nickel(II) species, should not be described as nickel(IV) complexes.[2,3,14,15]

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Received November 30, 1966

Alkylation of Chymotrypsin by α-Bromo-4-nitroacetophenone, a Charge-Transfer Acceptor

Sir:

We have observed that a new long-wavelength absorption band is formed when chymotrypsin (ChT)[1] is alkylated with α-bromo-4-nitroacetophenone (BrNAP).[1] In this communication, we present evidence that this relatively intense absorption band (λ_{max} 350 mμ (ϵ 7.55 × 10^3 M^{-1} cm^{-1})), which is not shown by either enzyme or the alkylating agent alone, is a "charge-transfer band" arising from the interaction of a tryptophan residue of the enzyme and the 4-nitroacetophenone moiety.

BrNAP reacts with ChT at methionine-192 (*vide infra*) to yield a monoalkylated derivative which is inactive toward N-acetyl-L-tyrosine ethyl ester. Evidence for the 1:1 stoichiometry is obtained from amino acid analysis and the absorption spectrum of ChT–NAP in the 260–290-mμ region. At these wavelengths, the absorption spectrum of ChT–NAP is essentially the sum of the spectra of ChT and BrNAP. The rapid rate of inactivation of ChT by BrNAP is closely paralleled by the rate of increase of the new absorption band at 350 mμ.

Amino acid analysis of ChT–NAP showed that neither of the two histidines of ChT had been modified. On the other hand, the methionine content of the alkylated enzyme was low (1.5 Met/mole) compared to that of the native enzyme (2.0 Met/mole). In order to prove that a single methionine had been alkylated, both ChT and ChT–NAP were oxidized with performic acid according to the method of Hirs.[2] Amino acid analysis of performic acid oxidized ChT (following acid hydrolysis) indicated that both methionines had been converted to methionine sulfone; no methionine could be detected. In contrast, similar treatment of performic acid oxidized ChT–NAP yielded only one methionine sulfone/mole; and, in addition, 0.5 methionine/mole of enzyme was found. Since alkylated methionines are stable to performic acid oxidation,[3] but are only partially regenerated to methionine on acid hydrolysis,[4] the above results show that BrNAP alkylates one of the enzyme's two methionines.

In order to determine which of the two methionines (192 or 180) had been alkylated, the enzyme and the alkylated enzyme were treated with hydrogen peroxide under conditions which convert methionine-192 to methionine sulfoxide.[5,6] Whereas H_2O_2 treatment of native ChT, followed by basic hydrolysis,[3] yielded equimolar amounts of methionine and methionine sulfoxide, identical treatment of ChT–NAP yielded no methionine sulfoxide and the same amount of methionine as ChT–NAP that was not treated with H_2O_2. These experiments show that methionine-192 of ChT–NAP is protected against oxidation by H_2O_2. Therefore, the methionine alkylated by BrNAP is methionine-192.

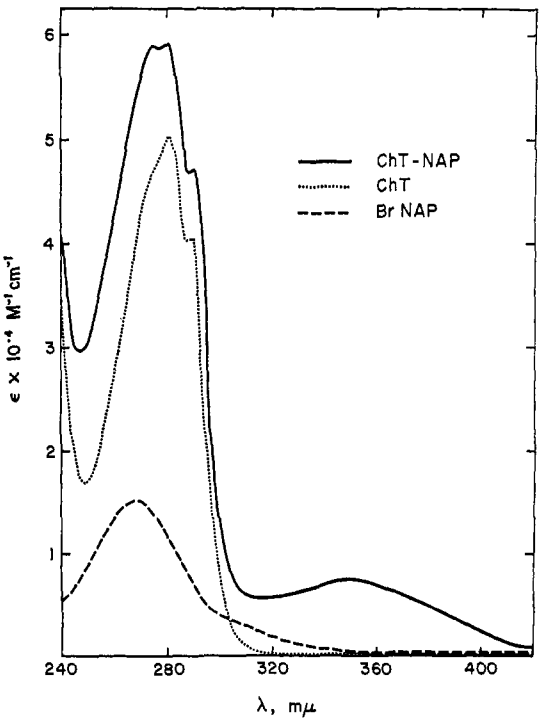

Figure 1. Absorption spectra of chymotrypsin (ChT), chymotrypsin alkylated with α-bromo-4-nitroacetophenone (ChT–NAP), and α-bromo-4-nitroacetophenone (BrNAP) at 25° in 0.001 *M* HCl. The spectrum of BrNAP was obtained in a solution containing 5% acetonitrile.

