chromium undergoes oxidation,[7] but apparently without blocking the chromium ion. The product III

$$\underset{\substack{O^{2-}\\ \text{I } (mag,\ diss)}}{O^{2-}Cr^{2+}OH^{-}} \xrightarrow[(H_2O)]{\text{room temp.}} \underset{\substack{O^{2-}\\ \text{III } (mag,\ ---)}}{O^{2-}Cr^{3+}O^{2-}} + H^{0}$$

has only slightly less magnetic activity than I, but it has little dissociative activity. The reaction shown is possibly catalyzed by water through the sequence of adsorption, conversion to $2OH^-$, and desorption. The atom of hydrogen may be expected to dimerize quickly, and especially so if, as proposed, active species migrate over alumina surface.[8]

Heating species III in hydrogen causes a complete loss of activity at 120° (or somewhat higher). This is represented

$$\underset{\substack{O^{2-}\\ \text{III } (mag,\ ---)}}{O^{2-}Cr^{3+}O^{2-}} + H_2 \longrightarrow \underset{\substack{O^{2-}\\ \text{IV } (---,\ ---)}}{\overset{H^{-}}{O^{2-}Cr^{3+}OH}}$$

This, rather than depletive reduction,[7] seems probable as the source of hydrogen chemisorption which occurs on chromia gel[9] in the 200° region.

Finally, above 300°, the chromium is reduced, with catalytic reactivation

$$\underset{\substack{O^{2-}\\ \text{IV } (---,\ ---)}}{\overset{H^{-}}{O^{2-}Cr^{3+}OH^{-}}} \xrightarrow{>300^{\circ}} \underset{\substack{O^{2-}\\ \text{I } (mag,\ diss)}}{O^{2-}Cr^{2+}OH^{-}} + H^{0}$$

The rapid recovery of activity above 300° is associated with renewed access of molecular hydrogen to the chromium.

The poisoning action of laboratory air on species I is probably due to a combination of oxidation of the chromium to Cr^{3+}, plus adsorption and conversion to OH^-, thus effectively blocking all access to the paramagnetic ion.[7] Oxidation to the diamagnetic hexapositive chromium seems less probable in the ruby surface.

Use of a dilute solid solution of reasonably well-characterized structure, and relative freedom from surface contamination, appears to bridge, in part, the gap between those studies made under conditions of exceptional surface cleanliness, and those on practical catalysts. The method is applicable to systems other than ruby.

Acknowledgment. Thanks are due to Poul Knudsen, Ernest P. Chock, and Michael J. Perona for assistance. The work was supported under contract with the Army Research Office (Durham). Some equipment was purchased under grant from the National Science Foundation. The author is indebted to Union Carbide Corporation, Linde Division, for a gift of the ruby and sapphire.

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Infrared Spectra of Adenosine Triphosphate Complexes in Aqueous Solution

Sir:

From a recent paper,[1] the impression could arise that coordination of metal ions to adenosine triphosphate (ATP) in aqueous solution would not produce any significant shifts in the infrared absorptions of the triphosphate group other than those which accompany loss of a proton, so that no information regarding the mode of binding in ATP complexes could be derived from their infrared spectra. In order to clarify that significant and structurally interpretable shifts indeed occur upon complex formation of ATP^{4-},[2] infrared absorption curves of ATP^{4-} and, as an example, of its zinc complex, measured in D_2O and H_2O solutions, are presented in Figure 1.

