E2-36: Beta-secondary and solvent kinetic isotope effects in beta-lactamase catalysis: detection of sticky substrates

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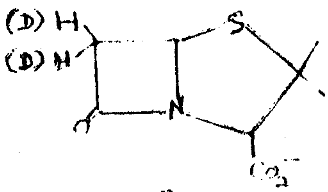
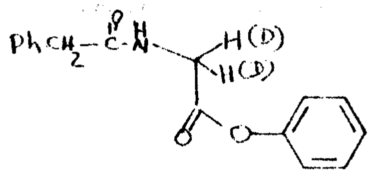
The beta-lactamase enzymes are responsible for much of the bacterial resistance to beta-lactam antibiotics and thus are important targets for mechanistic studies and inhibitor design. Little is however known about the kinetically important transition states involved in substrate turnover by these enzymes. These issues are being investigated by means of kinetic isotope effects. To this end, the protonated and deutrated substrates I and II were prepared.

First, for comparison with enzyme data and for the refinement of experimental method, measurements were made on the secondary and deuterium solvent isotope effects produced during acid and base catalysed hydrolysis of I and II. (For base hydrolysis of I $(k_H/k_D)_{\beta\text{-sec}}$ = (0.948 ± 0.012) and k_{H20}/k_{D20} = (0.763 ± 0.012). Acid hydrolysis of I yielded $(k_H/k_D)_{\beta\text{-sec}}$ = (0.978 ± 0.008) and k_{H20}/k_{D20} = (0.270 ± 0.002) for the beta- For the hydrolysis of II $(k_H/k_D)_{\beta\text{-sec}}$ = (0.942 ± 0.021) & k_{H20}/k_{D20} = (0.748 ± 0.018).

The turnover of these substrates by the class C beta-lactamase of *Enterobacter clocacae* p99 were then determined. The beta secondary isotope effects were (0.862 ± 0.035) and (1.002 ± 0.012) for I and II respectively. The corresponding solvent isotope effects were (1.5 ± 0.2) and (0.82 ± 0.03). These results indicate that the acylation of the enzyme is the first irreversible step in the steady state turnover of I, but that this step for II is likely to be some physical process. Most simply, this would be the binding of II to the enzyme, thus identifying II as a sticky substrate. The value of k_{cat}/K_M is too small however for this step to be diffusion controlled. Slow rearrangement of a non-covalent enzyme-substrate complex is indicated.



I



II