Co-operativity in ligand binding to dihydrofolate reductase

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The chemotherapeutic effectiveness of methotrexate arises from its extremely tight binding to dihydrofolate reductase. We are attempting to understand the interactions responsible for this tight binding in the case of *L. casei* dihydrofolate reductase. One potentially valuable approach to this problem is the study of the binding of 'fragments' of the ligands. The 'fragments' of methotrexate we have used are 2,4-diaminopyrimidine (DAP) and *p*-aminobenzoyl-L-glutamate (PABG), both of which bind relatively weakly ($K_a = 8 \times 10^3\,M^{-1}$ and $1 \times 10^3\,M^{-1}$, respectively). N.m.r. studies indicate that the fragments bind in a very similar way to the corresponding moieties of methotrexate. Not only do they bind simultaneously to the enzyme, but they bind *co-operatively*. Thus PABG binds 25-fold more tightly to the enzyme-DAP complex than to the enzyme alone. This co-operativity involves ligand-induced conformational changes in the protein, and indications of the amino-acid residues involved have been obtained from ^1H n.m.r. The conformational changes resemble those accompanying methotrexate binding.

The binding and co-operativity of a series of analogues of PABG and DAP have been studied. The structural requirements for binding and for co-operativity are clearly distinct; for example, N-valeryl-PABG binds some 70-fold tighter than PABG, but shows no co-operativity with DAP. Some of the differences in conformation of the complexes involved in these effects can be observed by ^1H n.m.r.

Nuclear magnetic resonance studies of ligand binding to dihydrofolate reductase

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Studies of the binding of inhibitors such as methotrexate and trimethoprim to dihydrofolate reductase will be used to illustrate some of the n.m.r. methods for the investigation of small molecule binding to proteins, and the kind of information which can be obtained.

Analysis of data by nonlinear regression analysis

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Many of the models used by pharmacologists for fitting experimental data are nonlinear e.g. in enzyme kinetics, and binding constant and pK determination. The most accurate, simple and unbiased way to fit experimental data to such models is by direct least squares estimation of the nonlinear parameters using a computer. By this method self-consistent values can be determined for all the unknown parameters of the problems together with statistical estimates of their accuracy. It is possible to produce a single computer programme which is simple to operate, yet can solve a very wide range of problems, both simple and complex.

The on-line use of such a programme to analyse kinetic and binding curve data will be demonstrated.