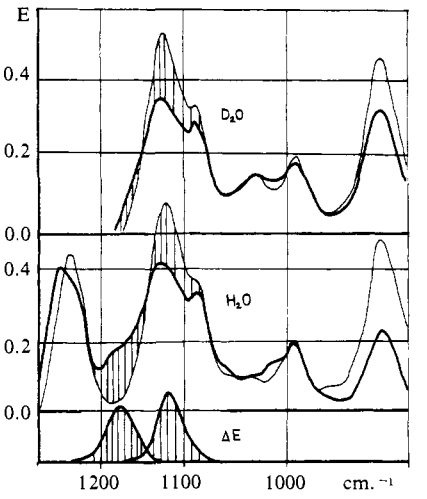

Figure 1. Effect of complex formation with Zn^{2+} on infrared absorption spectrum of ATP^{4-} in D_2O and H_2O solutions: thin lines, absorption of 0.2 *M* Na_4ATP at pH 8.8 and pD 8.8; heavy lines, absorption of 0.2 *M* Na_4ATP + 0.2 *M* $ZnCl_2$ at pH 7.7 and pD 7.9 (27 μ IRTRAN cells, Beckman IR 8).

All the triphosphate absorption bands of ATP^{4-} observable in the 900–1300-cm.$^{-1}$ region in D_2O and H_2O solution show, on complex formation with Zn^{2+}, characteristic changes. At the pH and pD given, no species other than ATP^{4-} and $ZnATP^{2-}$ (*i.e.*, no ATP protonated species) are present to measurable extents. Coordination of Zn^{2+} produces a pronounced change of the absorption band at 1120 cm.$^{-1}$. From the spectra in H_2O solution it is evident that the decrease of the main peak at 1120 cm.$^{-1}$ is accompanied by an equivalent increase of absorption at 1175 cm.$^{-1}$; complex formation with Zn^{2+} obviously shifts one of the components of the 1120-cm.$^{-1}$ ATP absorption to higher wave numbers by 55 cm.$^{-1}$. In D_2O very much the same features are observed as in H_2O except that the increase of absorption at 1175 cm.$^{-1}$ is indeed scarcely observable in D_2O. This is most likely due to the fact that the steep rise of D_2O absorption at 1150–1200 cm.$^{-1}$ obscures this spectral region.

The symmetric $>PO_2^-$ and asymmetric $-PO_3^{2-}$ stretching vibrations contribute to the triphosphate ab-

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sorption at 1120 cm.$^{-1}$,[3] the latter with two components which are approximately degenerate in free ATP^{4-}. The absorption which is shifted from 1120 to 1175 cm.$^{-1}$ by complex formation with Zn^{2+} is most likely one of these two asymmetric $-PO_3^{2-}$ stretching components. A splitting of the two asymmetric $-PO_3^{2-}$ vibrations by 50–60 cm.$^{-1}$ is also observed in other ligands containing $-PO_3^{2-}$ groups, *e.g.*, diphosphate, on complex formation with Zn^{2+} and also with Cu^{2+}.[4] This removal of degeneracy must be due to a reduction of the pseudo-C_{3v} symmetry of the terminal $-PO_3^{2-}$ group by its coordination to these metal ions. Significant information regarding the mode of binding in these complexes may therefore evolve from a closer study of their 1100–1200-cm.$^{-1}$ absorptions in aqueous solution.

Acknowledgment. Thanks are expressed to the Swiss National Foundation for Scientific Research for financial support of this research.

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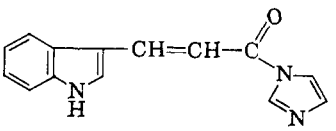
Acyl Intermediates in the α-Chymotrypsin-Catalyzed Hydrolysis of Indoleacryloylimidazole

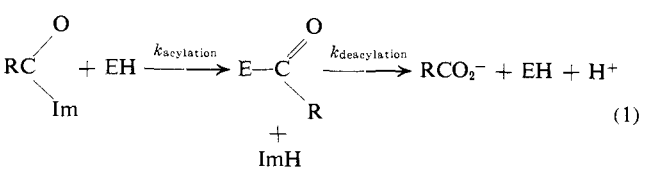
Sir:

