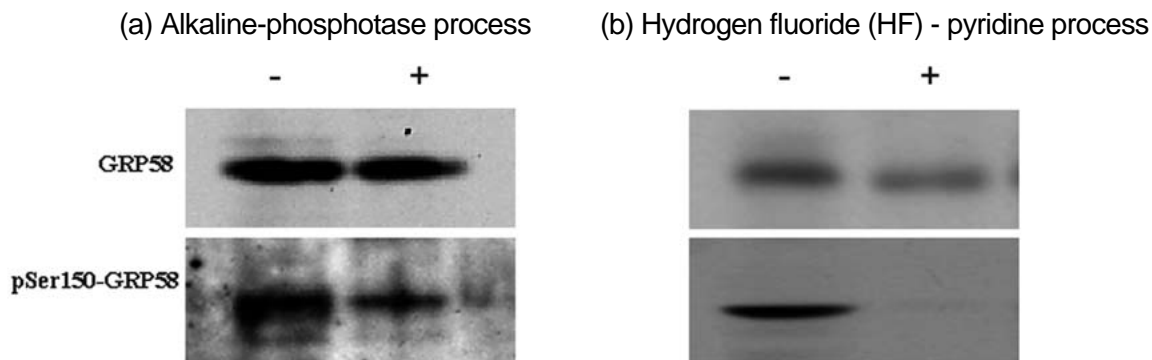
One well-used method of detecting phosphorylated proteins utilizes a technique of protein dephosphorylation. This analysis involves conducting electrophoresis before and after dephosphorylation, and detecting the corresponding protein band shift.

Up to now, the majority of protein dephosphorylation has been accomplished by using alkaline phosphatase to effect phosphoester cleavage. However, incomplete dephosphorylation has often been observed with this method due to specific enzyme-substrate interaction characteristics. We previously reported on a technique developed to resolve this problem, in which hydrogen fluoride is used to conduct chemical dephosphorylation. The hydrogen fluoride method goes to completion without any sub-reactions or any of the adverse effects due to differences in substrate characteristics observed in enzymatic dephosphorylation. We introduce here a recent application in which this method is used to conduct protein dephosphorylation using a biological sample (α GRP58)².

① Main process flow to protein dephosphorylation using hydrogen fluoride (HF) - pyridine (70%) (Fig. 1)



②Dephosphorylation of α GRP58 using hydrogen fluoride (HF) - pyridine (70%), alkaline-phosphatase (Fig. 2)



It was confirmed that whereas dephosphorylation by alkaline-phosphatase did not come to completion (Fig. 2 (a)), dephosphorylation by hydrogen fluoride was completed (Fig. 2 (b)). The α GRP58 and phosphorylated α GRP58 were detected with α GRP58 antibody and pSer150- α GRP58 antibody, respectively.

This example was the first application in which we used HF to perform chemical dephosphorylation of natural proteins. We believe this methodology may prove useful in future research on phosphorylated proteins.

*This data was kindly provided by Professor Katsuya Nagai, Laboratory of Proteins Involved in Homeostatic Integration, Institute for Protein Research, Osaka University.

Literature cited: 1)H.Kuyama , C.Toda, M.Watanabe, K.Tanaka, O.Nishimura,
" An efficient chemical method for dephosphorylation of phosphopeptides "
Rapid Commun.Mass Spectrom., 2003;17: 1493-1496.

2)K.Kita, N.Okumura, T.Takao, M.Watanabe, T.Matsubara, O.Nishimura, K.Nagai,
"Evidence for phosphorylation of rat liver glucose-regulated protein 58, GRP58/ERp57/ER-60,
induced by fasting and leptin"
FEBS Lett. 2006; 580:199-205



JQA-0376