groscopic). The free amino acid was obtained by ion-exchange chromatography and crystallized from water–acetone.

In preliminary studies with histidine ammonia lyase,[11] 4-fluoro-DL-histidine appears to have a higher affinity for the enzyme (K_m = 0.9 × 10^{-3} M) than does DL-histidine (K_m = 2.7 × 10^{-3} M); on the other hand, the fluoroamino acid is deaminated (to 4-fluorourocanic acid) much more slowly (V_{max} = 0.09 unit/mg of protein)[11] than is DL-histidine (V_{max} = 14.3 units/mg of protein) and serves as a competitive inhibitor (K_i = 1.27 × 10^{-3} M) for the normal substrate. The pharmacological testing of these and other ring-fluorinated imidazoles is in progress. The scope of the method, with respect to the preparation of other difficultly accessible fluoro organic compounds, is also being explored.

(11) C. B. Klee, *J. Biol. Chem.*, **245**, 3143 (1970); we are indebted to Dr. Klee for performing the enzymatic studies.

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Chitotriose, a "Tritium Exchange Probe" of the Active Cleft of Lysozyme

Sir:

We should like to report a "tritium exchange probe" technique for the study of protein active sites. A molecule which binds to the active site is labeled with tritium by means of exchange with tritiated water followed by freeze drying. The rate of exchange of the tritium back into water is measured in the presence and in the absence of the protein by means of a freeze-drying technique.[1] Comparison of these rates gives information about interactions between the molecule and the active site. As an example, we have studied the exchange of chitotriose in the presence and absence of hen egg-white lysozyme.

Samples of chitotriose in tritiated water (10^{-3} M, 10^{-3} Ci/ml) were frozen and dried to high vacuum. Addition of unlabeled water, followed by liquid scintillation counting, indicated a value of 11.8 ± 0.7 for the apparent number of hydrogens/molecule exchanged. This is consistent with complete exchange of all OH and NH groups. A buffer solution of succinate (0.01 M, pH 5.0) at 0° was added to such samples for varying periods of time followed by freezing and freeze-drying during which the ice temperature was maintained at −30°. Unlabeled water was then added and the samples counted. In experiments with lysozyme the buffer solution was replaced by lysozyme solutions (0°, pH 5.0) containing amounts of enzyme approximately equivalent to the chitotriose present. Exchange out was measured in the same way.

The results of these experiments are shown in Figure 1. In all cases exchange out of the tritium-labeled OH groups is too fast to measure. In the absence of lysozyme there appears to be some exchange too rapid for accurate measurement (see insert of Figure 1) and one hydrogen per molecule which exchanges fairly

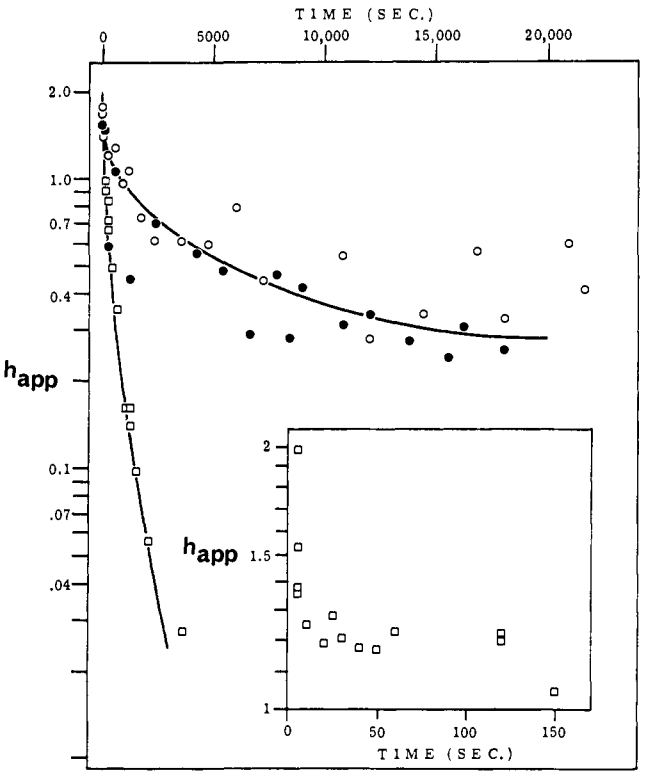

Figure 1. Semilogarithmic plot of the apparent number of unexchanged hydrogen atoms per molecule of chitotriose, h_{app}, *vs.* time, at pH 5.0 and 0°: □, 1.0 × 10^{-3} M chitotriose, 1.0 × 10^{-2} M succinate buffer; points at short times are shown on insert; ○, 0.98 × 10^{-3} M chitotriose, 1.54 × 10^{-3} M lysozyme; ●, 1.04 × 10^{-3} M chitotriose, 0.90 × 10^{-3} M lysozyme.

slowly. We can tentatively identify this hydrogen as the amide hydrogen of the reducing saccharide unit of chitotriose. The other two amide hydrogens appear to exchange more rapidly at pH 5.0. Control experiments indicate that points near the beginning of this exchange may be systematically low due to experimental artifacts[1] but that points defining the slower exchange are fairly accurate.

