solution 1 is

$$\text{amine(l)} + H_2SO_4(1) \longrightarrow \text{amine}\cdot H^+(1) + HSO_4^-(1)$$

and in solution 2

$$\text{amine(l)} + H_2SO_4(2) \longrightarrow \text{amine}\cdot H^+(2) + HSO_4^-(2)$$

Subtracting, we have for transferring the reaction from solution 1 to 2

$$\text{amine}\cdot H^+(1)HSO_4^-(1) + H_2SO_4(2) \longrightarrow \text{amine}\cdot H^+(2)HSO_4^-(2) + H_2SO_4(1)$$

Allowance must now be made for transferring from solution 1 to solution 2 the increment of H_2SO_4 needed to protonate the amine. This correction is made using data from the work of Giauque.[16]

When these enthalpies of transfer are combined with the corresponding free energies, the partial molar entropies of transfer (relative to the value in 10% H_2SO_4, *i.e.*, 1.085 *M*) result.[17] Tables I and II show that across all of the observable range of moderately concentrated sulfuric acid, the medium effect on the free energies of solution for the three kinds of ammonium ions relative to each other are almost completely controlled by the enthalpy term. *There is a nearly negligible difference in the way the entropies of solvation for the primary, tertiary, and quaternary anilinium ions respond to variation of acidity.* If these results may be generalized, it follows that the type of entropy argument which was a strong buttress of the original hydration interpretation for strong base ions in water is not applicable to similar ions in strong acid.

Table I. Thermodynamic Properties for Transfer of Anilinium Ion Relative to Tetraethylammonium[a]

Wt % H_2SO_4	$\Delta\Delta F$,[b] kcal/mole	$\Delta\Delta H$,[b,c] kcal/mole	$T\Delta\Delta S$, kcal/mole
10.00	0.00	0.00	0.00
20.00	0.46	0.53	0.07
30.00	1.08	1.43	0.35
40.00	2.05	2.40	0.35
50.00	2.70	3.29	0.59
60.00	3.19	3.50	0.31
70.00	...	3.53	...
80.00	...	3.79	...

[a] *E.g.*, $\Delta\Delta H$ (anilinium) − $\Delta\Delta H$ (tetraethylammonium). [b] $\Delta\Delta F$ and $\Delta\Delta H$ interpolated. To get original numbers (as read from graph), add 0.19 kcal/mole to $\Delta\Delta F$ and −5.77 to $\Delta\Delta H$. [c] Maximum error in ΔH measurements is estimated to be ±0.200 kcal/mole.

Table II. Thermodynamic Properties for Transfer of N,N-Dimethylanilinium Ion Relative to Tetraethylammonium[a]

Wt % H_2SO_4	$\Delta\Delta F$,[b] kcal/mole	$\Delta\Delta H$,[b,c] kcal/mole	$T\Delta\Delta S$, kcal/mole
10.00	0.00	0.00	0.00
20.00	0.22	0.30	0.08
30.00	0.45	1.01	0.56
40.00	1.00	1.49	0.49
50.00	1.37	1.82	0.45
60.00	1.72	1.73	0.01
70.00	1.58	1.46	−0.12
80.00	...	1.07	...

[a] *E.g.*, $\Delta\Delta H$ (N,N-dimethylanilinium) − $\Delta\Delta H$ (tetraethylammonium). [b] $\Delta\Delta F$ and $\Delta\Delta H$ interpolated. To get original numbers (as read from graph), add 0.105 kcal/mole to $\Delta\Delta F$ and −4.77 to $\Delta\Delta H$. [c] Maximum error in ΔH measurements is estimated to be ±0.200 kcal/mole.

A simple calculation shows that this is in accordance with expectation.

Between 10 and 70% sulfuric acid, the change in partial molal entropy of water[16] ($\Delta\bar{S}_{H_2O}$) is only −0.3 cal/deg mole. Employing the usual hydration formalism[9,11] and assuming no hydration of the tetraethylammonium ion through specific hydrogen bonds, we have for the entropy of transfer for a hydrated ion that loses Δn molecules of hydrating water over this acidity range

$$(\bar{S}_{BH^+}\cdot n_{70}H_2O) - (\bar{S}_{BH^+}\cdot n_{10}H_2O) = (n_{70} - n_{10})\Delta\bar{S}_{H_2O} = 0.3\Delta n$$

Therefore, even if the anilinium ion lost all of the three water molecules that make up the formal hydration difference between it and the tetraethylammonium ion, it would only result in an entropy of transfer difference of about 1 entropy unit or 0.3 kcal/mole in $T\Delta\Delta S$ across this range. The corresponding change in $\Delta\bar{F}_{H_2O}$ is a decrease of about 1.8 kcal/mole. This is of the right magnitude to account for the $\Delta\Delta F$ values of our ammonium ions, but seems very small for the removal of hydrating water molecules if they are in fact held by strong (say 5–15 kcal/mole) hydrogen bonds such as are implied by the $B—H^+\cdots OH_2$ model.

