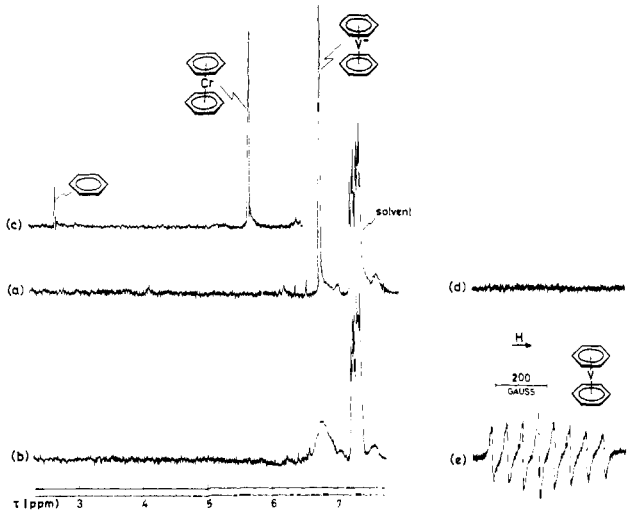

Figure 1. The 100-MHz ^{1}H NMR spectrum (a) of Bz_2V^- in HMPA-d_{18} as obtained at room temperature upon exhaustive reduction with K. After standing for ca. 30 min without exposure to K, this solution of Bz_2V^- gives rise to spectrum b. The NMR spectrum (c) results from Bz_2Cr under conditions analogous to (a). ESR records (d and e) are taken at room temperature of the solutions exhibiting the NMR spectra a and b, respectively.

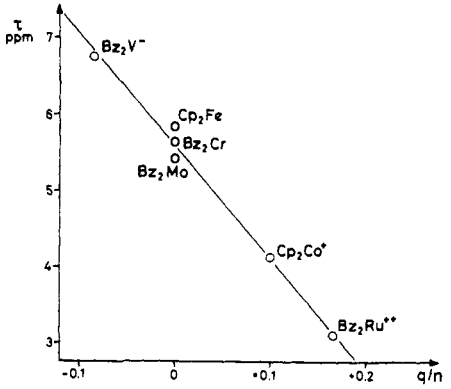

Figure 2. Plot of ^{1}H chemical shifts, τ, vs. normalized formal charges, q/n, for several 18-valence-electron sandwich complexes: Bz_2V^- in HMPA-d_{18}, this work; Cp_2Fe in DMSO-d_6, this work; Bz_2Mo in benzene-d_6, ref 16; Bz_2Cr in HMPA-d_{18}, this work; Cp_2Co^+ in DMSO-d_6, ref 17; Bz_2Ru^{2+} in DMSO-d_6, ref 18.

Figure 1a shows the ^{1}H NMR spectrum obtained upon exhaustive reduction of $Bz_2V\cdot$ with K in HMPA-d_{18}. It displays a sharp signal at τ 6.72 which is consistent with the structure of an axially symmetric anion $[Bz_2V^{-I}]^-$, in analogy with the corresponding absorption of the isoelectronic chromium complex Bz_2Cr^0 (τ 5.64; Figure 1c). Under the conditions of our experiment, the radical $Bz_2V\cdot$ slowly reforms when the solution of Bz_2V^- in HMPA-d_{18} is allowed to stand void of further contact with potassium. A concomitant buildup of the ESR spectrum due to $Bz_2V\cdot$ (Figure 1e) and a gradual broadening of the NMR signal are observed (Figure 1b). The fast electron exchange between Bz_2V^- and $Bz_2V\cdot$, as evidenced by such broadening,[11,12] corroborates our assignment of the NMR signal at τ 6.72 to the anion Bz_2V^-.

