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INDIVIDUAL PROFILES OF STAPHYLOCOCCAL PROTEIN A  
BINDING BY HUMAN SERUM

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**Abstract**--The molecular species in normal human serum that bind protein A were different from person to person. These different molecular species were resolved by agarose gel electrophoresis and detected by probing western blots with protein A conjugated with I-125. For each person, the number and avidity of the bands of molecules binding protein A persisted for the entire five month duration of the experiment.

Protein A, an envelope protein from the Cowan strain of Staphylococcus aureus, specifically binds to human immunoglobulins of several classes (for review see Langone, 1982). For the gamma class of immunoglobulins, protein A binds to the Fc region of the molecule (Kronvall and Frommel, 1970; Sjodahl, 1976; Lancet et al., 1978). Because it binds specifically to immunoglobulins, and conjugates are commercially available, protein A is frequently used in experimental protocols to detect antigen specific antibodies (Langone, 1980a). However, attempts to use these conjugates as reagents in place of a second antibody in commercial assays, as suggested by Surolia et al., 1982, have been generally unsuccessful. The failure of protein A to bind to serum antibodies to the same extent as to purified antibodies suggests that serum antibodies are in some way different from the form they assume after purification. That the conserved domains of immunoglobulins have multiple functional conformations is now being confirmed by X-ray crystallographic studies showing that the immunoglobulin binding site for protein A must be conformationally correct before binding can occur (Dorrington and Klein, 1982). The implications of different immunoglobulin conformers bear further examination, and their understanding may be crucial for elucidating how the humoral immune system interacts with the complement system as well as with the cellular immune system.

Recently, a nitrocellulose blotting technique was developed to study the hepatitis B surface antigen (HBsAg) (McMichael et

al., 1981). The method was a modification of western blotting (Towbin et al., 1979), with the critical difference being that, instead of blotting proteins out of acrylamide gels, the blotting was done from agarose electrophoresis gels. This difference allows large aggregates of proteins, even viruses, to migrate during electrophoresis. During the development of the technique for the HBsAg study, I-125 conjugated protein A was used in an attempt to detect the previously reported HBsAg associated immune complexes (Inman et al., 1981). The HBsAg band seldom coincided with a band that bound protein A, and there were an unexpectedly large number of bands that bound the protein A, even among normal control sera. The binding occurred primarily in the gamma region, but a large majority of the sera bound protein A as discrete bands in the beta region as well. These observations led to a further examination of the patterns of protein A binding for sera drawn from the same individuals over an extended time period.

Sera were drawn from healthy individuals once a month for five months and stored at  $-20^{\circ}\text{C}$  until assayed. The I-125 conjugated protein A was purchased from either New England Nuclear or Amersham Corp. Two different buffer systems were used. The reason for using two systems was that at pH 8.6, where most immuno-electrophoresis is performed, a significant amount of material either does not migrate or migrates toward the cathode due to electroendosmosis. Using a higher pH buffer abolished both of these effects and allowed a more definitive characterization of the protein A binding species. Resolution of the different protein bands, however, was less pronounced at the higher pH. For the low pH electrophoresis, a pH 8.6 Tris-barbital

buffer [4.43g tris(hydroxymethyl)aminomethane (TRIS), 2.24g diethylbarbituric acid per liter] was used, and for the high pH electrophoresis, a pH 10.25 sodium carbonate buffer (0.05 ionic strength) was used.

Autoradiographs of conjugated protein A bound to blotted electrophoresed sera are shown in Figure 1. For this experiment, four different individuals allowed their serum to be collected once a month for five consecutive months. Panels A and B represent two different autoradiograph exposure times of the sera blotted from the pH 8.6 buffer electrophoresis. The bottom panel (panel C) shows the results when the pH 10.25 buffer was used for electrophoresis. Note that each individual had a different protein A binding profile from that of the other persons. For individuals coded 'I' and 'II', the largest degree of protein A binding occurred within the gamma region. The opposite was seen for individuals 'III' and 'IV', whose sera bound the protein A predominately in the beta region. When followed monthly, each person's serum bound protein A to nearly the same extent and with the same banding profile for the five successive months.

Comparing results from the two different buffer systems showed that immunoglobulins, which are known to bind protein A, largely failed to migrate, or migrated toward the cathode due to electroosmotic conditions at pH 8.6. Electrophoresis at pH 10.25 allowed all the serum proteins that bound protein A to migrate toward the anode. Consistent with immunoglobulin occurring in the electroosmotic band was this band's great affinity for protein A (panels A and B). When the electrophoresis was performed at the high pH, the gamma region had a proportionate

increase in binding (panel C).

Previous reports indicate protein A preferentially binds immunoglobulins that are aggregated in some way, such as in immune complexes (Brunda et al., 1977; McDougal et al., 1979; Langone 1980b; Dobre et al., 1984). This would explain the existence of multiple discrete bands in the beta region. There were hints, however, that protein A binding was controlled by factors other than aggregation. Note in figure 1, that person 'IV' sera bound less protein A in the gamma region than the other sera. Related to this may be the fact that a healthy young woman was found whose serum contained no detectable protein A binding species using our detection technique. Repeated attempts were made to detect binding by this particular person's serum, such as using conjugated protein A from two different sources, different preparations of agarose, and different sources of nitrocellulose, all without success. It was only after this serum had been treated with the detergent sodium dodecyl sulfate (SDS) that protein A binding could be detected. It was also found that pretreating sera with 5 M potassium thiocyanate always increased the degree of protein A binding by several hundred fold (manuscript in preparation). These observations suggest that serum immunoglobulins can occur in a configuration or with a cofactor that blocks protein A binding.