The utility of acylimidazoles as acylating agents of serine proteases has been well established by Bender and co-workers.[1,2] Recently we have been concerned with the preparation of acyl-enzyme intermediates with strong absorptions in the ultraviolet, but outside of the absorption regions of the constituent tyrosine and tryptophan residues of the enzyme proteins. Toward this end the kinetics of the reaction of N-(indole-3-acryloyl)imidazole (I) with α-chymotrypsin



I

was investigated spectrophotometrically. A reaction intermediate with an absorption maximum (λ_{max} 360 mμ) lower than the acylimidazole reactant (380 mμ) and higher than the carboxylate product (313 mμ) was detected. When the course of reaction was followed at 350 mμ, there was an initial rise in optical density followed by a decline, suggesting a correspondence with the general pathway proposed by Bender and co-workers[3] (eq. 1).



(1)

A more detailed spectral analysis of the kinetics of the reaction of I with α-chymotrypsin revealed some quantitative additions to the acylation–deacylation mechanism of eq. 1. By following the reaction pathway at particular wave lengths and at various pH, a pH-dependent shift in the spectrum of the acyl-enzyme could be observed. These spectrophotometric changes are more pronounced with I than with cinnamoylimidazole because of the extremely high degree of susceptibility of the indoleacryloyl ultraviolet spectrum to the nature of the acyl substituents (and/or to the nature of the solvent environment).

When the reaction of I with α-chymotrypsin is followed spectrophotometrically near neutrality, two consecutive reactions can be distinguished. In this pH range the first reaction is too rapid to be followed with a conventional recorder. At lower pH, however, there is a measurable, and pH-dependent, rate for this first step. The second step in the reaction can be measured over a wide range of pH and concentrations and is clearly first order. The pH dependence of the rate constants is illustrated in Figure 1. The pH–rate profile for deacylation resembles that reported by Bender and co-workers[3,4] for various deacylation processes. It should be noted, however, that the apparent pK_A' for activation of this acyl-enzyme (7.7) is considerably higher than the various values quoted for other acyl-enzymes.[3] We have measured the apparent pK_A' for activation of cinnamoyl-chymotrypsin in the same buffer solvents and find a value of 7.15 pH, in excellent agreement with a previous report.[4] Hence the difference in pK_A' for the indoleacryloyl-enzyme is real and, we believe, significant.

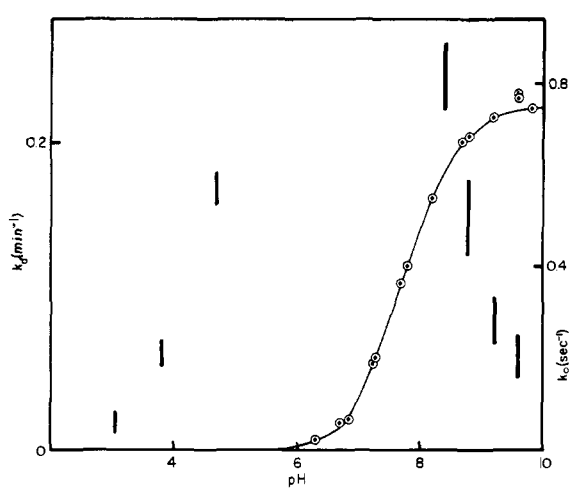


Figure 1. Rates of acylation and deacylation of α-chymotrypsin by indoleacryloylimidazole (I). ○, Deacylation rate (left-hand ordinate) of indoleacryloyl chymotrypsin in pyrophosphate and phosphate buffers, μ = 0.1 *M*, 25°. The solid line is calculated for pK_A' = 7.70. ▌, Acylation of chymotrypsin by I in acetate and pyrophosphate buffers under apparent pseudo-first-order conditions; $k = -\mathrm{d}\ln[\mathrm{I}]/\mathrm{d}t$ (right-hand ordinate). $E_0 = 1 \times 10^{-4}$ *M*, $[\mathrm{I}]_0 = 2 \times 10^{-5}$ *M*. The lack of precision in acylation rates is a consequence of the rapidity of the reaction.

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