In order to assess the electron affinity of $Bz_2V\cdot$, the complex was reduced in presence of equimolar amounts of naphthalene (Np), biphenyl (Ph-Ph) or benzene (Bz), respectively, the formation of the anions Bz_2V^-, $Np\cdot^-$, $Ph\text{-}Ph\cdot^-$ and $Bz\cdot^-$ being monitored by ESR spectroscopy. The result of these experiments (Chart I) point to a sequence, $Np > Bz_2V\cdot \approx Ph\text{-}Ph > Bz$, of decreasing electron affinity. The ready uptake of an additional electron by $Bz_2V\cdot$, as contrasted with the behavior of Bz_2Cr,[13] may be rationalized by the different nature of the frontier orbitals involved. Whereas reduction of $Bz_2V\cdot$ to Bz_2V^- introduces a second electron into a quasi-*nonbonding* orbital (an almost pure metal d_{z^2}-AO a_{1g}),[14] the formation of $Bz_2Cr\cdot^-$ from Bz_2Cr would lead to the single occupancy of an *antibonding* orbital (presumably an MO e_{2u} with a dominant π-ligand character).[5] That this simple one-electron model must be considered an over-simplification[15] is indicated by the plot of proton chemical shifts, τ vs. formal charges q/n (normalized according to the number n of ring protons). The slope of the line for several 18-valence-electron sandwich complexes (Figure 2) amounts to ca. +15 ppm per unit charge, thus being enhanced by a factor 1.5 relative to the uncoordinated cyclic π-systems.[19] This result is not anticipated by a naive orbital picture in which charge differences between the complexes would exclusively concern the central atom. Also rather unexpected is the high degree of correlation (Figure 2) if one bears in mind that the metals in the individual compounds belong to different transition series.[20]

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(12) In contrast to the signal of Bz_2V^-, there is essentially no broadening of the residual absorption due to the isotopic impurities in HMPA-d_{18} (Figure 1b).
(13) Bz_2Cr undergoes no reduction when allowed to react with solvated electrons produced by K in DME at −80°.[5] On the other hand, upon prolonged exposure of Bz_2Cr to a potassium mirror in HMPA-d_{18} at room temperature, a gradual cleavage of the complex must occur, since the ^{1}H NMR signal of free benzene is observed along with that of unreacted compound Bz_2Cr (Figure 1c).
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Ch. Elschenbroich,* F. Gerson

Physikalisch-Chemisches Institut der Universität
4056 Basel, Switzerland

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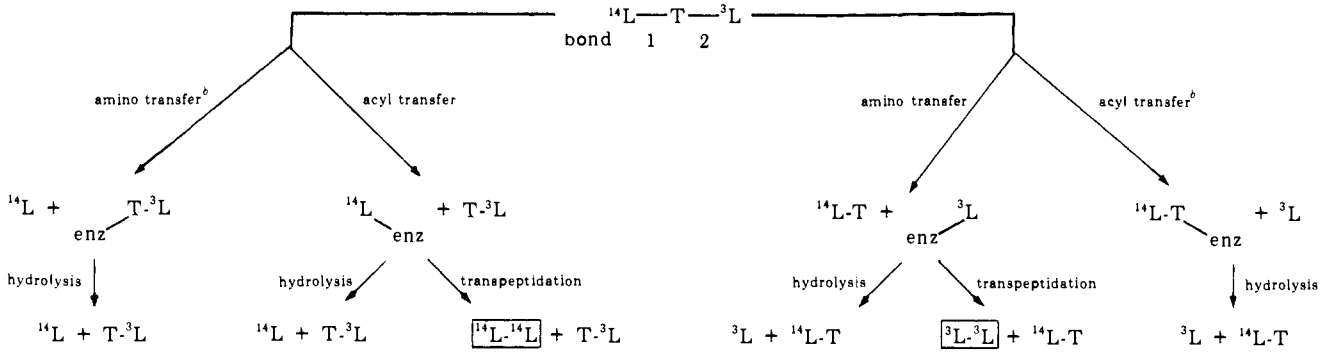
Acyl- and Amino-Transfer Routes in Pepsin-Catalyzed Reactions

Sir:

