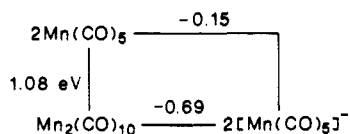
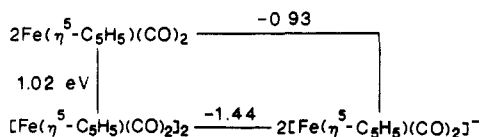


Scheme I^a

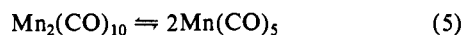
^a 0.5 M TBAH-CH₃CN solution, 22 ± 2 °C, in V vs SSCE.

Scheme II^a

^a 0.5 M TBAH-CH₃CN solution, 22 ± 2 °C, in V vs SSCE.

(NCCH₃)⁺, is formed by a two-electron oxidation of [Mn(CO)₅]⁻ which is in competition with the one-electron oxidation to give Mn(CO)₅. At scan rates lower than ~5000 V/s, dimerization of Mn(CO)₅ to give Mn₂(CO)₁₀ occurs, and the reductive wave at $E_{pc} = -0.30$ V is not observed. The large ΔE_p value for the [Mn(CO)₅]⁻/Mn(CO)₅ couple is a consequence of the high scan rates. Figure 2B shows that reduction of [Mn(CO)₅(NCCH₃)⁺] results in the appearance of the [Mn(CO)₅]⁻/Mn(CO)₅ couple. It also shows that a competition exists between the two-electron reduction of the cation [Mn(CO)₅]⁺ at $E_{pc} = -1.10$ V and one-electron reduction to Mn(CO)₅ followed by dimerization and reduction of Mn₂(CO)₁₀ at $E_{pc} = -1.50$ V.⁴

The results of the fast scan and equilibration experiments allow the construction of the Latimer-type diagram in Scheme I. A value of $\Delta G_{M-M}^{\circ} = 25(\pm 1)$ kcal/mol ($K = 5.3 \times 10^{-19}$) for the metal-metal bond equilibrium in reaction 5 can be calculated from the potentials for the monomeric and dimeric couples. Given the expected positive entropy change for reaction 5, this value is in a range consistent with ΔH values for the same equilibrium estimated by other techniques.⁵



The same electrochemical techniques have been applied to the carbonyl bridged dimer [Fe(η⁵-C₅H₅)(CO)₂]₂. For the [Fe(η⁵-C₅H₅)(CO)₂]₂/2[Fe(η⁵-C₅H₅)(CO)₂]⁻ couple an equilibrium is reached with the [Ru(bpy)₃]^{2+/+} couple ($E^{\circ} = -1.35$ V vs SSCE) with $K(22 \pm 2 \text{ } ^\circ\text{C}) = 7.8(\pm 0.6) \times 10^{-4}$. From that result and the results of fast scan cyclic voltammetry, $\Delta G^{\circ} = 22(\pm 1)$ kcal/mol ($K = 1.7 \times 10^{-17}$) for the associated metal-metal bond equilibrium in Scheme II.

Until now, the direct measurement of metal-metal bond strengths in solution has been confined to those cases where the metal-metal bond is sufficiently weak to allow the dimer and the associated radicals to establish a detectable equilibrium at room temperature.^{6,7} The combination of redox equilibration and fast scan cyclic voltammetry experiments promises to be a more general approach and to allow for the determination of metal-metal bond strengths in solution for cases which are otherwise immeasurable. It also seems clear that the same approach may be of value in establishing thermodynamic redox potentials for other kinetically irreversible organometallic couples.

Acknowledgment. We gratefully acknowledge the support of the Office of Naval Research. We also thank Drs. B. P. Sullivan, M. R. M. Bruce, and A. J. Downard for their helpful discussions.

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Methyl Chymotrypsin Catalyzed Hydrolyses of Specific Substrate Esters Indicate Multiple Proton Catalysis Is Possible with a Modified Charge Relay Triad¹

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Methyl chymotrypsin, alkylated at N^ε2 of His-57, still exhibits multiple proton catalysis for the hydrolysis of specific substrate esters even though the classical charge-relay mechanism, long thought to be important for serine protease catalysis, is clearly precluded from operating. The status of the mechanism of serine protease catalysis thus remains in question.^{2,3} The charge-relay mechanism, involving multiple proton transfer between Asp-102 and His-57 and between His-57 and Ser-195 or water, has previously been supported by proton inventory studies⁴ which have shown multiple proton catalysis for specific substrates.