The assignment of the new long-wavelength absorption band[7] of ChT–NAP (Figure 1) as a charge-transfer transition is supported by the following evidence. (a) Alkylation of chymotrypsin with α-bromo-2,4-dinitroacetophenone yields a modified enzyme possessing a new absorption band with λ_{max} 365 mμ. The red shift in λ_{max} (from 350 to 365 mμ) upon increasing the electron affinity of the acceptor (nitroacetophenone to dinitroacetophenone) is consistent with the assignment of the new absorption band as a charge-transfer transition.[8] (b) When ChT–NAP and ChT–DNAP are heated to 65°, they completely lose their characteristic long-wavelength transitions. Upon cooling the heat-denatured alkylated proteins, the long-wavelength absorption bands reappear. (c) The new absorption band of ChT–NAP can also be destroyed

(1) The abbreviations used are ChT, chymotrypsin; BrNAP, α-bromo-4-nitroacetophenone; ChT–NAP, chymotrypsin alkylated with BrNAP; and ChT–DNAP, chymotrypsin alkylated with α-bromo-2,4-dinitroacetophenone.
(2) C. H. W. Hirs, *J. Biol. Chem.*, 219, 611 (1956).
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(4) H. G. Gundlach, W. H. Stein, and S. Moore, *J. Biol. Chem.*, 234, 1761 (1959).
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(6) H. Schachter and G. H. Dixon, *ibid.*, 239, 813 (1964).
(7) This absorption band is optically active. It has a molecular ellipticity of 2.56 × 10^4 deg cm^2 $dmole^{-1}$ at 350 mμ.
(8) E. M. Kosower, "Molecular Biochemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 183.

and regained by reversibly denaturing the protein in 8 *M* urea at pH 3.0.

The reversible heat and urea denaturation of ChT–NAP shows the new absorption band is dependent on the conformation of the protein. Since charge-transfer interactions require the close proximity of the donor and acceptor moieties, a charge-transfer interaction in a protein should be destroyed when gross conformational changes of the protein occur (*e.g.*, such as those induced with heat and urea).

The above lines of evidence are therefore consistent with the identification of the new absorption bands in both ChT–NAP and ChT–DNAP as due to charge-transfer interactions. It is then pertinent to inquire as to the nature of the donor moiety of the enzymic charge-transfer complex.

To aid in identifying which aromatic amino acid residue (phenylalanine, tyrosine, or tryptophan) was serving as the donor in the enzymic charge-transfer interaction, reversible, noncovalent complexes composed of toluene, *p*-cresol, and indole[9] were prepared with both α-bromo-4-nitroacetophenone and α-bromo-2,4-dinitroacetophenone in acetonitrile as solvent. The charge-transfer complexes of these compounds with the nitroacetophenones showed new absorption bands in the region 300–400 mμ. For example, the absorption maxima of the charge-transfer complexes that toluene, cresol, and indole form with α-bromo-2,4-dinitroacetophenone are 312, 315, and 345 mμ, respectively. Since the absorption maximum of the charge-transfer complex with indole most closely corresponds to that of ChT–DNAP (λ_{max} 365 mμ),[10] these results suggest that the donor in the enzymic charge-transfer complex is a tryptophan residue.

