on neopentyl chloride as the abundance of the parent peak was found to be $\leq 10^{-4}\%$ relative to the base peak given by the *t*-butyl ion.

The A.P. of the lower chloroalkanes are given in Table I. Evidently they depend primarily on carbon

TABLE I

APPEARANCE POTENTIALS OF THE CHLOROALKANES, RCl

R	A.P.(RCl^+), e.v. E.I.[a]	A.P.(RCl^+), e.v. P.I.[b]	A.P. (CH_3CCl) − A.P. (RCl)	ΔH_f, kcal. mole^{-1} RCl[c]	ΔH_f, kcal. mole^{-1} RCl^+
CH_3	11.44 ± 0.02[d]	11.28	0	−21	243
C_2H_5	11.10 ± 0.06[e]	10.97	0.34	−26	230
n-C_3H_7	10.78 ± 0.04[f]	10.82	0.66	−32	217
i-C_3H_7	10.77 ± 0.03	10.78	0.67	−35	214
n-C_4H_9	10.50 ± 0.07	10.67	0.95	−37	205
i-C_4H_9	10.48 ± 0.1	10.66	0.94	−38	204
sec-C_4H_9	10.52 ± 0.1	10.65	0.91	−40	203
t-C_4H_9	10.3 ± 0.1	10.61	1.14	−43	195

[a] Electron impact, this work. [b] Photoionization, ref. 2. [c] $\Delta H_f(RCl)$ from ref. 5. [d] Mean value in ref. 2 is 11.37 e.v. [e] Mean value in ref. 2 is 11.19 e.v. [f] Mean value in ref. 2 is 11.83 e.v.

number, although *t*-butyl chloride shows an additional effect owing to chain branching. Heats of formation of the parent ions, $\Delta H_f(RCl^+)$, can be found by combining literature values[5] of $\Delta H_f(RCl)$ and our A.P. (RCl^+). Now it is well known that in homologous series, lengthening or branching the chain confers stability; that is, ΔH_f decreases. This is the trend found for the chloroalkane ions. Alternatively, one can regard the A.P.(RCl^+) relative to A.P.(CH_3Cl^+) as a measure of stabilization. These A.P. increments suggest that even if a localized electron on chlorine were removed in process 1, the charge on the resulting ion, $C_nH_{2n+1}Cl^+$, is then essentially delocalized as might be expected for the analogous ion, $C_nH_{2n+2}^+$.

Finally, it is usual that A.P. obtained by photoionization are lower than those found by electron impact.[2] As the reverse was found here for the chlorobutanes, this is a discrepancy which needs to be resolved.

(5) S. W. Benson and A. N. Bose, *J. Chem. Phys.*, **39**, 3463 (1963).

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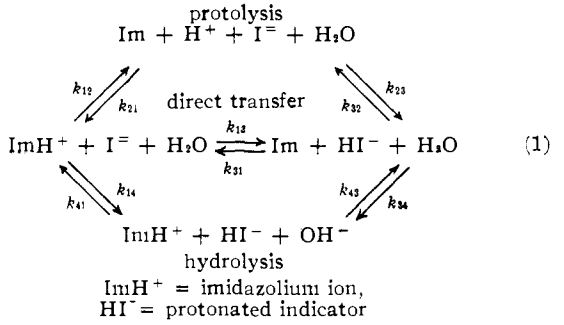
RECEIVED AUGUST 24, 1964

A Kinetic Study of the Imidazole Groups of Chymotrypsinogen, Chymotrypsin, and Some Derivatives, Using the Temperature-Jump Method

Sir:

Ionizable groups of proteins are best characterized by the rate constants of their protonic processes. It is of interest to learn through the values of these constants how the factors of protein structure modify the properties of these groups relative to those of small model compounds. Relaxation methods can provide rate constants when the protein has only a few groups of a given type. The two imidazole groups of chymotrypsin (CT) form such a case and in the presence of a pH indicator are the source of a large chemical relaxation transient measurable with a precision of 3% or better using the temperature-jump technique. The mechanism (eq. 1) is that reported for imidazole itself.[1]



(1)

The experimental data are quantitatively fit by eq. 2 and 3, derived using an appropriate steady-state treatment for $[H^+]$ and $[OH^-]$.

