An EPR Study of the Myeloma Protein MOPC 315 and the Hybridoma Proteins 29-22 and HPD-1 using a Dnp-Spin Label Hapten

Dean Oester Final Seminar August 22, 1983

Cellular and humoral components of the immune response protect the host from invasion by foreign substances referred to as immunogens. Immunoglobulins comprise the humoral response and are high-molecular weight proteins with varying amounts of attached carbohydrate. The basic structural unit of an immunoglobulin consists of two identical, high-molecular weight proteins, heavy (H) chains, and two identical low-molecular weight proteins, light (L) chains [1] (Figure These chains are held together in the immunoglobulin by a varying number of disulfide bonds as well as noncovalent domain interactions based on the characteristic β sheet structure of the protein [2]. Each immunoglobulin unit has two identical active sites.

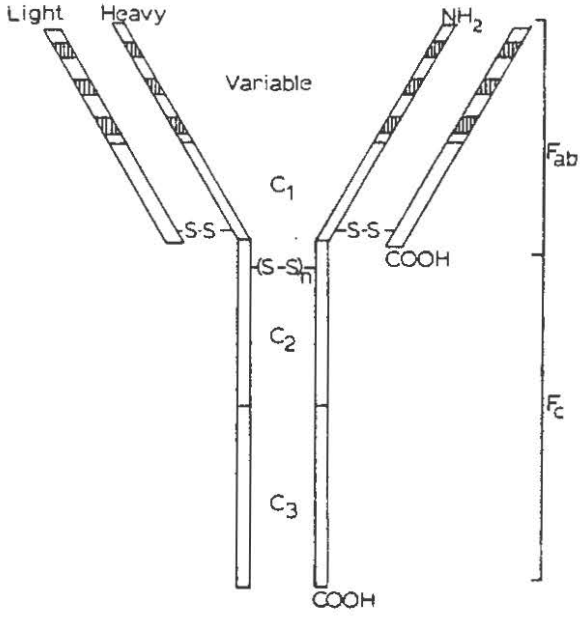
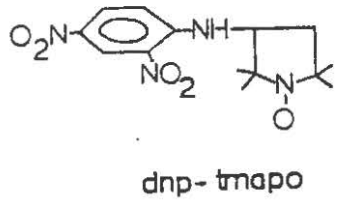


Figure 1



dnp-tmapo

Figure 2

Natural immune responses are characterized by a high degree of heterogeneity with respect to Ig class, affinity toward hapten, and specificity [3]. Affinity is described by the equilibrium between one active site and hapten (Equation 1). The equilibrium constant

$$Ab + Hp \rightleftharpoons \overline{AbHp}$$

increases over time during the immune response. This process is known as maturation with increases in affinity of 10^7 observed for some hapten systems [4].

Hsia and Piette pioneered spin label studies on natural heterogeneous immune responses specific to 2,4-dinitrophenyl (Dnp) deriva-

tached with varying length hydrocarbon spacers determined the average depth of the active site pocket to be 1.1-1.2 nm [5]. Myeloma proteins, homogeneous immunoglobulins of unknown specificity, were also investigated with this method by Dwek. Based on the degree of anisotropy in the spin label epr spectra, depth as well as lateral dimensions were obtained for the active sites [6].

We questioned the direct correlation of the active site model for Dnp binding to the myeloma MOPC 315 to naturally induced immunoglobulins. Two specifically induced anti-Dnp hybridoma (homogeneous) immunoglobulins, HPD-1 and 29-22, were compared to MOPC 315.

We found the three immunoglobulins to have similar affinities toward Dnp-glycine as measured with fluorescence quenching experiments. The affinity for MOPC 315 was 1.2×10^5 M^{-1}, while hybridoma 29-22 had an affinity of 2.3×10^5 M^{-1} and hybridoma HPD-1, 4.1×10^5 M^{-1} with the trend being HPD-1 > 29-22 $\geq$ MOPC 315.

Purification of the three immunoglobulins by affinity chromatrography showed that Dnp-glycine, used in the elution, could not be efficiently removed from either hybridoma, even after exhaustive dialysis. Dnp-glycine could, however, be easily removed from the active sites of MOPC 315. UV-visible spectroscopy and Scatchard analysis of the fluorescence data revealed only 52% and 50% of the active sites free for HPD-1, respectively, and only 42% and 43% of the active sites free for 29-22. The slight differences in measured affinities could not explain the drastic difference between the myeloma and the hybridomas.

Epr titrations of the hybridoma immunoglobulins with the paramagnetic probe Dnp-tmapo (Figure 2) revealed considerably less anisotropy associated with the spin probe when Dnp was bound in the active sites. Lateral dimensions for MOPC 315 with this spin label probe were determined by Dwek and coworkers to be 0.35 nm and 0.50 nm to either side of the spin label portion of the hapten [6]. Using a similar analysis of the epr spectra, the lateral dimensions for 29-22 were 0.50 nm, and >0.50 nm for HPD-1. Neither hybridoma distinguished between the enantiomers of the spin label. The rigidity of the spin label environment followed the trend MOPC 315 > 29-22 > HPD-1, just opposite the trend in affinities.

The immunoglobulin with the most rigid spin label environment had the lowest affinity toward Dnp-glycine and also released Dnp-glycine most readily. HPD-1 had the greater affinity while providing the more open environment for the spin label. In comparing the two hybridomas, the more open active site of HPD-1 released Dnp-glycine to a greater extent than 29-22. The narrowness of the active site pocket for MOPC 315 suggests that Dnp is structurally similar to a portion of a larger site-filling hapten so that the active site does not express any specificity to the immunogen backbone.

References

1. (a) Hobart, M. J. In "The Immune System" Hobart, M. J.; McConnell, I., Eds.; Blackwell Scientific Publications: Oxford, 1978.
   (b) Eisen, H. N. In "Immunology," 2nd Ed.; Harper and Row: New Yo 1980.

2. (a) Edelman, G. M.; Cunningham, B. A.; Gall, W. E.; Gottlieb, P. D.; Rutishauser, U.; Waxdal, M. J. Proc. Natl. Acad. Sci.,USA 1969, 63, 78.
   (b) Fleischman, J. B.; Porter, R. R.; Press, M. M. Biochem. J. 1963, 88, 220.

3. (a) Eisen, H. N.; Siskind, G. N. Biochemistry 1964, 3, 996.
   (b) Clem, L. W.; Small, P. A. J. Exp. Med. 1970, 132, 385.
   (c) Voss, E. W., Jr.; Sigel, M. M. J. Immunol. 1972, 109, 665.

4. Watt, R. M.; Herron, J. N.; Voss, E. W., Jr. Mol. Immunol. 1980, 17, 1237.

5. Hsia, J. C.; Piette, L. H. Arch. Biochem. Biophys. 1969. 129, 296.

6. (a) Dwek, R. A.; Krott, J. C. A.; Marsch, D.; McLaughlin, A. C.; Press, E. M.; Price, N. C.; White, A. I. Eur. J. Biochem. 1975, 53, 25.
   (b) Sutton, B. J.; Gettins, P.; Givol, D.; Marsh, D.; Wain-Hobson, S.; Willian, K.; Dwek, R. A. Biochem. J. 1977, 165, 177.
   (c) Willian, K.; Marsh, D.; Sunderland, C. A.; Sutton, B. J.; Wain-Hobson, S.; Dwek, R. A.; Givol, D. Biochem. J. 1977, 165, 199.