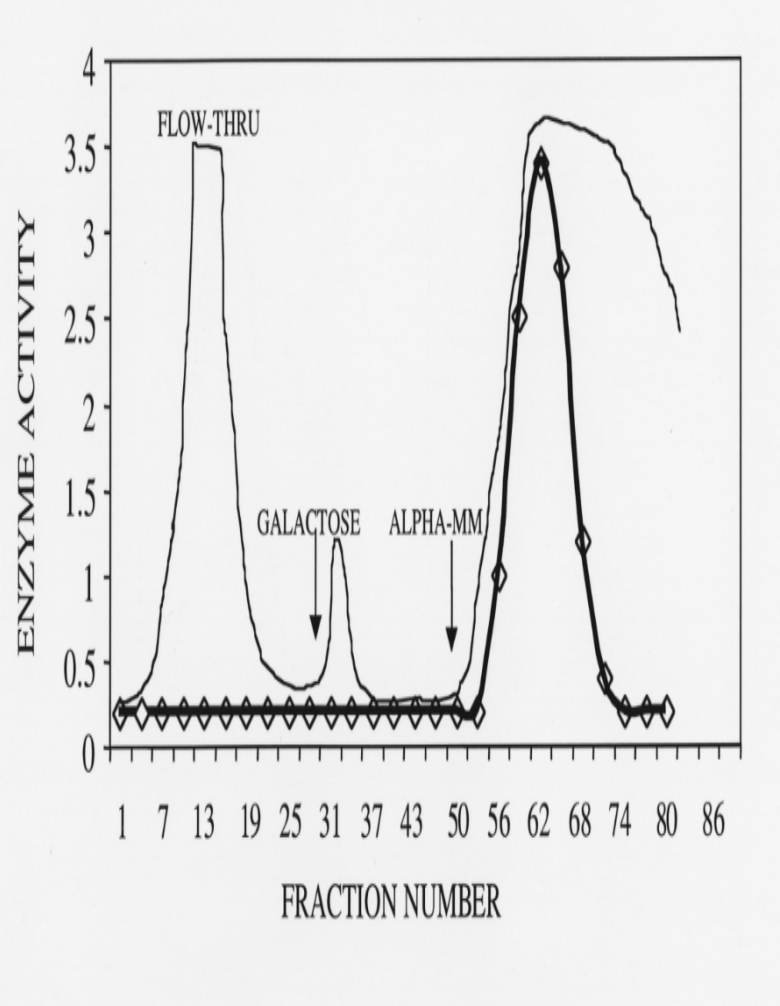

Figure 1. Concanavalin A affinity chromatography. The prepared protein solution in 1% Triton X-100 was applied to a Concanavalin A affinity column. The absorbance at 280 nm (indicated by the thin line) was monitored continuously and reflected protein concentration as well as Triton X-100 levels. The column was eluted stepwise with buffer A containing 1% Triton (Flow thru), buffer A containing 0.1% Triton and 0.3M galactose and buffer A containing 0.1% Triton and 50 mM alpha methyl mannoside (alpha-MM). The enzyme activity was assayed in a reaction mix containing *p*NPP (indicated by the thick line).