For many years, it has been accepted that the pepsin-catalyzed hydrolysis of oligopeptide substrates, of the general type X-CO-NH-Y, involves the formation of a so-called "amino-enzyme" (enzyme-NH-Y or its noncovalent equivalent, enzyme:$^+NH_3$-Y), with the ordered release of the two halves of the substrate: $X\text{-}COO^-$ then $^+NH_3\text{-}Y$.[1] This view has been based upon studies of the pepsin-catalyzed

Scheme I. Transpeptidation in [^{14}C] Leu-Tyr-[^{3}H] Leu (^{14}L-T-^{3}L)[a]



[a] An amino enzyme is represented by enz^{X}, and an acyl enzyme is represented by ^{X}enz. There is no unequivocal evidence requiring that either of these intermediates involve covalent bonds between x and the active site, and the question of whether they are covalent or non-covalent species must remain open. [b] Transpeptidation processes for enz$^{T\text{-}^{3}L}$ or from $^{^{14}L\text{-}T}$enz will give rise to ^{14}L-T-^{3}L-T-^{3}L or to ^{14}L-T-^{14}L-T-^{3}L, hydrolysis of which will not yield L-L.

transpeptidation reaction in which, in the presence of an acceptor X′-COO$^-$, synthesis of X′-CO-NH-Y could be observed during the hydrolysis of X-CO-NH-Y,[2] and upon kinetic studies concerning the orderedness of product release.[3] Further, despite a careful search, no direct evidence for an acyl-enzyme (X-CO-enzyme, or X-COO$^-$:enzyme) could be obtained.[4] Yet the presumption of a unique amino-enzyme pathway met with some difficulties,[2d] which culminated in the observation by Takahashi and Hofmann[5] that pepsin was also capable of acyl group transfer. Examination of the early evidence for amino group transfer showed that, while the existence of this transfer pathway was not in doubt, a fair test of a system where *both* transfer modes were equally feasible had not been made. We report here the investigation of such a system, from which we conclude that *acyl transfer may—for appropriate substrates—be just as important in pepsin catalysis as amino transfer and that each may occur concurrently.*

The substrate chosen was the tripeptide Leu-Tyr-Leu, which on incubation with pepsin gives rise to the transpeptidation product Leu-Leu, as first shown by Hofmann.[5] In order to distinguish between the two pathways of amino and acyl transfer, doubly radiolabeled substrate[6] was used: [^{14}C]Leu-Tyr-[^{3}H]Leu. As is evident from Scheme I, transpeptidation by acyl transfer will give rise to [^{14}C]Leu-[^{14}C]Leu and amino transfer will produce [^{3}H]Leu-[^{3}H]Leu. The relative importance of the two pathways may be determined from the distribution of the label in the transpeptidation product, Leu-Leu. The results of this experiment are shown in Table I, and indicate that the product Leu-Leu is derived from both amino transfer *and* from acyl transfer pathways. At all pH values at which transpeptidation was detectable, the acyl transfer route accounts for three to four times as much Leu-Leu as does the amino transfer route (see Table I).

It is possible, of course, that the intrinsic susceptibilities of the two peptide bonds in the tripeptide substrate are very different, and that the large proportion of [^{14}C]-dipeptide arises from the fact that pepsin shows an overwhelming preference for attack at bond 1 (see Scheme I). The hydrolytic susceptibilities of the two bonds were therefore measured from the ^{14}C:^{3}H ratio in the first-formed Leu, from incubations containing relatively dilute tripeptide in which transpeptidation is undetectable. The results of this experiment are shown in Table II, from which it is evident that bond 1 of Leu-Tyr-Leu is attacked only about twice as fast as bond 2, in rough agreement with the results of Takahashi et al.[5] This makes it probable that the contributions of acyl transfer and amino transfer to the cleavage of either bond