The distinctive profile of each human's serum was highly reproducible by the agarose electrophoresis-western blot method. Because of their uniqueness for the individual, there may be some potential clinical, or even forensic applications for this method of examining protein A binding profiles. One immediate use might

be as an assay for monitoring cancer patients receiving the type of perfusion therapy in which their plasma is passed over immobilized protein A (Steele et al., 1974; Bansal et al., 1978; Terman et al., 1981; Ray et al., 1982). The clearest lesson from this discovery is that immunoglobulins, as they occur in situ, can have different properties from those that have been purified or otherwise treated.

## REFERENCES

- Bansal S.C., Bansal B.R., Thomas H.L., Siegal P.D., Rhoads J.E., Cooper D.R., Terman D.S., and Mark R. (1978) Ex vivo removal of serum IgG in a patient with colon carcinoma. Cancer **42**, 1-18.
- Brunda M.J., Minden P., Sharpton T.R., McClatchy J.K. and Farr R.S. (1977) Precipitation of radiolabeled antigen-antibody complexes with protein A-containing Staphylococcus aureus. J. Immunol. **119**, 193-198.
- Dobre M.-A., Mota G., Ghetie V. and Sjoquist. 1984. Selective affinity of protein A containing staphylococci for monomeric and polymeric IgG. J. Immunol. Meth. **66**, 171-178.
- Dorrington K.J. and Klein M.H. (1982) Binding sites for F<sub>C</sub> receptors of immunoglobulin G and factors influencing their expression. Mol. Immunol. **19**, 1215-1221.
- Inman R.D., McDougal J.S., Redecha P.B., Lockshin M.D., Stevens C.E. and Christian C.L. (1981) Isolation and characterization of circulating immune complexes in patients with hepatitis B systematic vasculitis. Clin. Immunol. Immunopath. **21**, 364-374.
- Kronvall G. and Frommel D. (1970) Definition of staphylococcal protein A reactivity for human immunoglobulin G fragments. Immunochem. **7**, 124-127.
- Lancet D., Isenmen D., Sjudahl J., Sjoquist J. and Pecht I. (1978) Interactions between staphylococcal protein A and immunoglobulin domains. Biochem. Biophys. Res. Comm. **85**, 608-614.
- Langone J. J. (1980a) <sup>125</sup>I-labeled protein A: Reactivity with IgG and use as a tracer in radioimmunoassay. Meth. Enzymol. **70**, 356-357.
- Langone J. J. (1980b) Immune complex formation enhances the binding of staphylococcal protein A to immunoglobulin G. Biochem. Biophys. Res. Comm. **94**, 473-479.
- Langone J. J. (1982) Protein A of Staphylococcus aureus and related immunoglobulin receptors produced by streptococci and pneumococci. Adv. Immunol. **32**, 157-252.
- McDougal J. S., Redecha P. B., Inman R. D. and Christian, C. L. (1979) Binding of immunoglobulin G aggregates and immune complexes in human sera to staphylococci containing protein A. J. Clin. Invest. **63**, 627-636.
- McMichael J. C., Greisiger L. M. and Millman I. (1981) The use of nitrocellulose blotting for the study of hepatitis B surface antigen electrophoresed in agarose gels. J. Immunol. Meth. **45**, 79-94.

- Ray P.K., Idiculla A., Mark R., Rhoads J.E., Thomas H., Bassett J.G. and Cooper D.R. (1982) Extracorporal immunoadsorption of plasma from a metastatic colon carcinoma patient by protein A-containing nonviable Staphylococcus aureus. Cancer 49, 1800-1809.
- Sjodahl J. (1976) Repetitive sequences in protein A from Staphylococcus aureus: Three highly homologous Fc-binding regions. FEBS Let. 67, 62-67.
- Steele G., Ankurst J. and Sjogren H.O. (1974) Alterations in in vitro antitumor activity of tumor-bearing serum by adsorption with Staphylococcus aureus Cowan I. Intl. J. Cancer 15, 83-92.
- Surolia, A., Pain D. and Khan, M. I. (1982) Protein A: Nature's universal anti-antibody. Trends Biochem. Sci. 7, 74-76.
- Terman D.S., Young J.B., Shearer W.T., Ayus C., Lehane D., Mattioli C., Espada R., Howell J.F., Yamamoto T., Zaleski H.I., Miller L., Frommer P., Feldman L., Henry J.F., Tillquist R., Cook G., and Daskal, Y. (1981) Preliminary observations of the effects on breast adenocarcinoma of plasma perfused over immobilized protein A. New Eng. J. Med. 305, 1195-1200.
- Towbin H., Staehelin T. and Gordon J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. 76, 4350-4354.



### Figure legend

Fig. 1. Autoradiographs of healthy human sera electrophoresed in agarose gels, blotted onto nitrocellulose, and probed using  $^{125}\text{I}$ -conjugated staphylococcal protein A. Panels 'A' and 'B' are two different film exposures of the same nitrocellulose blot for which the sera were electrophoresed in pH 8.6 Tris-barbital buffer. Panel 'B' was exposed approximately 16 hr and panel 'A' for 48 hr. Panel 'C' is of the same sera in the same sequence electrophoresed in pH 10.25 carbonate buffer. This panel was exposed for approximately 16 hr. The four individuals are designated as 'I,' 'II,' 'III,' and 'IV.' The lanes marked 1 through 5 are sera drawn sequentially from each person one month apart. The anode is at the top of the figure. The loading sites are indicated on the right side by the letter 'L.' The electroendosmotic band by 'EEO.' The alpha, beta and gamma regions are indicated at the left of each panel.