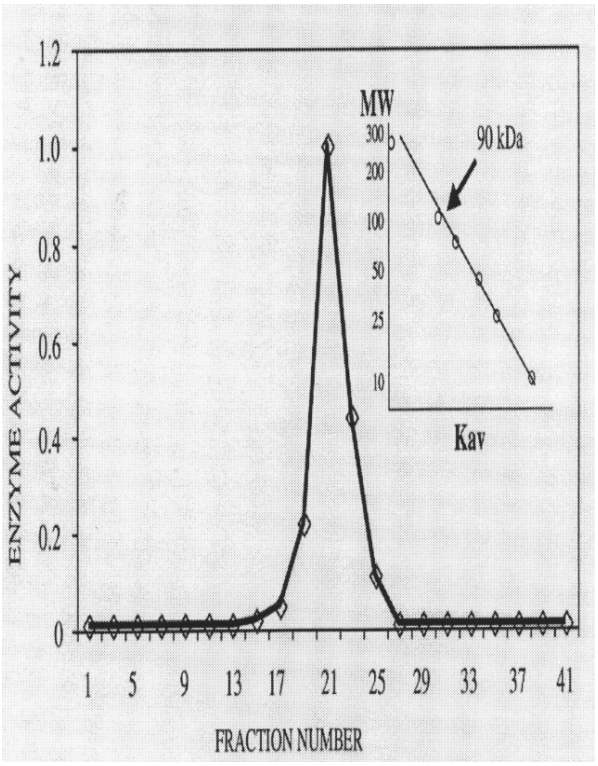

Figure 3. 300SW gel filtration. The pooled active fractions from the DEAE column were concentrated by a YM100 column and applied to a 300SW gel filtration column. The column was equilibrated with buffer A containing 0.1% Triton X-100 and 0.3M NaCl and eluted with the same buffer. 5NU activity was determined in each fraction and compared to standard molecular weight markers. The protein eluted at a position corresponding to ~90 kDa.

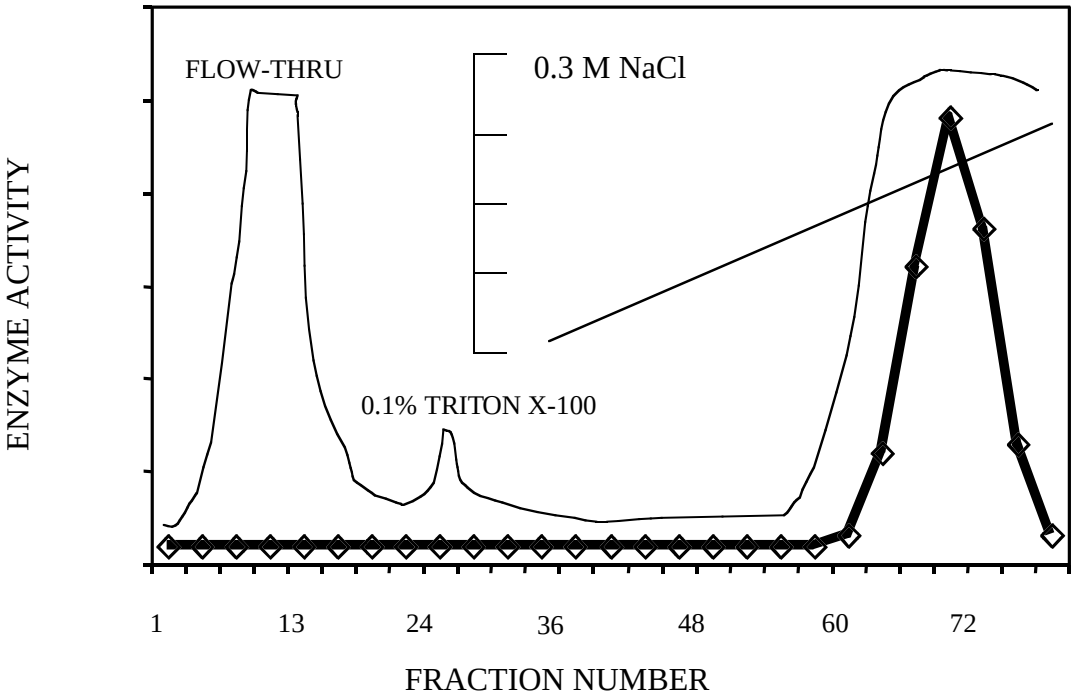

Figure 2. DEAE HPLC. The pooled active fractions from Concanavalin A chromatography were applied to a DEAE sephadex column. The column was washed with buffer A containing 0.1% Triton X-100 (0.1% Triton X-100), then, eluted with a linear gradient of buffer A containing 0.1% Triton and 0.3M NaCl. The thick line shows the peak of enzyme activity. About 35 total units were obtained from the column. The absorbance at 280 nm (indicated by the thin line) was monitored continuously and reflected protein concentration as well as Triton X-100 levels.

