in early isotope studies. In current investigations related to the present report, a net synthesis of acetyl-L-phenylalanine by slices of liver, kidney, or brain of the rat was easily demonstrated. Since a primary metabolic pathway for excess acetylphenylalanine—deacetylation by appropriate acylase systems—would be of little value to the phenylketonuric, its excretion might be anticipated.

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SC 2211

On the titration behavior of dimethylaminonaphthalene–protein conjugates

It has been suggested that the unusual displacement of the pK_a of certain groups conjugated to proteins is due to masking of these groups by an ice-like structure surrounding the protein[1]. For example, the pK_a of the dimethylamino group of 1-dimethylaminonaphthalene-5-sulfonyl chloride conjugates of glycine in water is 3.99, whereas the same group attached to bovine serum albumin exhibits a pK_a of 1.67 (ref. 2). Interpretation of these results in terms of frozen-water layers in the vicinity of the protein, however, has been questioned[3].

The present communication reports the titration behavior of two DNS–protein conjugates: bovine pancreatic RNAase and egg-white lysozyme.

RNAase A was prepared by chromatography on Amberlite IRC-50 of Sigma Chromatographic Grade RNAase by the procedure of HIRS, MOORE AND STEIN[4]. Lysozyme was obtained from Worthington Biochemical Corp. (Lot No. 583). Both proteins were coupled to DNS by reaction for 24 h at 4° in 0.1 M $NaHCO_3$ (pH 8.2). RNAase was freed from unreacted dye by 3 precipitations with acetone at 0°, lysozyme, by passage over G-25 Sephadex (Pharmacia, Uppsala) in 0.08 M acetic acid, followed by CG-50 type II chromatography in 0.3 M phosphate buffer (pH 7.18)[5]. The latter procedure revealed a single peak absorbing at 280 mμ. Both proteins were labelled to the extent of 1.0 mole dye/mole protein as calculated from the molar absorbancies of RNAase[6], lysozyme[7], and DNS[8]. Both conjugates retained full enzymic activity when assayed using RNA and *Micrococcus lysodeikticus* cell walls, respectively. Re-

Abbreviation: DNS, 1-dimethylamino-5-naphthalene-sulfonyl chloride.

duced RNAase was prepared from its DNS conjugate by the method of ANFINSEN AND HABER[9].

Titrations were performed at 25° using the Radiometer pH meter, Model TTTl, standardized with National Bureau of Standards potassium phosphate buffer (pH 6.86). Protonation of the dimethylamino group was followed by measurement of fluorescent intensity using a Brice-Phoenix light-scattering photometer employing a mercury light source. Corning filters No. 5970 and No. 3385 intercepted the excitation and emission beams, respectively. The degree of dissociation, α, was computed from the difference in intensities between pH 7–8 and pH 1.0 and the intensity at the given pH. The apparent pK_a was determined at $\alpha = 0.50$.

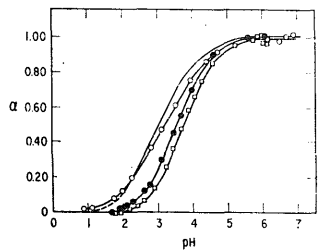

Fig. 1. Titration curves of the DNS–RNAase conjugate. O—O, in 0.10 M KCl; ———, calculated for RNAase in 0.10 M KCl; ● —●, reduced RNAase in 0.10 M KCl; □ —□, in 8 M urea.

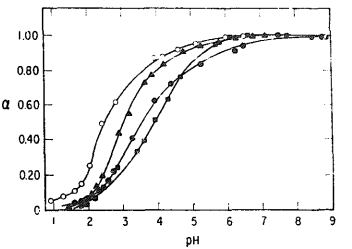

Fig. 2. Titration curves of the DNS–lysozyme conjugate. O—O, in water; ▲—▲, in 8 M urea; ●—●, in 0.10 M KCl; ■—■, in 8 M urea, 0.10 M KCl.

The results are shown in Fig. 1 for RNAase and in Fig. 2 for lysozyme. The dissociation curves are all fully reversible except for the case of lysozyme in 8 M urea*. Table I summarizes the pK_a values. Fig. 1 also shows the theoretical titration curve of RNAase–DNS computed from the intrinsic pK of DNS[2] and the titration data of TANFORD AND HAUENSTEIN[11]. The value 3.20 for native RNAase in 0.10 M KCl is in agreement with the value 3.05 computed from electrostatic considerations.

KLOTZ[12] has reported $pK_a = 2.37$ for DNS–lysozyme in water, in agreement with the value 2.50 shown in Table I. However, the pK_a of this conjugate in 0.10 M KCl was observed in the present study to be 3.55. Since chloride binding by lysozyme has been shown to be significant in the acid region[13], it seems reasonable to attribute the difference in pK_a in water and 0.10 M KCl to such binding. The small alkaline shifts in pK_a observed with 8 M urea solutions of both conjugates appear to be explainable on the basis of both molecular expansion and the effect of urea in decreasing the electrostatic field. Molecular expansion may also account for the alkaline shift observed after reduction of the four disulfide bonds of RNAase. Therefore, the DNS derivatives of these two proteins exhibit "normal" titration behavior, and an

* Full reversibility in this case was observed only at pH values above 3.5. Below pH 3.5, a slow time-dependent increase in fluorescent intensity occurred in agreement with the observation[10] that lysozyme in 8 M urea undergoes a structural transition below pH 3.7. Therefore, although the plots of α *versus* pH in this instance cannot be considered to represent thermodynamic titration curves, the pH of half fluorescent quenching was determined rapidly prior to the occurrence of the time-dependent effects.

ice-like lattice surrounding these molecules need not be invoked to explain the acid shifts in pK_a. Moreover, if such lattices are responsible for lowering of the pK_a of the DNS conjugate of bovine serum albumin, it seems reasonable to expect similar behavior in the case of other protein conjugates—a conclusion unsupported by the present study.

TABLE I

pK_a VALUES FOR DNS CONJUGATES OF RNAASE AND LYSOZYME

Preparation	*Solvent*	pK_a
RNAase	0.10 M KCl	3.20
RNAase (calculated) *	0.10 M KCl	3.05
Reduced RNAase	0.10 M KCl	3.55
RNAase	8 M urea	3.75
Lysozyme	water	2.50
Lysozyme	0.10 M KCl	3.55
Lysozyme	8 M urea	3.05
Lysozyme	8 M urea, 0.10 M KCl	3.90

* Calculated from data of TANFORD AND HAUENSTEIN[11].

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SC 2184

Isolation of 1-methylimidazole-4-acetic acid, A metabolic product of histamine, from human urine

The major urinary excretory product of histamine in man is 1-methylimidazole-4-acetic acid; it has been identified by isotope-dilution techniques in urine of humans injected with radioactive histamine[1–3]. No reliable non-isotopic analytical method