A POSSIBLE MODEL FOR THE ABNORMAL TYROSYL RESIDUES OF RIBONUCLEASE*

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Received May 18, 1964

Half the tyrosyl residues of bovine pancreatic ribonuclease have abnormal properties which include low dissociation constants, low fluorescence, resistance to attack by iodine and perturbations in the ultraviolet spectrum (Scheraga and Rupley, 1962). Several of these atypical properties are explicable on the assumption that the abnormal tyrosyl residues are buried in a hydrophobic region of the molecule and thus shielded from attack by reagents. However, a non-polar environment is not sufficient to account for the low fluorescence, since peptides of tyrosine invariably show enhanced fluorescence in organic solvents of low dielectric constant. During a search for a model system which might mimic the properties of these abnormal tyrosyl residues, the author has observed that phenols combine with organic acid amides in non-polar solvents, and the hydrogen-bonded complexes which form have a number of properties that are similar to those of the abnormal tyrosyl residues.

METHODS

Fluorescence and the absorption spectra were measured by conven-

* This investigation was supported by a PHS research grant (GM10515-02) from the National Institute of General Medical Sciences.

tional methods (Cowgill, 1964).

Phenol (Allied Chem.) and N,N'-dimethylacetamide (Eastman Org. Chem.) were purified by fractional distillation. Solvents were supplied by Matheson, Coleman and Bell as spectro-grade quality and were found free of fluorescing or light-absorbing impurities in the wavelength region of interest.

RESULTS

The fluorescence of phenol in n-hexane is decreased markedly upon addition of small amounts of N,N-dimethylacetamide (DMA). This is illustrated by the curve labelled hexane in Figure 1. The loss of

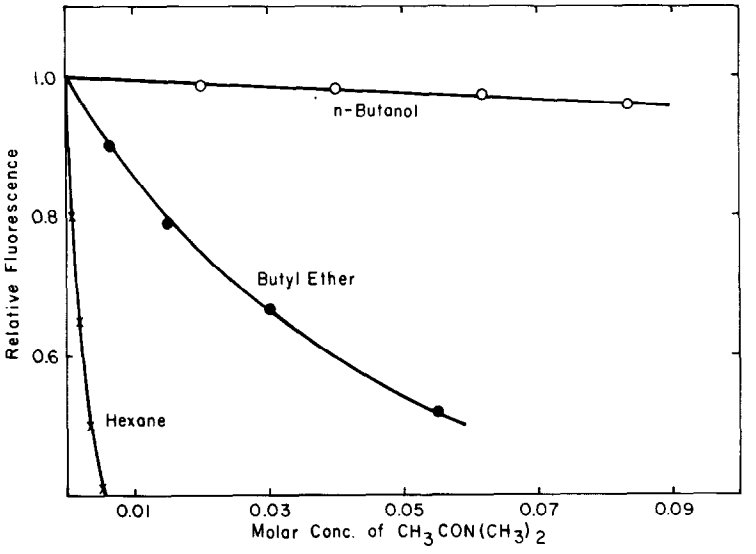

Figure 1. Effect of N,N'-dimethylacetamide on the relative fluorescence emission of 6 x 10^{-5} M phenol at 25°C in the solvents indicated in the figure.

fluorescence does not occur by a collisional quenching process; instead, it is due to the formation of a complex between the phenol and DMA. The formation of this complex is revealed by the change in the absorption spectrum of phenol in the presence of DMA (Figure 2). The spectroscopic properties of similar phenol-amide complexes have been described by S. Mizushima et al., 1955.

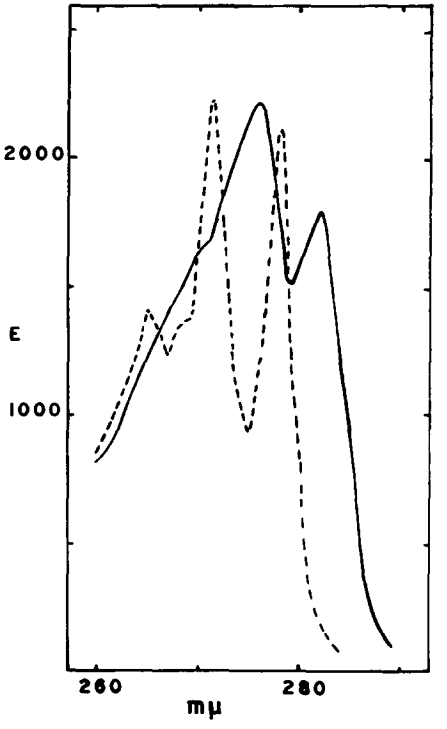

Figure 2. Absorption spectra in n-hexane of phenol (----) and of phenol plus excess (0.2 M) DMA (———); E designates the molar extinction coefficient.

The data suggest that a hydrogen-bonded complex forms by the following reaction:

$$C_6H_5\text{-}O\text{-}H + O{=}C\begin{matrix}CH_3\\N(Me)_2\end{matrix} \rightleftharpoons C_6H_5\text{-}O\text{-}H\cdot\cdot O{=}C\begin{matrix}CH_3\\N(Me)_2\end{matrix}$$

The association constant (K) can be determined from either the spectrophotometric or fluorometric data (for the latter calculation the complex is assumed to be non-fluorescent), and the value for the system phenol plus DMA in hexane at 25°C is 300 liter/mole when calculated with either set of data. The value of K decreased markedly in more polar solvents (see Figure 1) and the complex could not be detected in aqueous solution.

DISCUSSION

The proposal that some tyrosyl residues of proteins are hydrogen-bonded to carboxyl groups has been made by a number of investigators in order to account for abnormal properties of these residues. For example, Scheraga, 1957, proposed that disruption of hydrogen bonds between carboxyl and tyrosyl residues might account for changes in the ultraviolet spectrum of ribonuclease in acid solution. Similar proposals have been advanced to account for the low fluorescence of tyrosyl residues in polypeptides (Rosenheck and Weber, 1961) and native ribonuclease (Gally and Edelman, 1962) on the basis that ionized carboxylic acids do quench the fluorescence of tyrosine in aqueous solution (Teale, 1960).

The observ ations described in this Communication provide additional evidence that tyrosyl residues can form hydrogen bonds with carboxyl groups. In addition, the capacity of the phenolic group for hydrogen-bond formation is shown to include, not only carboxyl groups in hydrophilic regions of the protein, but also carbonyl groups in hydrophobic regions (the carbonyl group of the peptide bond as well as unionized carboxyl and carboxamide side-chains). The formation of such hydrogen bonds in hydrophobic regions of ribonuclease might account for a number of the abnormal properties such as low fluorescence, spectral shift to higher wave-lengths and lack of interaction with reagents such as iodine. Furthermore, the free energy change (ΔF^{o} = -3.38 Cal/mole) associated with the formation of the hydrogen bond could contribute significantly to the forces which mold the protein into its native configuration.

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