ABSORBANCE CHANGES IN THE 275 mμ REGION ASSOCIATED WITH EXPOSURE OF BURIED TRYPTOPHANYL RESIDUES TO AQUEOUS ENVIRONMENT

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Perturbations of the UV absorption spectra of proteins in the 230-260 mμ and 280-320 mμ regions have been thoroughly discussed by various authors (Wetlaufer, 1962; Donovan, 1964 and 1968; Mihalyi, 1968; Polet and Steinhardt, 1968). It is agreed that the appearance of a 291-292 mμ peak in the difference spectrum is due to the exposure of tryptophanyl residues, normally buried in the interior of proteins, to aqueous solvent. Changes in absorbance in pH difference spectra have been frequently observed in the 270-280 mμ region (Tanford, et al, 1956; Inada, et al, 1964; Tachibana and Murachi, 1966), but have not been discussed. The present investigation reports the effects of pH on the UV absorption spectrum of apo-conalbumin, a metal-binding protein from hen's egg white, as observed by difference spectral studies, with particular attention paid to changes in the 270-280 mμ region. From the results of these studies, we propose that the transfer of a buried tryptophanyl residue from the interior of a protein to an aqueous environment is accompanied by an increase in molar absorbance at 275 mμ, $\Delta \epsilon_{275}$, of about 10^3.

MATERIALS AND METHODS

Conalbumin was prepared as previously reported (Woodworth and Schade, 1959) with the modification that the protein was chromatographed on CM-Sephadex C-50 rather than on CM-cellulose. The preparation was found to be pure by spectrophotometric and metal-binding criteria and homogeneous on electrophoresis in polyacrylamide gel (Woodworth and Clark, 1967). The protein preparation had an A_{280} of 18, equivalent to a concentration of 15 mg/ml.

The spectra were recorded with a Cary 15 spectrophotometer at an ambient temperature of 22°. The slit width was kept between 0.05 and 0.2 mm; the optical path length of the samples was 0.50 mm.

The pH of the solution was adjusted with 1N NaOH. Difference spectra were recorded within 5 minutes of pH adjustment unless stated otherwise. Fisher certified reagent grade urea was used without further purification.

RESULTS AND DISCUSSION

The difference spectra of apo-conalbumin at various alkaline pH's up to 11.2 vs. 7.0 are identical in shape with that shown in Fig. 1. These difference spectra are characterized by two absorption maxima at 245 and 295 mμ, with an absorbance ratio of 4.7:1, and a negative minimum at about 275 mμ. The close similarity, if not identity, of these difference spectra with the pH difference spectra of tyrosine and tyrosyl peptides assures us that we are observing a pure tyrosyl dissociation spectrum in apo-conalbumin up to pH 11.2.

The shape of the pH difference spectra of apo-conalbumin at pH > 11.2 vs. 7.0 deviates from the tyrosine ionization difference spectrum. In Fig. 2, the dashed curve, the difference spectrum of apo-conalbumin at pH 13.0 vs. 7.0 was recorded within 5 minutes after the pH was adjusted,

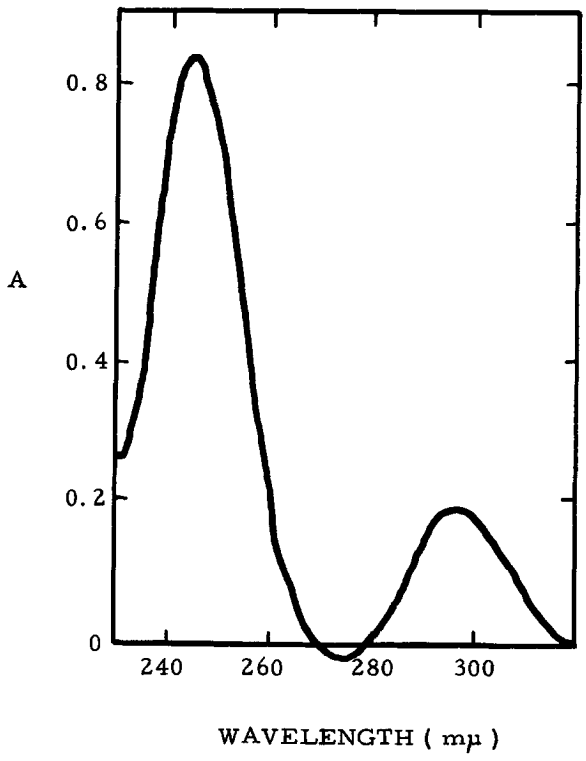

Fig. 1. Difference spectrum of apo-conalbumin at pH 11.0 vs. 7.0. Conalbumin concentration = 15 mg/ml. Optical path length = 0.5 mm.

It differs from Fig. 1 in that the two maxima have shifted to about 242 and 297 mμ, with an absorbance ratio of 5.4:1, a shoulder at 291-292 mμ has appeared, and the minimum in the 275 mμ region has become positive. In Fig. 2, the solid curve, recorded one hour after the pH was adjusted to 13.0, is similar in shape to the dashed curve except that increases in absorbance of varying degree have occurred in all regions. The increase in molar absorbance at 275 mμ, $\Delta\epsilon_{275}$, is about 1.5×10^4; if one assumes that the increase in absorbance owing to turbidity at 275 mμ is the same as that at 320 mμ, the net $\Delta\epsilon_{275}$ equals 1.0×10^4.