$$\tau^{-1} = \tau_{DT}^{-1} + \tau_P^{-1} + \tau_H^{-1} \qquad (2)$$

$$\left.\begin{aligned} \tau_{DT}^{-1} &= k_{13}(\overline{[ImH^+]} + \overline{[I^{-2}]}) + k_{31}(\overline{[Im]} + \overline{[HI^-]}) \\ \tau_P^{-1} &= \frac{k_{12}k_{23}(\overline{[ImH^+]} + \overline{[I^{-2}]}) + k_{21}k_{32}(\overline{[Im]} + \overline{[HI^-]})}{k_{23}\overline{[I^{-2}]} + k_{21}\overline{[Im]}} \\ \tau_H^{-1} &= \frac{k_{14}k_{43}(\overline{[ImH^+]} + \overline{[I^{-2}]}) + k_{41}k_{34}(\overline{[Im]} + \overline{[HI^-]})}{k_{41}\overline{[HI^-]} + k_{43}\overline{[ImH^+]}} \end{aligned}\right\} \qquad (3)$$

where τ = over-all relaxation time (10 to 70 μsec.), τ_{DT} = direct transfer relaxation time, τ_P = protolysis relaxation time, and τ_H = hydrolysis relaxation time. The bars over the concentrations indicate equilibrium concentrations at the upper temperature (10°). Our experimental system consisted of imidazole (or protein), phenol red (indicator), and 0.1 *M* KNO_3. The pH dependence of τ for imidazole and several proteins (Fig. 1) is well fitted by eq. 2 and shows a dominance of τ_{DT}. There was no complication from carboxyl or ammonium protonic processes in the pH range studied.

The dependence of τ^{-1} on total protein concentration is linear with an intercept value of $(1.00 \pm 0.05) \times 10^4$ sec.$^{-1}$ for all the proteins at pH 7.5 and 4.6×10^{-5} *M* total phenol red. According to eq. 3, the intercept is a function of all the rate constants and the indicator concentration. Although small variations in the protolytic and hydrolytic rate constants would not be detected, the constancy of the intercepts demands approximate constancy of all the rate constants or a set of accidental compensations yielding a constant intercept. Accidental compensation appears improbable and if it is rejected, k_{13}, k_{31}, k_{12}, k_{21}, k_{34}, and k_{43} are essentially invariant for all the "available" imidazole groups of chymotrypsin and its derivatives. The slope of a τ^{-1} *vs.* protein concentration plot thus measures the "available" concentration of imidazole groups, defined as those groups which are capable of reacting with indicator. The direct transfer constants k_{13} and k_{31} are then a measure of the extent to which the environment provided by the protein hinders the direct transfer reaction in eq. 1. Although the rate constants have not yet been refined with statistical methods, estimates of k_{13} and k_{31} are 2.9×10^8 and 5.8×10^7 M^{-1} sec.$^{-1}$,

(1) M. Eigen, G. G. Hammes, and K. Kustin, *J. Am. Chem. Soc.*, **82**, 3482 (1960).

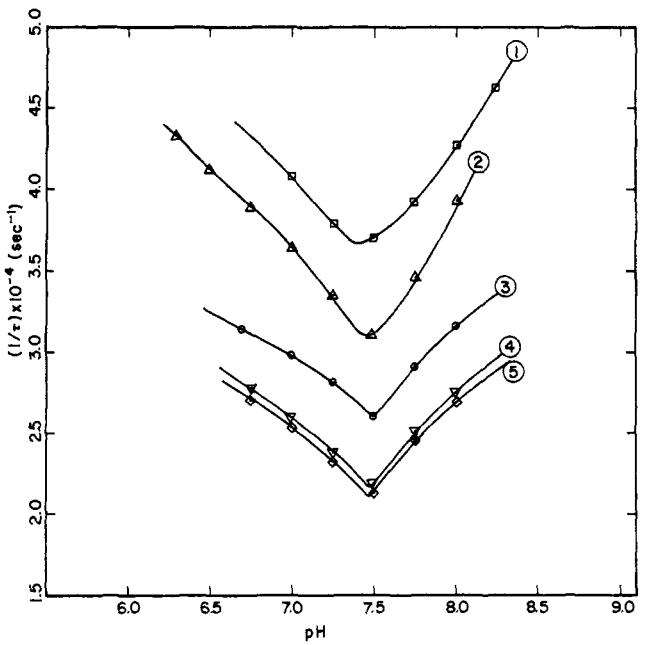


Fig. 1.—pH dependence of τ^{-1}. All concentrations are total concentrations; temperature 10 ± 1°, ionic strength 0.1 (KNO_3) Curve 1, ⊡, 8×10^{-5} *M* model imidazole, 2.3×10^{-5} *M* phenol red; curve 2, △, 8×10^{-5} *M* CT, 4.6×10^{-5} *M* phenol red; curve 3, ⊙, 8×10^{-5} CGN, 4.6×10^{-5} *M* phenol red; curve 4, ▽, 8×10^{-5} *M* TPCK -CT (94.5% inactive), 4.6×10^{-5} *M* phenol red; and curve 5, ◇ 8×10^{-5} *M* DIP-CT, 4.6×10^{-5} *M* phenol red.

respectively, for the proteins, to be compared with $k_{13} = 7 \times 10^8$ and $k_{31} = 1.4 \times 10^8$ M^{-1} sec.$^{-1}$ for model imidazole. These results suggest that the "available" imidazole groups of these proteins experience relatively slight environmental restrictions.

