

**10. When mixed at certain concentrations, an antibody and its antigen can react to form insoluble “immunoprecipitate”. (This reaction can be used analytically to detect the presence of the antigen.) (a) Why does this reaction not occur when the antibody is present in large excess over the antigen (or *vice versa*)? (b) Why does this reaction not occur when F<sub>ab</sub> fragment is used instead of antibody?**

(a) Immunoprecipitate forms because each (divalent) Ab can bind two antigen molecules, and an antigen molecule can bind multiple antibodies (multiple epitopes). However, with a large excess of antigen, there is unlikely to be more than one antibody bound per antigen molecule. Similarly, with a large excess of antibody, there is unlikely to be more than one antigen bound per antibody molecule. (b) F<sub>ab</sub> fragment has only one antigen-binding site per molecule.

**11. With the help of a MedLine search, identify an affinity column which is suitable for the purification of the following enzymes, and, in each case, explain the biochemical basis of the interaction between the ligand and the enzyme.**

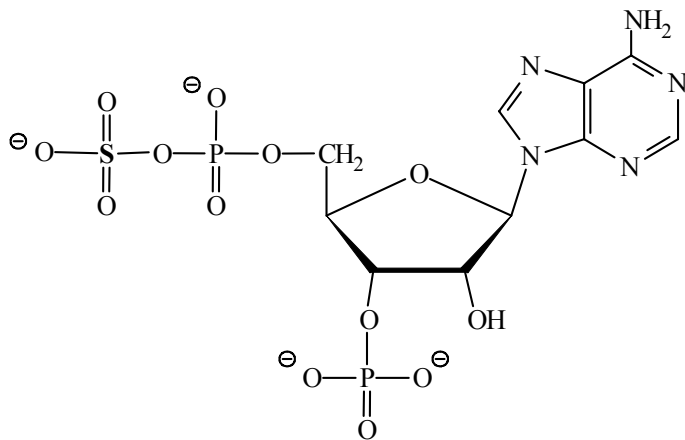
**(a) phenol sulfotransferase**

Tong, Z., and James, M.O., Purification and characterization of hepatic and intestinal phenol sulfotransferase with high affinity for benzo[*a*]pyrene phenols from channel catfish, *Ictalurus punctatus*, *Arch Biochem Biophys* 376: 409-419, 2000.

Cytosol from channel catfish liver and intestinal mucosa has high sulfotransferase activity with low concentrations of 3-, 7-, or 9-hydroxybenzo[*a*]pyrene. To further investigate this conjugation pathway, sulfotransferase activity toward 9-hydroxybenzo[*a*]pyrene was isolated from catfish intestinal and hepatic cytosol by chromatography on anion exchange and PAP-agarose affinity columns. ...

PAPS (substrate)

PAP is the corresponding molecule but without the sulfate



*Purification of liver enzyme:*

	<b>Protein (mg)</b>	<b>Tot. Act. (nmol/min)</b>	<b>% yield</b>	<b>Sp. Act. (nmol/min/ mg)</b>	<b>Purification (-fold)</b>
<b>Cytosol</b>	688	413	100	0.6	1
<b>Dialysis</b>	505	409	98.9	0.81	1.4
<b>DEAE</b>	34	251	61	7.4	12.3
<b>Affinity</b>	0.41	81.7	20	201	335

*Note the effectiveness of the affinity purification step.*

### **(b) serine proteinase**

Sardana, V.V., Blue, J.T., Zugay-Murphy, J., Sardana, M.K., and Kuo, L.C., An uniquely purified HCV NS3 protease and NS4A(21-34) peptide form a highly active serine protease complex in peptide hydrolysis, *Protein Expr Purif* 16: 440-7, 1999

The N-terminal domain of the hepatitis C virus (HCV) polyprotein containing the NS3 protease (residues 1027 to 1206) was expressed in *Escherichia coli* as a soluble protein under the control of the T7 promoter. The enzyme has been purified to homogeneity with cation exchange (SP-Sepharose HR) and heparin affinity chromatography in the absence of any detergent. ...

Note: Heparin, a proteoglycan, inhibits blood clotting by inhibiting the serine proteases of the clotting cascade, such as thrombin (see Stryer p. 257).

*Purification of protease:*

	<b>Protein mg)</b>	<b>Sp. Act. (nmol/min/ mg)</b>
<b>Supernatant</b>	1380	n.d.
<b>Sepharose</b>	47.6	3.1
<b>Heparin</b>	11.1	40.7

**(c) 5'-nucleotidase.**

Spychala, J., and Fox, I.H., The application of affinity chromatography for the separation of "high  $K_m$ " and "low  $K_m$ " 5'-nucleotidase and other AMP metabolizing enzymes, *Adv Exp Med Biol* 253B:119-27, 1989.

**AMP-sepharose 4B** has been widely used as a general ligand affinity chromatography for purification of AMP deaminase, 5'-nucleotidase, adenosine kinase and other adenine nucleotide metabolizing enzymes. Since these enzymes generally **differ in their kinetic properties related to the values of  $K_m$  for AMP** and analogous compounds, it was assumed that there may be a specific elution pattern of some of the enzymes which would enable sequential elution from the column during a single run. **Using 0.5 M NaCl, 10 mM ATP and 5 mM adenosine as eluting agents, it was possible to separate on AMP-sepharose column,** AMP deaminase "high  $K_m$ " and "low  $K_m$ " 5'-nucleotidase and adenosine kinase. ...