α-Chymotrypsin has been chemically modified by using methyl *p*-nitrobenzenesulfonate which has been shown to be specific for methylation at N^ε2 of His-57.⁵ The catalytic activity of the methylated enzyme is reduced by a factor of 10³-10⁴ relative to native enzyme.⁶ Any catalytic contribution from native enzyme was avoided through the use of the proflavin displacement technique.⁶ Since this method allows one to observe only decay of the acyl methyl chymotrypsin there is no possible interference from any contaminant of native enzyme. Alkylation was expected to disrupt the charge-relay triad in such a way as to prevent the multiple proton catalysis indicated by proton inventories with the native enzyme.⁴ However, the proton inventory for the deacylation of *N*-acetyl-L-Tyr-methyl chymotrypsin is clearly bowl-shaped (Figure 1 and Table I) and indicates multiple proton catalysis is still being employed ($Dk_3 = 3.7$). Proton inventories (not shown) for the deacylation of *N*-Cbz-L-Tyr-methyl chymotrypsin and *N*-acetyl-L-Phe-methyl chymotrypsin are also bowl-shaped and exhibit equally large solvent isotope effects. These proton inventories do not become linear, as one might have predicted, with the alkylated enzyme. This indicates that the alkylated enzyme does not simply react by a mechanism in which the Nδ1 of imidazole has moved into a position to abstract a proton from the nucleophilic water molecule involved in hydrolysis of the acyl enzyme. Such a mechanism would produce a linear proton inventory. The observation of multiple proton catalysis suggests that other mechanisms must be considered. The proton inventory can be successfully reproduced⁷ (Table I) by using models involving either two-proton or three-proton catalysis. A possible two-proton mechanism is shown in Figure 2, and a three-proton mechanism is shown in Figure 3.

We favor the three-proton model with contributions from H_a and two H_b protons from the oxyanion hole in Figure 3. Fink² has recently discussed the importance of the so-called oxyanion hole in catalysis, and this result lends support to his arguments. Arguments⁴ which have been used to explain the activation of the charge-relay mechanism dependent on substrate structure may also be used to explain the activation of a mechanism involving the amide protons of the oxyanion hole. We have previously

(1) Abbreviations: methyl chymotrypsin: N^ε2-His-57 methyl α-chymotrypsin; Tyr: tyrosine; Phe: phenylalanine; Cbz: carbobenzoxy; Asp: aspartic acid; His: histidine.

(2) Fink, A. L. In *Enzyme Mechanisms*; Page, M. I., Williams, A., Eds.; Royal Society of Chemistry: London, 1987; Chapter 10.

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(7) While the exact fractionation factors (ϕ) may be varied slightly to give a better numerical fit, the curvature of the inventory plot is the most interesting result, and the exact magnitude of the fractionation factors is not a key issue here. Other models may also reproduce the data.

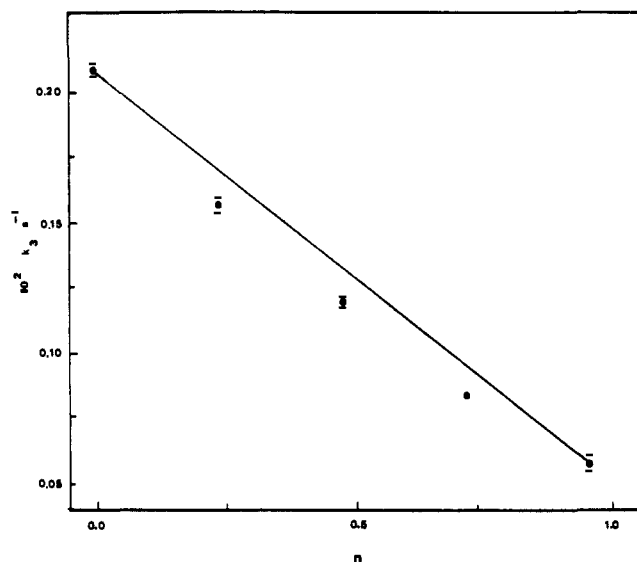


Figure 1. Proton inventory for methyl chymotrypsin catalyzed hydrolysis of *N*-acetyl-L-Tyr-ethyl ester was determined by using the proflavin displacement technique. Kinetic data are deacylation (k_3) rate constants. Rate constants were determined by using the proflavin displacement technique (Bernhard, S. A.; Lee, B. F.; Tashjian, Z. H. *J. Mol. Biol.* 1966, 18, 405) in a procedure similar to that of Henderson.⁶ Methyl chymotrypsin was purified according to a modified procedure of Matta (personal communication to J. D. S.). Phenylmethylsulfonyl fluoride was added to the methyl enzyme stock solution to destroy any residual activity due to native enzyme. The proflavin displacement technique gave reproducible results with different batches of methyl enzyme. Native α -chymotrypsin was added to the cuvettes, after initial acylation of the methyl enzyme, to rapidly hydrolyze any remaining substrate. This procedure obviates any potential problems with contamination by native enzyme.

