Molecular Orbital Study of Hapten–Antibody Interactions

THERE is considerable interest in the application of quantum chemical calculations to pharmacology¹. Quantum mechanical methods can be used to compute submolecular properties, such as charge distribution in a molecule. For a series of compounds eliciting the same type of response a correlation is sought between activity and some function of computed parameters.

All the work of this type presupposes that the activity in the biological environment is a simple function of the binding strength between the effector and receptor molecules, and that no other complicating features influence the pharmacological activity of the molecule.

We have sought a situation where there are unequivocal data on intermolecular binding in a biological medium in order to test whether simple calculations can yield parameters which do, in fact, correlate with binding. We need a set of data covering the binding of a series of rather similar molecules bound to something which resembles a pharmacological receptor.

A system which satisfies all these requirements is the hapten–antibody series investigated by Pressman *et al.*². In this study, antibody was obtained against an antigen (hapten) consisting of the phenyl trimethyl ammonium ion attached to protein by a para-azo linkage. The binding energies of a series of ammonium ions with this antibody were determined by precipitation experiments.

It seems likely that the binding between this particular hapten series and the antibody receptor site is electrostatic, and chemical investigation suggests the presence of a carboxyl group in the receptor which might attract the positive ammonium ion. The latter is quaternary substituted, so that hydrogen-bonding and charge-transfer are unlikely to be involved. Thus it should be possible to obtain a straight line correlation between the calculated charge density on the nitrogen and the free energy of binding.

The graph shows such a relationship for the series of hapten shown in the table. The correlation is expected to follow the relation

$$\Delta G = k \frac{e_1 e_2}{r} + C$$

where e_1 is the carboxylate charge, e_2 is the nitrogen charge, and r is the distance between the charges in the complex.

It is assumed that all values on the right hand side of the equation, except e_2 , are constant. C contains interactions other than electrostatic binding, in particular the entropic

Fig. 1 Correlation between nitrogen charge and free energy for interaction between trimethyl ammonium hapten groups on ovalbumin and antibody. The line shows a least squares fit to the experimental points, numbered as in Table 1. Correlation coefficient 0.95, significance level 0.1 %.

Table 1 Free Energy of Binding between Antibody and Hapten for Different Carrier Proteins

Hapten* trimethyl ammonium ions	Mulliken charge on nitrogen	Free energies in calories†		
		A	B	C
1 α -naphthyl	0.1667	-435	-178	-388
2 <i>p</i> -tolyl	0.1424	-105	-105	-206
3 <i>n</i> -tolyl	0.1431	0	291	84
4 <i>o</i> -tolyl	0.1469	123	77	41
5 Phenyl	0.1431	0	0	0
6 <i>p</i> -amino phenyl	0.1424	90	77	41
7 <i>p</i> -amino benzyl	0.1344	430	406	256
8 Triethyl phenyl	0.1175	766	1,013	789

* Binding energies for each hapten are measured by the extent to which the unattached ammonium ions inhibit the precipitation of antibody with trimethyl phenyl ammonium ion attached to carrier protein.

† A refers to data for the hapten protein carrier ovalbumin; B and C are for horse serum albumin at pH 6 and pH 8 respectively.

term, which will vary only slightly along the series, because of the strong structural similarity between the different molecules.

The quantum chemical procedure employed was the extended Hückel molecular orbital method³. Wave functions were computed for each of the molecular species in the table. Charge distributions were then found using the Mulliken population analysis formalism⁴. The quantitative expression for charge density on atom a is

$$Q_a = \sum_i \sum_k P_{ik} S_{ik}$$

where P_{ik} is the density matrix, S_{ik} is the overlap matrix, and i and k are atomic orbitals, i being an atom a .

This method calculates charge from the products of the coefficients in the orbital wave functions and the electronic charge between two atoms being divided equally between them. Although this gives only a crude indication of charge density, any trends in the series of molecules should be satisfactorily reproduced.

Since the same antibody is used in each case, and the conformation of the methyl groups is unchanged throughout the series, solvation effects should not complicate the situation. Very similar results to those reproduced in the figure have also been obtained for two other sets of biological data of Pressman *et al.*² using horse serum albumin as the hapten carrier protein instead of ovalbumin.

The correlations between experimental binding free energies and computed charges seem reasonable when account is taken of the heterogeneity of the antibody and the relatively crude calculation of charge. The results provide some justification for the wealth of work being published on correlation between drug activity and molecular orbital calculated parameters. We intend to pursue this problem further both by improving the quality of wave function calculated and the method of calculating charge distribution, and also by extending the present type of investigation to hapten–antibody interaction where binding other than coulombic attraction seems important.

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¹ Kier, L. B., *Molecular Orbital Theory in Drug Research* (Academic Press, New York, 1971).

² Pressman, D., Grossberg, A. L., Pence, L. H., and Pauling, L., *J. Amer. Chem. Soc.*, **68**, 250 (1946).

³ Hoffmann, R., *J. Chem. Phys.*, **39**, 1, 397 (1963).

⁴ Mulliken, R. S., *J. Chem. Phys.*, **23**, 1833 (1955).