Results of exchange at two different lysozyme concentrations are also shown. There is a dramatic difference due to marked slowing of exchange. Data for the different lysozyme concentrations are badly scattered but seem in approximate agreement. One of the amide hydrogens of chitotriose apparently exchanges at a rate too fast to measure. A second hydrogen exchanges more slowly and can be observed. The third hydrogen exchanges quite slowly. It should be noted that the portion of the exchange curve representing this slow exchange is not linear. This portion of the curve should be linear if all the hydrogens involved in this slow exchange are chemically equivalent.

The interactions between chitotriose and the active cleft of lysozyme have been studied by Phillips and coworkers[2] by means of X-ray crystallography. They divide the cleft into six sites, A–F. Chitotriose occupies sites A, B, and C. The reducing terminus is in site C. The amide hydrogen in site A is thought to form a hydrogen bond with the carboxylate group of aspartic acid 101, the amide hydrogen in site B does not interact with the enzyme, and the amide hydrogen

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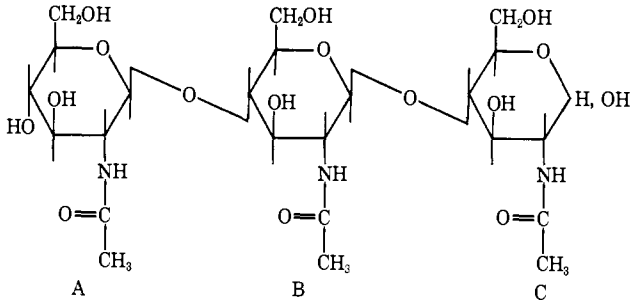
(2) C. C. F. Blake, *et al.*, *Proc. Roy. Soc., Ser. B*, **167**, 378 (1967).



in site C forms a strong hydrogen bond with the carbonyl group of alanine 107.

Our exchange results are consistent with the X-ray structure. The amide hydrogen which exchanges too rapidly for measurement in the presence of lysozyme can be associated with site B. The other two amide hydrogens exchange much more slowly since they cannot exchange with water while hydrogen is bonded to the enzyme. The more slowly exchanging hydrogen can be associated with site C since the lack of linearity of the exchange curve in this region can be explained by the presence of the α and β anomers of chitotriose. If we assume that mutarotation cannot occur while chitotriose is bound we would expect that the extent of opening of the hydrogen bond in site C would be different depending upon whether the α or β anomer were bound. This difference would be reflected in a difference in exchange rate. By elimination the more rapidly exchanging hydrogen can therefore be associated with site A.

The equilibrium between lysozyme, P, and chitotriose, M, can be represented as PM $\rightleftarrows$ P + M, where K = (P)(M)/(PM). The dissociation constant for the complex has been measured by a number of methods[3] and has a value of about 0.5×10^{-5} at pH 5. The kinetics of the above reaction have been measured by temperature-jump and stopped-flow techniques.[4-6] Association and dissociation rates are much faster than the exchange rates observed here.

From the total concentrations of lysozyme and chitotriose present and the above value of K, it is possible to calculate (M)/[(PM) + (M)] for each kinetic experiment. For experiments at the higher lysozyme concentration used, the ratio has a value of 0.0088. At the lower lysozyme concentration the value is 0.16. Reference to Figure 1 shows that there is approximate agreement between the exchange curves at these two lysozyme concentrations. Consequently, we can infer that exchange may occur mainly through PM rather than through M. More specifically, exchange apparently occurs through states in which the chitotriose is bound to the enzyme but the amide hydrogen bonds at sites A or C are broken. However, this conclusion must be preliminary until the unpleasant scatter of these points can be eliminated.

We can offer the following tentative explanations for the scatter of exchange points when lysozyme is present. This molecule is known to bind strongly about nine molecules of water during freeze-drying.[7] This water could be bound in the active cleft. Incomplete removal of this water from clefts in which chitotriose is present could give exchange points which are too high. An alternative possibility would be that water in a cleft could still slowly exchange with chitotriose after freezing. Removal of this water during drying could give points which are too low. More work is being carried out to settle the question.

It is clear that the "tritium exchange probe" method should be useful in gaining new understanding of interactions between protein active sites and the molecules which bind to them. The method will be most useful in situations where binding is strong since under such conditions, differences in exchange behavior in the presence and absence of the protein will be most marked.

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Catalytic Reduction of Olefins with a Polymer-Supported Rhodium(I) Catalyst

Sir:

Many biological systems are capable of selecting substrates for reaction from solution on the basis of bulk molecular properties. These same systems, which are generally catalytic, also allow a large turnover of substrates without loss of the catalytic site. This is accomplished in most cases by attaching the reagent to the inside of large, semiordered, insoluble polymer. Solvent channels in the polymer allow soluble substrates to enter and leave the stationary reagent site. In this way, the substrates are easily separated from reagents and the overall geometric and polar properties of the solvent channels determine the substrates that are able to enter into the catalytic center.

We now wish to report that this rather simple principle has been used to develop a new class of olefin hydrogenation catalysts which demonstrates many of the best properties of homogeneous and heterogeneous catalysts.[1] In addition they are capable of selecting olefins from solution on the basis of overall molecular size.

Polystyrene beads (200–400 mesh) with 1.8% cross-linking of divinylbenzene were chloromethylated on 10% of the aromatic rings by the procedure of Pepper, Paisley, and Young.[2] The chloromethylated polymer was treated with a 1 M tetrahydrofuran solution of lithiodiphenylphosphine[3] for 1 day to replace 80% of the chlorines with diphenylphosphine groups.[4] These beads were then equilibrated with a twofold excess

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