Edward M. Arnett, John J. Burke
Department of Chemistry, University of Pittsburgh
and Mellon Institute, Pittsburgh, Pennsylvania 15213

Received February 21, 1966

---

(16) W. F. Giauque, E. W. Hornung, J. E. Kunzler, and T. R. Rubin, *J. Am. Chem. Soc.*, **82**, 62 (1960).

(17) In our work, the common anion for the various amines salts is formally the bisulfate anion, while in Boyd's work[13] it is the pentacyanopropenide anion. In each case, the anion contribution cancels out from comparisons of one salt with another.

The Ability of Anionic Detergents to Fold Disordered Polypeptide Chains of Histones in the α-Helical Form[1]

Sir:

According to previous optical rotatory dispersion studies,[2–4] anionic detergents increase the α-helix content of nonhelical proteins. This was concluded on the basis of the observed changes of the Drude constants (λ_c) and the Moffitt constants (b_0). Troitskii recently published a paper[5] in which an extended Moffitt equation[6] of Wada, *et al.*,[7] was applied to proteins, and Troitskii concluded that most of the ordinary globular proteins contain large amounts of the β conformation in addition to the α-helix and disorder. The possible existence of the β form in globular pro-

---

(1) Supported in part by Grants CA-01785 and CA-07746 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, and by Grants G-051 and G-138 from the Robert A. Welch Foundation, Houston, Texas.

(2) B. Jirgensons, *Arch. Biochem. Biophys.*, **94**, 59 (1961).

(3) B. Jirgensons, *ibid.*, **96**, 321 (1962).

(4) B. Jirgensons, *J. Biol. Chem.*, **238**, 2716 (1963).

(5) G. V. Troitskii, *Biofizika*, **10**, 895 (1965).

(6) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci. U. S.*, **42**, 596 (1956).

(7) A. Wada, M. Tsuboi, and E. Konishi, *J. Phys. Chem.*, **65**, 1119 (1961).

teins has been considered also by others.[8-12] Imanishi, *et al.*,[12] concluded that detergents facilitated destruction of the β form and promoted formation of the α-helix in Bence-Jones proteins. According to Troitskii,[5] the negative shift of b_0, *e.g.*, of serum γ-globulin, upon treatment with sodium decyl sulfate, is caused not by the formation of the α-helix but by destruction of the β conformation. This contention led to the experiments described in this communication.

Since the conformation of the nonhelical proteins, such as γ-globulin, is unknown, an example of a completely disordered protein should be found. The calf thymus histone fractions at very low ionic strength were found to behave as fully disordered linear polyelectrolytes. At low concentrations of the histone, its reduced specific viscosity increased exponentially with dilution,[13] and its rotatory dispersion curve in the Cotton effect zone of 190–240 mμ was very similar to the curve of the disordered poly-α-L-glutamate.[14,15] While some histone fractions, *e.g.*, F2b, became partially α-helical on addition of 0.02 to 0.1 *M* NaCl or other electrolytes, the lysine- and proline-rich histone F1 did not become ordered on such treatment.[14] To test the helix-forming ability of detergents, 0.10–0.32% aqueous solutions of the lysine-rich histone F1 were treated with 0.0012–0.05 *M* sodium dodecyl or decyl sulfate at 25° for at least 24 hr or at 50° for 2 hr. Small amounts of the aqueous detergent, when added to the histone solution, produced a precipitate (due to electrostatic interaction between the positively charged macromolecules of the histone with the negatively charged micelles of the detergent), but the precipitate dissolved in an excess of detergent. The dodecyl sulfate was more potent in forming a soluble complex than the decyl sulfate. The conformational transitions were determined by measuring the optical rotatory dispersion in the far ultraviolet with the Rudolph Model RSP-3 spectropolarimeter equipped with a double prism monochromator, as described previously.[14-16]

