

Enzyme motifs in antibodies

SIR—The promise of protein crystallography for understanding the structural basis for enzyme mechanisms is being realized, in part, by the growing recognition of a few basic catalytic motifs. Examples include the structurally related Asp-His-Ser and Asp-His-Zn catalytic triads, recently discussed in News and Views¹ and in Scientific Correspondence².

Conventional studies of enzyme active sites use site-directed mutagenesis to probe the chemical function of amino-acid residues. We have developed a complementary approach involving transplantation of catalytic motifs into antibody-combining sites which should clarify the function of residues in enzyme catalytic motifs and improve the design of catalytic antibodies by allowing the addition of appropriate residues and metal cofactors.

Although other proteins with catalytic sites have been designed *de novo*, such as a helix bundle with serine protease activity³, the use of antibodies has several advantages. First, the structure of the antibody framework is strongly conserved, providing a well-defined protein backbone. Second, portions of the complementarity-determining regions are strongly structurally conserved but allow for sequence variability⁴, so catalytic models can be built in a predictable manner. Third, the



Transplantation of a cofactor-binding site into the antibody light-chain complementarity-determining regions (CDRs). The framework Fv regions (dark grey) for the antibodies McPC603, D1.3, HyHEL5, HEd10, Newm, Kol, J539, 4-4-20 and R19.9 are superimposed to show the C_H backbones of the light-chain variable region (CDR1, medium grey; CDR2, white; and CDR3, light grey) with the zinc site from carbonic anhydrase (three histidines and the zinc atom) superimposed onto residues 34, 89 and 91. Binding site (top) faces forward. Although the CDRs show much more structural variation than the framework regions, all the antibodies share the β -strand structure that extends from the framework into the CDRs.

antibody sequence can be varied in these structurally conserved regions to test the specific requirements of the environment surrounding the catalytic motif. Fourth, the immune system can be used to create antibodies with appropriate binding sites for a given antigen. Finally, as techniques are now available for the combination of separate light- and heavy-chain libraries, chains with different binding specificities can be matched to complementary chains with different catalytic motifs.

Structural templates can be recognized, dissected from their environment in the enzyme and transplanted into any of a set of general sites within the antibody-combining site⁴ where their resulting metal binding or catalytic activity can be tested outside the original enzyme. Because these general sites are in the structurally conserved, sequence variable areas of complementarity-determining regions, this transplantation process is likely to be applicable to all antibodies.

An example of a structural template is the three-dimensional pattern of catalytic zinc sites, which include a main-chain hydrogen bond joining two of the zinc ligands⁴. The template for carbonic anhydrase, in which the three zinc ligands lie on two hydrogen-bonded antiparallel β -strands, can be accommodated in five structurally conserved sites general to all known antibody structures⁴. For one such antibody light-chain general site, the replacement of three amino acids in the complementarity-determining regions with histidine residues formed a zinc-binding site with an open coordination position at the bottom of the antigen-binding pocket (see figure). Creation of this site in the anti-fluorescein antibody 4-4-20 gave a mutant antibody that binds copper, zinc and cadmium with the same preferences as carbonic anhydrase⁵.

Thus, multisite design based on structural templates can be used to remodel an antibody to contain a cofactor-binding site, without requiring further mutagenesis and screening. Our new approach for designed antibody mutants could be used to evaluate features such as the Asp-His linkage in Asp-His-Zn and Asp-His-Ser catalytic triads independent of other features in the evolved enzyme active site.

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Alcohol sensitivity

SIR—Luddens *et al.*, in their paper¹ on the importance of the $\alpha 6$ subunit in the binding of the benzodiazepine Ro15–4513 to a subtype of the GABA_A receptor, stated: “most importantly, the newly characterized GABA_A receptor seems to be functionally involved in mediating the alcohol-induced impairment in motor performance that can be antagonized at the same receptor by Ro15–4513”. Because the authors found this subunit only in cerebellar granule cells, their conclusion is not consistent with the fact that the authors of the original study showing antagonism of ethanol on GABA_A receptors by Ro15–4513 (ref. 18 in ref. 1) used rat cortex, a tissue that does not contain the $\alpha 6$ subunit. Furthermore, we have shown² that Ro15–4513 is equally effective as an ethanol antagonist with GABA_A receptors from cortex or cerebellum.

In the cerebellum, ethanol-induced motor impairment is genetically linked with the action of ethanol on Purkinje cells (which do not contain the new receptor)^{3–5}. This action is antagonized by R015–4513 (ref. 6). It is possible that the $\alpha 6$ subunit is expressed in other brain regions and cell types and that the techniques used by Luddens *et al.* were not sufficiently sensitive to detect this expression, or that it is developmentally regulated (the authors did not specify the age of the rats used) — but these hypotheses remain untested.

Finally, although the Ro15–4513 binding site does differ in one pair of lines (AT/ANT) selected for ethanol sensitivity (ref. 17 in ref. 1), no differences were detected in this site in eight other lines (LS/SS, HAS/LAS, FAST/SLOW, HOT/COLD) selected for alcohol sensitivity (E. R. Korpi, personal communication), although differences in ethanol sensitivity of the GABA_A receptor have been shown for several of these lines^{7,8}. Thus, the difference in receptors between AT/ANT rats may result from gene fixation during inbreeding and may be unrelated to alcohol sensitivity. In view of these data, it seems premature to attempt to link the $\alpha 6$ subunit to alcohol sensitivity.

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