Table I. ^{14}C:^{3}H Ratio in [^{14}C] Leu-Tyr-[^{3}H] Leu and in the Leu-Leu Product Derived Therefrom after Incubation with Pepsin[a]

pH[b]	% ^{14}C in Leu-Tyr-Leu substrate[c]	% ^{14}C in Leu-Leu product[c]	% product from acyl transpeptidation[d]
2.1	No hydrolysis observed		
2.5	34.4	83.2	91
3.0	38.2	82.6	89
3.75	49.2	84.6	85
3.75	29.8	69.6	85
3.75	59.5	80.4	75
3.75	53.2	77.6	76
3.75	52.5	87.1	86
3.85	51.7	82.0	80
3.85	69.4	87.0	79
4.8	56.7	77.7	73
4.8	59.0	84.3	79
5.2	60.5	84.3	78
5.2	59.0	84.0	77
5.8	No hydrolysis observed		

[a] Porcine pepsin (after gel filtration on Sephadex G-25) (final concentration 2 μM) was incubated with [^{14}C] Leu-Tyr-[^{3}H] Leu (60 μM) (after each of the radioactive species had been purified by preparative high-pressure liquid chromatography) at 37° for times from 1 to 24 hr. [b] The buffers used were: pH 2.0–4.5, citric acid (0.1 M) in NaOH (0.2 M) plus HCl (0.1 M), diluted fivefold; pH 4.6–5.8, 0.02 M sodium acetate–acetic acid. [c] Leu-Leu was isolated either by two-dimensional paper electrophoresis (pH 1.9 then pH 3.5) or by high-pressure liquid chromatography on a column of C-18 "Porasil", eluted with water–acetonitrile–NH_4HCO_3. % ^{14}C is the number of ^{14}C counts as a percentage of the total counts. [d] Calculated from $q = 100y(100 - x)/[(100 - x)y + (100 - y)x]$ where q is the % reaction following the acyl transfer pathway, x is the % ^{14}C observed in the substrate Leu-Tyr-Leu, and y is the % ^{14}C observed in the transpeptidation product Leu-Leu.

are within a factor of two of each other. Even though the necessarily different pathways producing the Leu-Leu (i.e., via Leu-Leu-Tyr-Leu for the [^{14}C]-dipeptide and via Leu-Tyr-Leu-Leu for the [^{3}H]-dipeptide) preclude a quantitative comparison, the fact remains that the transpeptidation product is derived both from amino transfer *and* from acyl transfer, under the same conditions.

Finally, we must be sure that the isolated Leu-Leu is a mixture only of the doubly [^{14}C]-labeled dipeptide and the doubly [^{3}H]-labeled dipeptide. It is conceivable that the tripeptide substrate could bind directly to the enzyme and form either the [^{14}C]Leu-Tyr-[^{3}H]Leu acyl enzyme (via the terminal carboxyl group) or the corresponding amino enzyme (via the terminal amino group). Transpeptidation of *either* of these species could result in [^{3}H]Leu-[^{14}C]Leu.

Table II. ^{14}C:^{3}H Ratio in Leu Derived from the Pepsin-Catalyzed Hydrolysis of [^{14}C]Leu-Tyr-[^{3}H]Leu[a]

% ^{14}C in Leu-Tyr-Leu substrate[b]	% ^{14}C in Leu product[b]	Hydrolytic susceptibility of bond 1:bond 2[c]
26.5[d]	50.2	2.3 ± 0.5
49.5[d]	64.3	
51.6[e]	67.9	2.4 ± 0.5
53.5[e]	76.6	

[a] For conditions, see Table I. [b] % ^{14}C is the number of ^{14}C counts as a percentage of the total counts. [c] Calculated from (^{14}C:^{3}H ratio in Leu)/(^{14}C:^{3}H ratio in Leu-Tyr-Leu). See Scheme I. [d] [Leu-Tyr-Leu] = 4 μM: transpeptidation is undetectable under these conditions. [e] [Leu-Tyr-Leu] = 60 μM: cf. the substrate concentrations used in the experiments reported in Table I. In these experiments, comparable amounts of Leu and of Leu-Leu were found on the amino acid analyzer.[9]