Another fact which is consistent with (but not proof of) the identification of tryptophan as the donor in the enzymic charge-transfer complex is that the characteristic tryptophan fluorescence of chymotrypsin is 60% quenched in ChT–NAP and ChT–DNAP. The quenching of the fluorescence of indole residues involved in charge-transfer complexes has been observed in other systems.[11]

In summary, α-bromo-4-nitroacetophenone alkylates methionine-192 of chymotrypsin and forms a conformationally dependent charge-transfer complex with a vicinal tryptophan residue. Because BrNAP performs two functions, it can be termed a chemical–optical bifunctional reagent. Further, it can be noted that this reagent (and other similar reagents) provides a method for determining the maximum distance between the site of alkylation and the donor of the charge-transfer complex. In chymotrypsin, for example, we conclude that the methionine-192 sulfur can be no more than 8 A from the center of the indole moiety of a tryptophan residue.

(9) Toluene, *p*-cresol, and indole serve as models for the aromatic nuclei of phenylalanine, tyrosine, and tryptophan, respectively.

(10) The charge-transfer complex indole forms with α-bromo-4-nitroacetophenone has λ_{max} 330 mμ (*cf.* ChT–NAP, λ_{max} 350 mμ). The absorption maxima of the charge-transfer transitions of ChT–NAP and ChT–DNAP are each shifted 20 mμ to the red of the maxima of the corresponding model charge-transfer complexes containing indole. This red shift may be due to a solvent effect or to the presence of the positive charge on methionine-192 which results from sulfonium salt formation as a consequence of alkylation.

(11) S. Shifrin, *Biochim. Biophys. Acta*, 81, 205 (1964).

Acknowledgments. We are pleased to acknowledge the support of this work by U. S. Public Health Service Grant No. AM-07300. Important initial experiments were ably performed by Mr. P. John Flory, Jr. Professor William White of the University of Vermont had kindly informed us of the inactivation of chymotrypsin by α-bromoacetophenone prior to the start of this work.

(12) National Institutes of Health Postdoctoral Fellow, 1965–1966.

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Deoxygenation by Carbon Atoms

Sir:

The large value for the heat of combination of a carbon atom with an atom of oxygen (256.7 kcal/mole)[1] suggests that the deoxygenation of oxygen-containing compounds by carbon atoms should be a thermodynamically favored process. We are able to report several examples of this process in the condensed phase.

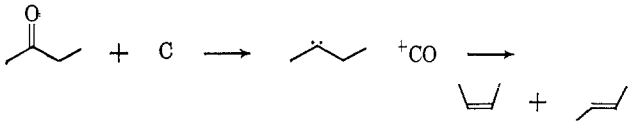
Reactions of carbon atoms, produced by nuclear transformation, with carbon dioxide, oxygen, and ethylene oxide have been reported to cause deoxygenation with production of radioactive carbon monoxide.[2,3]

The reaction system employed in this study has been reported previously.[4] Simultaneous deposition of carbon vapor with acetone at −196° results in the formation of carbon monoxide and propylene. This observation may be understood on the basis of an oxygen abstraction by the atomic carbon to form carbon monoxide and a dialkylcarbene. The resultant carbenes are known to rearrange to isomeric olefins.[5,6]

$$CH_3\overset{\overset{O}{\|}}{C}CH_3 + C \longrightarrow CH_3\ddot{C}CH_3 + CO$$

$$CH_3\ddot{C}CH_3 \longrightarrow CH_3CH{=}CH_2$$

Other ketones show similar behavior; the reaction with 2-butanone gave *cis*-2-butene and *trans*-2-butene in yields[7] of 67 and 16%, respectively. Small amounts of 1-butene and 1,3-butadiene are also observed in the 2-butanone reaction. A determination of the amount of carbon monoxide formed showed it to be roughly equimolar with the 2-butenes.



The reaction with cyclopentanone gave cyclopentene in 42% yield and a small amount of cyclopentadiene.

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(2) C. MacKay and R. Wolfgang, *Radiochim. Acta*, **1**, 42 (1962).
(3) C. MacKay and R. Wolfgang, *Science*, **148**, 899 (1965).
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(6) W. Kirmse and B. V. Bülow, *Chem. Ber.*, **96**, 3316 (1963).
(7) Yields are calculated in the following manner: (mole of product formed/mole of C_1 vaporized) × 100. The amount of C_1 in these vaporizations has been estimated at 30% of the weight of vaporized carbon (P. S. Skell and R. F. Harris, unpublished results).