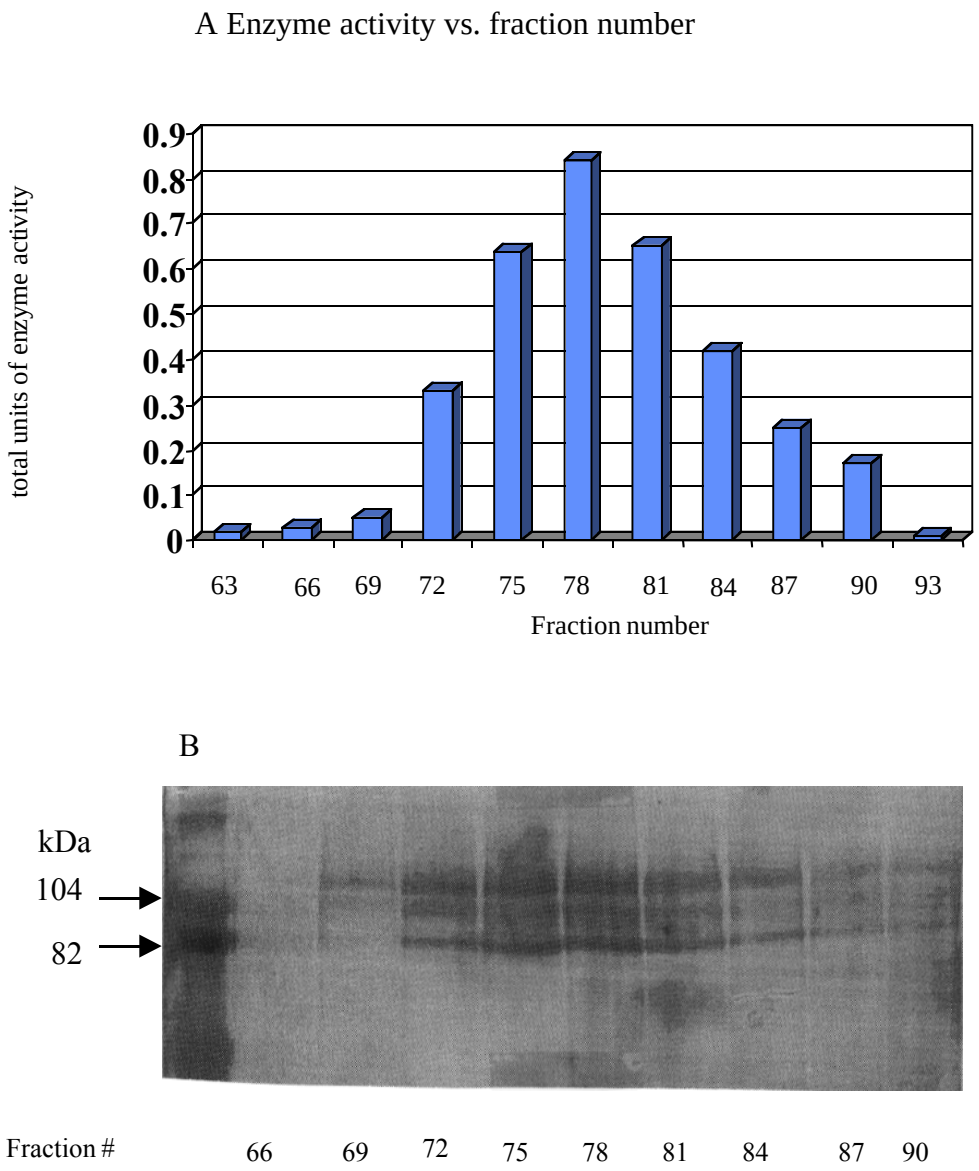

Figure 4. Comparison of 5NU enzyme activity and SDS PAGE analysis in fractions from the 300SW column. A. Enzyme activity vs. fraction number. B. 20 ml of each active fractions was mixed with 3 ml of 10xSDS loading dye and loaded on 5% SDS gel. After electrophoresis, the gel was silver stained. Three bands of 90, 120 and >120 kDa were seen. The first lane of the gel contained standard marker protein.