This possibility was eliminated by the finding that the ^{14}C:^{3}H ratio in the *N*-dansyl-Leu, derived from the N-terminal residue of the Leu-Leu product, was the same as in the *N*-dansyl-Leu derived from a previously hydrolyzed sample of the Leu-Leu product, within experimental error. The Leu-Leu predominantly derives, therefore, from the pathways outlined in Scheme I.[7]

The implications of these findings in the formulation of mechanistic proposals for the action of pepsin will be discussed fully elsewhere, but the requirement that transpeptidation products arise from both amino and acyl transfer suggests that after cleavage of the peptide link, *either* half of the substrate may leave first. Thus for such materials as Z-Tyr-Tyr,[2a] Z-Glu-Tyr,[2b] acetyl-Phe-Phe-Gly,[2c] and acetyl-Phe-Tyr,[2d,8] but not acetyl-Phe-Tyr-amide nor acetyl-Phe-Phe-ethyl ester,[2d] amino transfer has been observed. Yet, as reported here, transpeptidation from Leu-Tyr-Leu is predominantly (though not exclusively) via acyl transfer. While many earlier mechanistic proposals for pepsin that postulated an exclusive amino transfer pathway[1] now need some modification, it is probable that the relative importance of amino transfer and acyl transfer simply depends upon the ease with which the amino and acyl moieties of the cleaved substrate leave the active site.

Acknowledgment. We are grateful to the Science Research Council for support.

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Ann K. Newmark, Jeremy R. Knowles*
Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138
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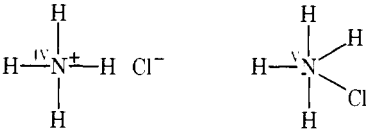
Onium Ions. XVI.[1] Hydrogen–Deuterium Exchange Accompanying the Cleavage of Ammonium (Tetradeuterioammonium) Trifluoroacetate by Lithium Deuteride (Hydride) Indicating SN2 Like Nucleophilic Displacement at Quaternary Nitrogen through Pentacoordinated NH_5

Sir:

A major stepping stone in the development of structural chemistry in the early part of the century was the realization of the ionic rather than covalent nature of ammonium compounds.[2] Ammonium compounds, as is well known, are tetravalent ionic (I), and not pentacovalent nitrogen com-



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pounds (II). It is, therefore, of substantial interest to report experimental evidence obtained in studies directed toward the investigation of ammonium hydride showing nucleophilic hydrogen exchange of the ammonium ion involving pentacoordinated NH_5. To our knowledge this study for the first time shows the pentacoordinating ability of nitrogen.

Our interest in NH_5 was aroused following successful studies in solution chemistry of five-coordinated CH_5^+[3] and its isoelectronic neutral boron analog, BH_5.[4] Both of these species have eight electrons surrounding the central atom involving two-electron three-center bonding. The ten-electron carbon analog (with a four-electron three-center bond) would be CH_5^- (or the fluorine analog CF_5^-) in a SN2 like reaction. Whereas nucleophilic substitution reactions at sp^3 carbon are well studied, no similar reactions were previously considered for tetrahedral nitrogen compounds (ammonium ions). We thought that the most obvious approach to inquire into the possibility of five-coordinated nitrogen pentahydride was to study the preparation and behavior of ammonium hydride, $^+NH_4H^-$. Whereas all other alkali hydrides are stable and well characterized, study of ammonium hydride has not been reported. Ammonium borohydride was studied in metathetic reactions and found unstable, liberating H_2[5] and forming the ammonia-borane complex.[6]

We considered that the most convenient way to approach the preparation of $NH_4^+H^-$, free of any solvent interference, was to carry out a metathetic reaction of a low melting ammonium salt with an alkali hydride. We, therefore, prepared ammonium trifluoroacetate ($^+NH_4{}^-O_2CCF_3$, mp 130°), and allowed it to react with lithium hydride in the melt. H_2 and NH_3 are formed besides lithium trifluoroacetate in the cleavage of the ammonium ion by the hydride through ammonium hydride (eq 1). The reactions were carried out in glass vessels, kept in an oven and flame dried