The "available" concentration of imidazole groups in CT is twice the total protein concentration as expected from titration data.[2] The L-1-tosylamido-2-phenylethyl chloromethyl ketone derivative of CT (TPCK-CT) has only one "available" imidazole group by the present method of analysis, in agreement with other evidence indicating that the catalytic-site imidazole in TPCK-CT is eliminated.[3]

The relaxation time profiles in the several variables were identical for diisopropylphosphorylchymotrypsin (DIP-CT) and TPCK-CT when corrected for a CT impurity of 5.5% in our TPCK-CT samples. This suggests that the DIP group blocks indicator attack on imidazole, consistent with a close approach of the imidazole and serine groups at the catalytic site. Since TPCK-CT has lost the protonic processes of the catalytic-site imidazole, it is possible that this group is also not free for protonic reactions in DIP-CT. By these tests TPCK-CT and CT differ only in the concentration of "available" imidazole groups. Rejecting accidental compensation, it is possible to conclude that both imidazoles of CT are essentially identical in behavior with the single "available" imidazole group of TPCK-CT and, consequently, that the catalytic-site imidazole group shows no special ionization properties.

The behavior of chymotrypsinogen (CGN) is peculiar and will require additional study. The intercept on a τ^{-1} *vs.* CGN concentration plot is identical with that obtained for CT, TPCK-CT, and DIP-CT. This suggests that the rate constants for "available" imidazoles are the same for CGN as for the other proteins. If so, the "available" imidazole concentration in CGN is about 1.5 (rather than 2 or 1) times the total protein concentration.

The effects of substrates and competitive inhibitors on the rate constants and "available" imidazole concentrations are currently being investigated. We have found that 2×10^{-3} *M* indole increases τ by 38% when total [CT] = 10^{-4} *M*. Indole is a competitive inhibitor having a dissociation equilibrium constant of 7.0×10^{-4} *M*. The increase in τ expected if indole binding prevents the direct transfer reaction at one of the two CT imidazoles can be calculated to be 37%. Consequently, it appears that the binding of indole causes some type of protective infolding of one imidazole group, reducing its "available" concentration. This finding strongly suggests that the binding of substrate side chains, through changes in folding, exerts direct control on a functional group participating in acylation or deacylation. The method is thus able to provide detailed information about the catalytic process even at this early stage of development.

DIP-CT and TPCK-CT were prepared from Worthington α-CT, and Worthington CGN was used.

Acknowledgment.—This work was supported by the National Science Foundation.

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RECEIVED AUGUST 14, 1964

(2) M. A. Marini and C. Wunsch, *Biochemistry*, **2**, 1454 (1963).
(3) G. Schoellmann and E. Shaw, *ibid.*, **2**, 252 (1963).

Phenylbis(dimethylamino)fluorophosphonium Phenylpentafluorophosphate[1]

Sir:

A recent report[2] on some aryldialkylaminotrifluorophosphoranes, $ArPF_3NR_2$, led us to include this type of compound in a general study of five-coordinate stereochemistry, conducted by means of F^{19} n.m.r. spectroscopy.[3] Both the F^{19} and P^{31} n.m.r. spectra for any R_2PF_3 species, except the perfluoroalkyl derivatives, $(R_f)_2PF_3$, could be interpreted in terms of a trigonal bipyramidal arrangement of the ligands around the phosphorus atom, the fluorine atoms occupying the two axial and one equatorial positions.

Among a number of alkyl- (aryl-) dialkylaminotrifluorophosphoranes, which we have investigated, one clearly stands out with respect to an unusual transformation occurring upon storage of this compound. Phenyldimethylaminotrifluorophosphorane, $C_6H_5PF_3N(CH_3)_2$, not previously reported, was prepared using the method of the Russian workers,[2] *via* $C_6H_5PF_3H$ and $C_6H_5PF_3Cl$, the latter being allowed to react with dimethylamine. We have also obtained the compound less expediently by the reaction of phenyldimethylaminochlorophosphine with arsenic or antimony tri-

(1) Phosphorus-Fluorine Chemistry. XI. For X in this series see: R. Schmutzler, *Z. Naturforsch.*, in press.
(2) Zh. M. Ivanova and A. V. Kirsanov, *Zhur. Obshch. Khim.*, **32**, 2592 (1962).
(3) (a) E. L. Muetterties, W. Mahler, and R. Schmutzler, *Inorg. Chem.*, **2**, 613 (1963); (b) E. L. Muetterties, K. J. Packer, W. Mahler, and R. Schmutzler, *ibid.*, **3**, 1298 (1964); (c) R. Schmutzler, *Angew. Chem.*, **76**, 570 (1964); *Angew. Chem., Intern. Ed. Engl.*, **3**, 513 (1964).
(4) J. F. Nixon and R. Schmutzler, *Spectrochim. Acta*, in press.