The $\Delta\epsilon_{275}$ may be explained in terms of the freeing of buried tryptophanyl residues from the interior of the conalbumin molecule

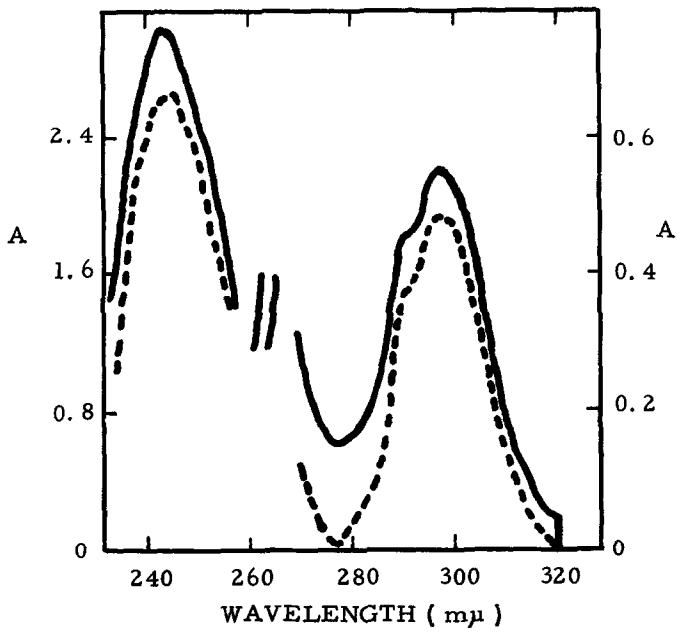

Fig. 2. Difference spectrum of apo-conalbumin at pH 13.0 vs. 7.0. Conalbumin concentration = 15 mg/ml. Optical path length = 0.5 mm.
--- recorded within 5 min. after the pH was adjusted to 13.0
___ recorded one hour after the pH was adjusted to 13.0

concomitant with the conformational change of the protein. The reasons for this conclusion are: (1) out of a total of 11 tryptophanyl residues per conalbumin molecule (based on molecular weight of 76,600 for conalbumin (Lewis, et al 1950; Spies, 1967)), the Koshland reagent only modified 1.8 residues in apo-conalbumin in aqueous solvent, and 7.4 residues in 100% chloroethanol (Gaffield, et al 1966). This indicates that a large number of tryptophanyl residues are buried in the interior of the native conalbumin molecule. The exposure of these buried tryptophanyl residues at pH > 11.2 is demonstrated by the appearance of a 291-292 mμ peak in the difference spectrum (Fig. 2). (2) Positive absorption in the region of 275 mμ in the alkaline vs. neutral pH difference spectrum appeared in tryptophan-

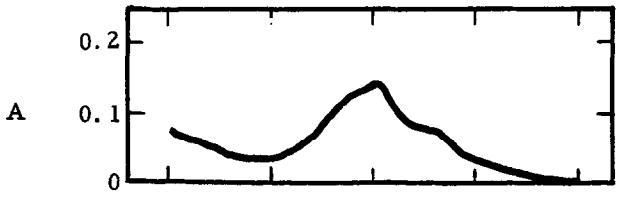

Fig. 3. Difference spectrum of N^{α}-acetyl-L-tryptophanamide at pH 13.0 vs. 7.0, determined at a concentration of 1.2 mM. Optical path length = 10 mm.

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containing protein such as bovine serum albumin (Tanford and Roberts, 1952), chymotrypsinogen, α-chymotrypsin, and trypsin (Chervenka, 1959, Inada, et al, 1964), but not in proteins containing tyrosine but no tryptophan, such as insulin (Crammer and Neuberger, 1943) and ribonuclease (Tanford, et al, 1956; Sage and Singer, 1962). (3) The $\Delta\epsilon_{275}$ of N^{α}- acetyl-L-tryptophanamide for pH 13.0 vs. 7.0 is about 100 (Fig. 3), which does not account for the large change of 10^4 found in the case of conalbumin. Assuming there are 9 buried tryptophanyl residues exposed at pH 13, and subtracting the value of 100/tryptophanyl residue owing to pH change, one obtains a value of about 10^3 associated with the exposure of each tryptophanyl residue. This is the same order of magnitude (700-1000) as the $\Delta\epsilon_{287}$ resulting from the exposure of a tyrosyl residue from the interior of a protein (Bigelow and Geschwind, 1960, Bigelow, 1961). (4) After the apo-conalbumin was incubated in 8M urea for two days, the pH 13.0 vs. 7.0 difference spectrum was normal, i.e., similar to the dissociation spectrum of tyrosine and to that shown in Fig. 1. Presumably the urea had exposed all tryptophanyl residues, so that the only effect of pH on the difference spectrum resulted from the ionization of tyrosyl residues.

The $\Delta\epsilon_{275}$ of 10^3 per mole of tryptophanyl residues exposed from the interior of protein to aqueous solvent is a very rough estimate. Because of the differences in environment of buried tryptophanyl residues, this value must be used with caution. Nevertheless, based on the results of the present investigation and the published data on other proteins, the association of positive absorption in the 275 mμ region in the alkaline vs. neutral pH difference spectrum of proteins with the exposure of buried tryptophanyl residues seems fairly certain, and appears to be uncomplicated by contributions from other chromophores.

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