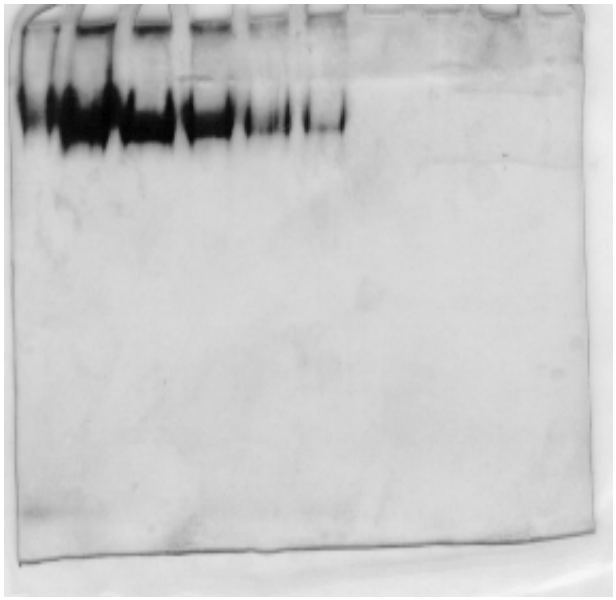

Figure 5. Activity stain of alkaline phosphatase on non-denaturing gel electrophoresis. Each lane contained an active fraction from Concanavalin A affinity chromatography. The gel was stained with reaction containing NBT and BCIP.

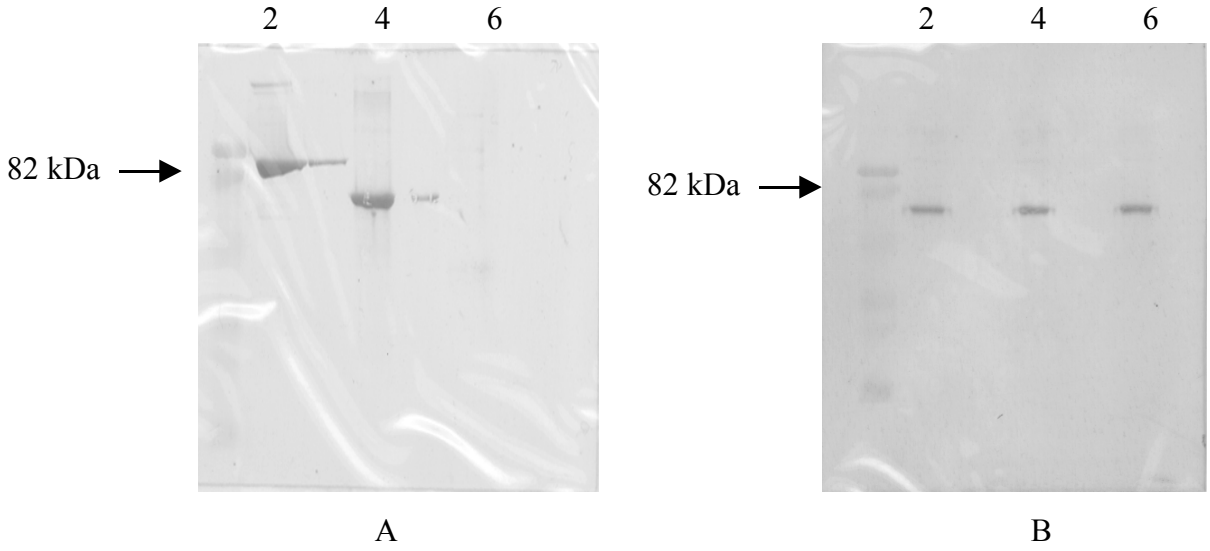

Figure 6. Test of the efficiency of protein transfer onto PVDF membrane. 7.5% SDS gel was prepared. Figure 6A, lane 1 contained standard protein marker. Lane 2 contained 1 microgram of glycogen phosphorylase A suspended in stacking buffer. Lane 3 contained 0.1 microgram of glycogen phosphorylase A. Lane 4 contained 1 microgram of BSA suspended in stacking buffer. Lane 5 contained 0.1 microgram of BSA (as did lane 2, 4 and 6 from figure 6B). Nothing is in lanes 3 and 5 in B. Lane 6 of Fig 6A contained 20 microliters of the most active fraction from 300SW column after concentration by a YM100 spin column. After electrophoresis, the gel was electroblotted onto PVDF membrane and stained with amido black.

