One Step Purification of a Yeast Protein Tyrosine Phosphatase, YPTP1, Overexpressed in *E. coli*

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Protein tyrosine phosphatases (PTPases) are enzymes that hydrolyze phosphoryl moiety from the phosphotyrosine residue of cellular proteins.[1,2] Much attention has recently focused on PTPases as well as protein tyrosine kinases, because the reversible protein tyrosine phosphorylation plays an essential role in various cellular processes. Previously we overexpressed a yeast PTPase, YPTP1, in *E. coli* expression system and studied its substate specificity.[3] Purification of overexpressed YPTP1 was accomplished by conventional chromatography technique which requires four purification steps.[3]

Development of a good affinity ligand for PTPases might circumvent the laborous steps for the purification of PTPases thus facilitating the studies on PTPases. Because phosphotyrosine itself is hydrolyzed by PTPases, nonhydrolyzable phosphotyrosine analogs have to be used for PTPase purification. Phosphonic acid, in which the O atom between the P atom and the benzene ring was substituted with a CH_2 group, can be a choice of such affinity ligands. *para*-Aminobenzylphosphonic acid immobilized on agarose (pABPA-agarose) was successfully used as an affinity ligand in a later stage of the purification of a PTPase which dephosphorylates the nicotinic acetylcholine receptor.[4] When we tested pABPA-agarose for the purification of YPTP1, purified enzyme binds to pABPA-agarose but, in the presence of excess of other proteins, YPTP1 does not bind to the resin.

A different version of phosphonic acid derivative, L-histidyldiazobenzylphosphonic acid-agarose (HBPA-agarose, SIGMA) (Figure 1), was employed for the purification of YPTP1 overexpressed in *E. coli*. HBPA-agarose had previously been used for the purification of alkaline phosphatase[5] but its use for the purification of PTPases had not been precedented. HBPA-agarose affinity chromatography of the crude lysate from *E. coli* cultures overexpressing YPTP1 afforded YPTP1 of apparent homogeneity. Typically, chromatography of the crude lysate obtained from 50 mL of *E. coli* culture on HBPA-agarose (bed volume 7.4 mL) gave with >90% yield YPTP1 of the specific activity[6] of 35 μmol/min/mg, higher than the value of 31 μmol/min/mg obtained with the enzyme purified by conventional techniques.[3] As the quantity of total proteins applied to the column increases, the purity of YPTP1 tends to decrease. Application of 100 mL of *E. coli* culture to the same size column afforded YPTP1 of about 80% purity.

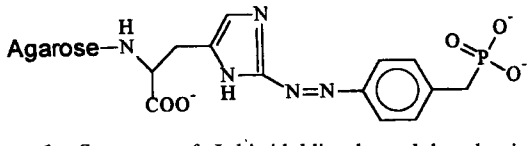

Figure 1. Structure of L-histidyldiazobenzylphosphonic acid-agarose (HBPA-agarose).

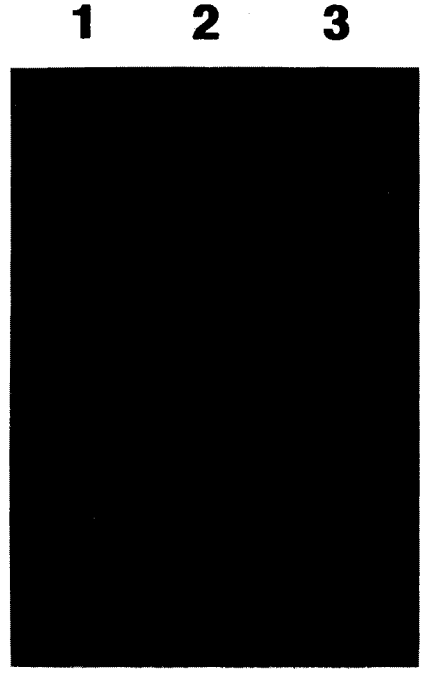

Figure 2. SDS-PAGE analysis of the results of affinity chromatography of YPTP1. Lane 1, molecular weight standards, from top to bottom: 205, 116, 97, 84, 66, 55, 45, 36 kD. Lane 2, crude cell lysate. Lane 3, after affinity chromatography on HBPA-agarose column.