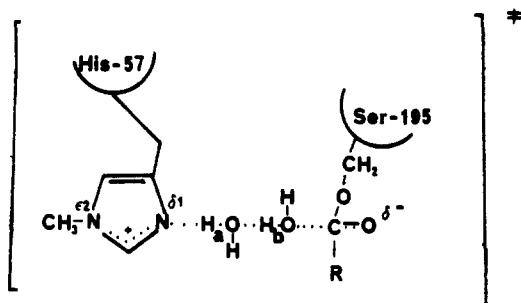


Figure 2. Two-proton mechanism for the methyl chymotrypsin deacylation reaction.

Table I. Methyl Chymotrypsin Catalyzed Hydrolysis of *N*-Acetyl-L-Tyr-ethyl Ester^a

atom fraction deuterium ^b n	experimental ^c $10^2 k_3, s^{-1}$	two-proton fit ^d $10^2 k_3, s^{-1}$	three-proton fit ^e $10^2 k_3, s^{-1}$
0.000	0.207 ± 0.002	0.207	0.207
0.239	0.155 ± 0.003	0.162	0.162
0.479	0.118 ± 0.002	0.123	0.123
0.718	0.082 ± 0.001	0.089	0.089
0.957	0.056 ± 0.003	0.061	0.061

^a At pH 7.64 or the equivalent pD, 0.05 M phosphate buffer, 20 μ M proflavin, methyl chymotrypsin = 9.24 μ M, native chymotrypsin = 0.76 μ M, $[S] = 4.9$ mM, 1.6% v/v CH_3CN . ^b Atom fraction deuterium was determined by the falling drop method by Josef Nemeth. ^c Experimental rate constants are the average of three runs for the deacylation rate. ^d Calculated by using ${}^n k_3 = 0.00207(1 - n + 0.520n)^2$ based on the Gross-Butler equation.⁴ ^e Calculated by using ${}^n k_3 = 0.00207(1 - n + 0.418n)(1 - n + 0.804n)^2$ based on the Gross-Butler equation.

reported the detection of the acyl enzyme intermediate for methyl chymotrypsin with *N*-acetyl-L-Phe-[1-¹³C]ethyl ester at room temperature.⁸ This is additional support for acylation on Ser-195

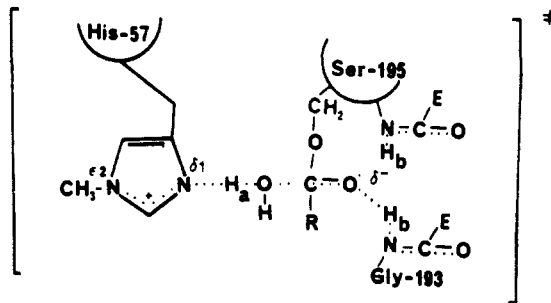


Figure 3. Three-proton mechanism for the methyl chymotrypsin deacylation reaction.

of the modified enzyme. The results clearly indicate that one need not invoke charge-relay catalysis to explain multiple proton catalysis in the mechanism of action of serine proteases. The most important result of this study may be the observation that one can obtain bowl-shaped proton inventories with a modified charge-relay system. This weakens the arguments that the observation of such proton inventories with native enzyme is strong support for classic charge-relay catalysis. Further proton inventory studies with various substrates and with modified and mutant enzymes will be important, as has been suggested by Schowen.³

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Spectroscopic and Structural Investigation of the Unbridged Dirhodium Cation $[Rh_2(CH_3CN)_{10}]^{4+}$

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Twenty years ago Maspero and Taube reported the synthesis of the dirhodium aquo species $[Rh_2(H_2O)_{10}]^{4+}$ from a reduction of $[Rh(H_2O)_5Cl]^{2+}$ by $[Cr(H_2O)_6]^{2+}$.¹ Subsequent claims² that the $Rh_2(aq)^{4+}$ species could also be prepared by the action of strong acids on solutions of dirhodium tetraacetate remain unsubstantiated,³ although the reverse reaction of $Rh_2^{4+}(aq)$ with sodium acetate to produce $Rh_2(O_2CCH_3)_4(H_2O)_2$ proceeds in fairly high yield.¹ The dimeric formulation of the aquo cation is based upon solution spectroscopic and magnetic properties as well as by its ion exchange behavior, but attempts to precipitate or crystallize the material have failed to produce a solid form of $[Rh_2(H_2O)_{10}]^{4+}$.^{1,4}

Since this early work, numerous binuclear rhodium(II) compounds have been prepared and studied in solution as well as by X-ray crystallography.⁵ Among these are the partially solvated cations of general formula $[Rh_2(O_2CR)_2L_6](X)_2$ ($L = CH_3CN, py$; $X = BF_4, PF_6, CF_3SO_3$) prepared by treating acetonitrile solutions of $Rh_2(O_2CCH_3)_4$ with strong acids⁶ or alkylating

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