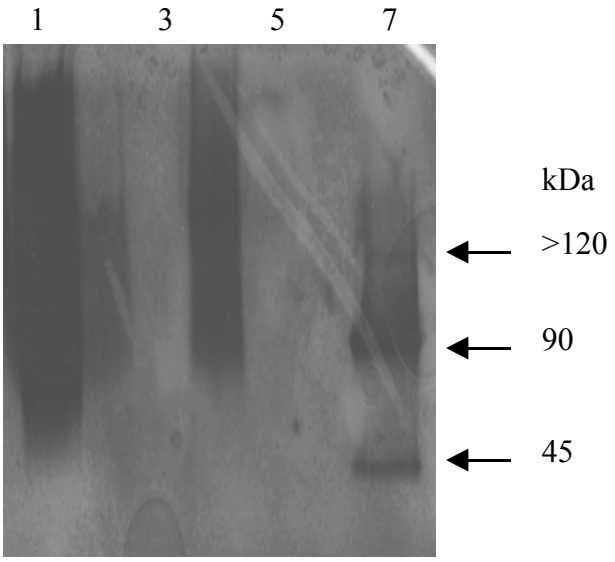

Figure 7. Activity stain of commercial AP and a sample from an active fraction of the 300SW column after transfer to a nitrocellulose membrane. Lane 1-3 contained commercial AP from Calbiochem (calf intestine) with no dilution, 1: 100 and 1: 1,000 dilution, respectively. Lane 4-6 contained commercial AP from Sigma (hog intestinal mucosa) with no dilution, 1: 100, 1: 1,000 dilution, respectively. Lane 7 contained 20 microliters purified AP. The reaction mixture contained NBT and BCIP.