Typical purification procedure is as follows. Crude lysate obtained from 50 mL cultures of *E. coli* BL21(DE3) carrying pT7-7-YPTP1 plasmid[3] was diluted with 10 mL of buffer A (30 mM TrisHCl, 2.5 mM EDTA, 10 mM β-mercaptoethanol, 1mM benzamidine, pH 7.0). Resulting solution was loaded on a HBPA-agarose column (13 mm × 56 mm) by gravity and unbound materials were washed with 5 mL of the same buffer. Elution of the column with buffer A containing 1 M NaCl and collection of active fractions afforded YPTP1 with apparent homogeneity when analyzed by polyacrylamide gel electrophoresis and Coomasie blue staining of the gel (Figure 2).

The potential applicability of the use of HBPA-agarose for the purifications of YPTP1 from *S. cerevisiae* and other PTPases from other organisms are now under investigation.

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3418).

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6. For PTPase assay, p-nitrophenyl phosphate (pNPP) was used as a substrate. The enzyme was added to the reaction mixture containing pNPP in reaction buffer (10 mM pNPP, 100 mM HEPES, 10 mM DTT, 5 mM EDTA, pH 7.0). The reaction was quenched by addition of 1.0 mL of 0.5 N NaOH solution and the absorbance was measured at 405 nm. *p*-Nitrophenol released was quantitated using a standard curve determined for *p*-nitrophenol.

New Excitation Technique using Jet Collision in a Supersonic Expansion

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The spectroscopic studies of highly excited transient molecules are of considerable interest in both theoretical and experimental chemists because they are believed to play very important roles in explaining the reaction mechanism.[1] One of the most powerful methods for observing these molecules is to use the technique of emission spectroscopy which has greatly contributed to the understanding of molecular structure.[2] In emission spectroscopy, the molecules are excited to high energy states by taking energy from the external sources such as microwave discharge, electric discharge, chemical reaction, photolysis, etc.[1] Of these, the method of electric discharge has been long employed for the generation and excitation of transient molecules.

The supersonic free jet expansion has been proven to be a powerful spectroscopic tool for obtaining the spectrum of molecular species in the gas phase since the early work on NO_2 by Smalley *et al.*[3] The spectral simplification and stabilization associated with the inert gas expansion usually cannot be obtained in any other ways. The combination of supersonic expansion technique with the emission method has had an enormous impact on the repertoire of spectroscopic molecular studies that can be carried out. Of the emission sources developed so far for these purposes, the only one giving enough continuous photon intensity for high resolution studies of weak transitions in a jet is the Engelking-type corona discharge which has been widely used for the observation of the vibronic emission spectrum of transient molecules.[4,5] This has been applied for the observation of vibronic emission spectra of rotationally cooled transient molecules in the gas phase.[6,7,8] Nevertheless, this method is only suitable for the transitions of large Franck-Condon factor as well as of small excitation energy.

A technique using a jet collision in a expansion chamber has been devised as a method of more effective energy transfer. Recently, Cossart and Cossart-Magos[9] have succeeded the observation of the emission spectra of highly excited CO^+ employing jet collision of metastable Ne atom and CO molecule generated from Geissler-type electric discharge. The same technique has been applied for the generation of CS^+ from the collision of metastable He atom with long-lived CS radical.[10] Similar methods have been used for the study of the energy transfer reaction between N_2 and CO under molecular beam conditions.[11] Very recently, Tokeshi *et al.* employed the ion-molecule collisions to generate the unstable molecular ion for observing the emission spectra of CH ($A^2\Delta$-$X^2\Pi$) produced in collisions of Ar^+ with aliphatic compounds.[12]

Recently, we have determined to develop in our laboratory new excitation technique which is useful for the vibronic emission spectra of highly excited transient molecules using jet collision in a corona excited supersonic expansion.

Figure 1 shows the schematic diagram of the jet collision in a corona excited supersonic expansion. The experimental apparatus used in this experimemt are similar to those reported previously.[13] The collision chamber was made of six-way cross Pyrex tube of 50 mm inner diameter. The nozzle was made of thick walled quartz tube of 12 mm outer diameter and 2 mm thickness, narrowed one end by flame heating to a capillary of the desired pinhole size, and connected with threaded adaptor (Ace glass model 5027-05). The anode located in the center of the nozzle tube with teflon holder was connected to the high voltage electric dc power supply (Bertan model 210-05R). The cathode made of a copper rod of 1.5 mm diameter and 100 mm length was placed to the parallel with target molecular jet through the Pyrex glass joint tube. Two nozzles perpendicular to each other were placed inside the chamber to produce the target and colliding jets. In this experiment, pinhole nozzles of 0.3 mm and 0.5 mm opening have been employed for the generation of metastable helium atomic and excited nitrogen molecular jets, respectively. The distance between the head of both nozzles was adjusted for the maximum excitation of the nitrogen molecules by the metastable He