Antigenicity of Myohemerythrin

Geysen *et al.* (*1*) report information bearing on the antigenicity of the protein myohemerythrin. Data assembled from 113 hexapeptides competing with the parent protein for binding to seven different rabbit polyclonal antisera to myohemerythrin allowed assignment of relative antigenicities to segments of primary structure. Their result is compared with sequence profiles of properties such as backbone mobility, atomic packing density, solvent accessibility, shape-accessibility radius, and electric charge distribution of myohemerythrin. The authors conclude that "the most frequently recognized sites form three-dimensional superassemblies characterized by high local mobility, convex surface shape, and often by negative electrostatic potential." They also take issue with our previous work (*2*) and with the work of others (*3*) which implies that antibody accessibility is the primary intrinsic determinant of antigenicity. We wish to demonstrate that the large probe accessibility model accounts for the antigenicity data of Geysen *et al.* at least as well as, and perhaps even better than, any of the structural properties investigated by these authors. In our model, the 1-nm radius probe is rolled over the protein surface in order to identify, relatively sparsely, its most protruding atoms. Antibody binding sites, with more complementary shapes, are known to contact up to six amino acid residues surrounding these most protruding points; data smoothing has been introduced to take account of the difference between the elementary nature of our model and the convolutions of the contact surface.

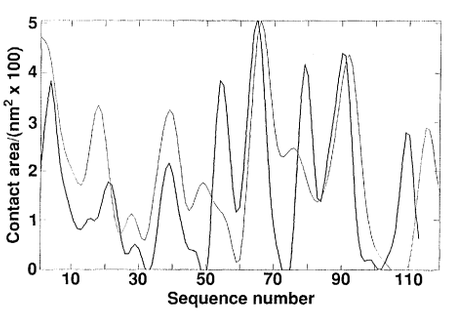

Fig. 1. Antigenicity (heavy line) and areas of contact with a spherical probe (probe radius = 1 nm, light line) of myohemerythrin. The antigenicity data are those given in figure 1C of (*1*), smoothed by three passes of the moving window algorithm (*2*). Nine passes of the same smoothing protocol were applied to the computed large probe contact areas [see (*2*) for details of contact area computations].

In Fig. 1, we have reproduced myohemerythrin antigenicity [data taken from figure 1C of (*1*)] as a smooth curve and have compared this curve with a profile of myohemerythrin large probe contact area computed and smoothed as described (*2*). The similarity of the two curves is striking. They both consist of nine maxima, six of which coincide in the two curves (that is, those at residues 4, 20, 29, 39, 64, and 90) and three of which partially overlap (antigenicity peak at residue 54 and contact area peak at 49; antigenicity peak at residue 79 and contact area peak at 77; antigenicity peak at residue 109 and contact area peak at 114). This degree of accord compares favorably with any of the correlations between peptide antigenicity and structural properties discussed by Geysen *et al.*, as depicted in figures 1, D and E, of their article. For example, Geysen *et al.* classify primary structural segments into three broad categories—most, average, and least antigenic (their table 1). The average (and total) large probe contact areas for the most and the least antigenic sites, evaluated according to this classification, are 2.27 Å^2 (109 Å^2 total) and 1.29 Å^2 (40 Å^2 total), respectively. These values, once again, differentiate better between the antigenic and nonantigenic segments than, for example, data for mobility of such segments favored by Geysen *et al.*, namely, 30 Å^2 and 23.2 Å^2.

The relative shift of antigenicity and large probe accessibility maxima at positions 109 and 114 (and, to a lesser extent, at some other positions) are of potential interest with regard to the large probe accessibility model. The phenomenon either indicates a genuine "frameshift" displacement of the two properties along the amino acid sequence, or it may be a by-product of partial cross-inhibition between peptides with similar sequences (such as the sequence Glu-Glu-His at positions 23 to 25 and the sequence His-Glu-Glu at positions 58 to 60); of competition at sites near to, but not coincident with, the antigenic epitope; or of any other similar "noise" necessarily inherent in the experimental method employed. Each of the rabbit polyclonal sera investigated by Geysen *et al.* probably consists of many partially overlapping antibody specificities; and boundaries of antigenic epitopes, as defined by such mixtures, will be necessarily more diffuse than those defined by individual monoclonal antisera. Examination of molar ratios at which the peptides competed with the parent protein might provide valuable hints; but such data are not easily obtained from assays described in (*1*), where peptides used in immunoassays were not purified subsequent to their chemical synthesis. We note that in experimental systems where the most important antigenic residues were identified in a sharp and unequivocal way [for example, single residue mutants of virus coat proteins (*4*)], the correlation between the most antigenic and the largest probe-accessible sites has been virtually absolute.

Structural attributes listed by Geysen *et al.* as characteristic of antigenic sites are properties known (*5*) to be associated with the most protruding, and therefore the most antibody-accessible, parts of protein surfaces. The relative importance of these various attributes for antigenicity is still being debated, but a review of the available experimental data lead us to conclude (*6*) that antigenicity and antibody accessibility are virtually synonymous, while the other properties listed by Geysen *et al.* are dispensable in at least some antigenic sites (*7*).

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Response: Using our experimental data, Novotny *et al.* purport to find a better correlation of antigenic sites with their calculated parameter "antibody accessibility" than with the factors we identified (*1*). We address three basic problems in their technical comment and other published papers (*2*, *3*) that deserve clarification both for this work on antigenicity and for the general field of structural analysis.

1) Methodology to correlate structure and activity should be described with informative terminology and, more important, should be compatible with the relevant experimental data. The "antibody accessibility" nomenclature implies that the area actually measured is that exposed to an antibody binding site, rather than that exposed to a large sphere (radius usually 1 nm). More-