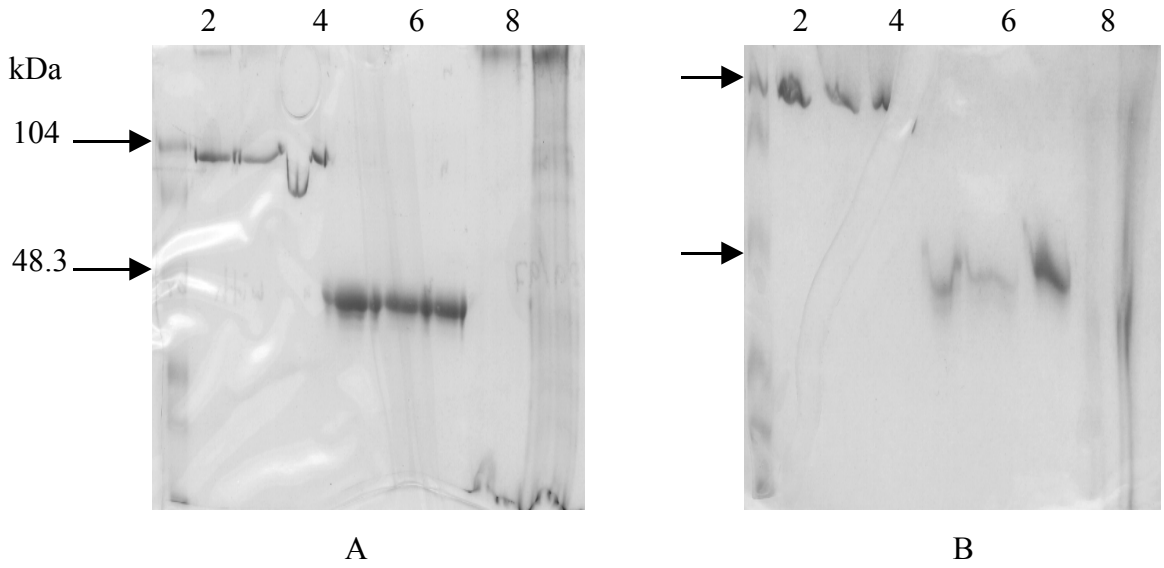

Figure 8. The effect of a stacking gel on protein handing. Figure 8A contained 4% SDS stacking gel and 5% SDS separating gel while figure 8B contained the gel with 5% SDS separating gel only. In both A and B, lane 1 contained prestained SDS PAGE standard; lane 2-4 contained 1 microgram of glycogen phosphorylase A in (1) buffer A, (2) buffer A containing 0.1% Triton X-100 and (3) buffer A containing 0.1% Triton X-100 and 50 mM alpha methyl mannoside, respectively. Lane 5-7 contained 1 microgram of ovalbumin in (1) buffer A, (2) buffer A containing 0.1% Triton X-100 and (3) buffer A containing 0.1% Triton X-100 and 50 mM methyl mannoside, respectively. Lane 8 and 9 in both A and B contained pooled active fractions from ConA column.

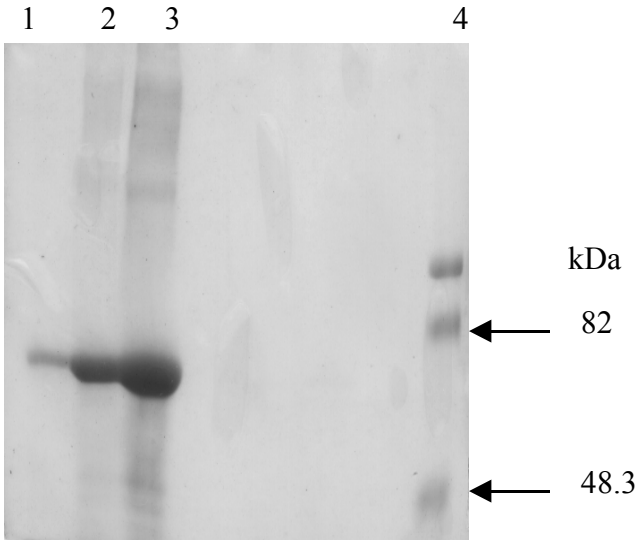

Figure 9. To test whether the amount of protein causes the smearing. 1, 5 and 20 micrograms of BSA suspended in buffer A was mixed with 5 microliters of 10 xSDS loading dye, loaded on 5% SDS gel(lane 1, 2 and 3, respectively) and Coomassie blue stain. Lane 4 contained standard protein marker. The result did not show that the amount of protein can cause smear.

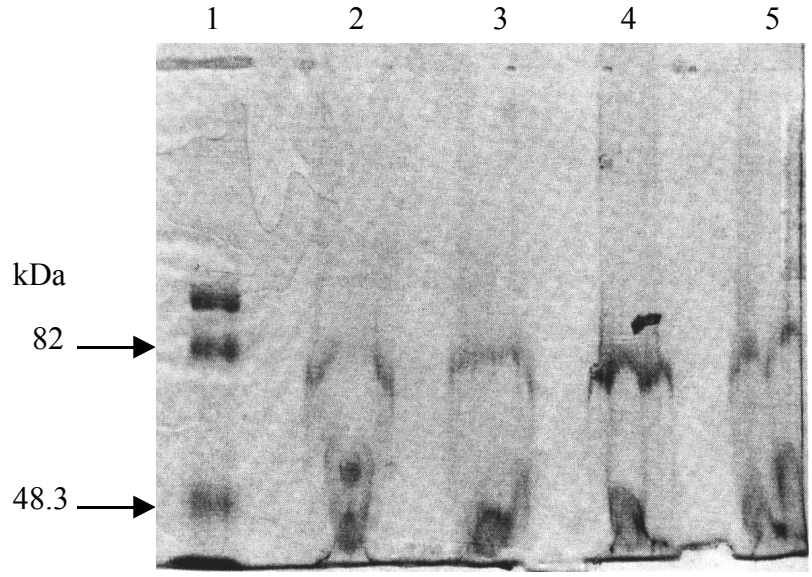

Figure 10. Test to determine if concentrating the protein resulted in smearing. Lane 1 contained standard protein marker. Lane 2 and 3 contained 5 micrograms of BSA in 12 ml buffer A containing 0.3M NaCl and 0.1% Triton X-100. The samples were concentrated by a YM10 column. Lane 4 and 5 contained the same samples as above but concentrated by amicon cell. The result showed that the concentrated sample could cause smear.