It was found that both decyl and dodecyl sulfates converted the disordered histone F1 partially into the α-helical form. While the pure aqueous histone displayed curves with a deep minimum at 206 and 209 mμ, the detergent-treated histone had curves with a minimum at 230 to 232 mμ and a positive maximum at 197 to 200 mμ as do proteins having a high α-helix content.[16-19] The results are summarized in Table I. An accurate estimation of the α-helix content is impossible, especially from the amplitude of the negative trough, because factors other than the α-helix are involved.[20,21] If the amplitudes of the positive peak are used,[15] the helix content is $[R']_{198}/75{,}000$, *i.e.*, 19,000/75,000 or 25%. Circular dichroism studies have shown that the rotational strength of the 190-mμ transition is about four times greater than that of the 222-mμ transition.[22] This favors the $[R']_{198}$ values for the estimation of the α-helix content. The b_0 values of the detergent-treated histone were between −157 and −190°, as determined from measurements in the 240- to 300-mμ zone using a λ_0 of 216, whereas the b_0 of the aqueous F1 was near zero. The other lysine-rich histone fraction F2b, which could be made 20% helical by NaCl and other simple electrolytes,[14] was converted to 36% α-helical form by a similar treatment with dodecyl sulfate. This higher efficiency of the detergent can be explained by the lesser content of proline in F2b (3.4%) in comparison to the 9.4% in F1. Complete amino analyses of the histones used have been reported.[14]

Table I. Cotton Effect Amplitudes of Histone F1 Treated with Decyl or Dodecyl Sulfates[a]

Mole ratio, F1 : detergent	Time, hr, and temp, °C, of treatment	$[R']^{25}_{231}$, deg	$[R']^{25}_{198}$, deg
1:5000 C_{10}	24, 25	−4500 (±500)	+17,800 (±1500)
1:5000 C_{10}	2, 50	−4900	+18,500
1:5000 C_{12}	2, 50	−6500	+18,000
1:1250 C_{12}	2, 50	−6500	+19,200
1:250 C_{12}	2, 50	−5300	+18,600
1:125 C_{12}	2, 50	−5700	+16,300

[a] The molecular weight of F1 was approximately 20,000. The $[R']$ values were computed from $[R'] = [\alpha]3M/100(n^2 + 2)$, where $[\alpha]$ is the specific rotation, *M* the mean residual molecular weight of the amino acids, and *n* the refractive index of the solvent. An approximation of $[R'] = 0.9[\alpha]_{231}$ and $[R'] = 0.85[\alpha]_{198}$ was used.

The observations prove that decyl and dodecyl sulfates do indeed facilitate the α-helix formation. The mechanism of this interaction is unknown, but it is likely that the bound detergent provides a hydrophobic environment around the peptide bonds, thus protecting them from hydrogen bonding with water.[23] These observations do not disprove the claim of other investigators[5,11] that detergents destroy the β structure. However, it seems that this is not the only transition the detergents are able to incite. Since the disordered histones are converted in part into the α-helical form, it is possible that the same may happen in some other proteins.

(8) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961).

(9) J. A. Schellman and C. G. Schellman, *J. Polymer Sci.*, **49**, 129 (1961).

(10) K. Imahori and H. Momoi, *Arch. Biochem. Biophys.*, **97**, 236 (1962).

(11) P. Callaghan and N. H. Martin, *Biochem. J.*, 87, 225 (1963).

(12) A. Imanishi, Y. Momotani, and T. Isemura, *J. Biochem.* (Tokyo), **57**, 417 (1965).

(13) B. Jirgensons, L. Hnilica, and S. Capetillo, *Makromol. Chem.* (in press).

(14) B. Jirgensons and L. Hnilica, *Biochim. Biophys. Acta*, **109**, 241 (1965).

(15) B. Jirgensons, *J. Biol. Chem.*, **241**, 147 (1966).

(16) B. Jirgensons, *ibid.*, **240**, 1064 (1965).

(17) E. R. Blout, I. Schmier, and N. S. Simmons, *J. Am. Chem. Soc.*, 84, 3193 (1962).

(18) E. Breslow, S. Beychok, K. D. Hardman, and F. R. N. Gurd, *J. Biol. Chem.*, **240**, 304 (1965).

(19) Y. Tomimatsu and W. Gaffield, *Biopolymers*, **3**, 509 (1965).

(20) M. A. Ruttenberg, T. P. King, and L. C. Craig, *J. Am. Chem. Soc.*, **87**, 4196 (1965).

(21) R. Biltonen, R. Lumry, V. Madison, and H. Parker, *Proc. Nat. Acad. Sci. U. S.*, **54**, 1018 (1965).

(22) G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).

(23) Ch. Tanford, P. K. De, and V. G. Taggart, *ibid.*, **82**, 6028 (1960)

B. Jirgensons, L. S. Hnilica
The University of Texas
M. D. Anderson Hospital and Tumor Institute
Department of Biochemistry, Houston, Texas 77025
Received January 8, 1966

A Novel Method for the Formation of the Tetraphenylbismuthonium Cation

Sir:

The metathetical reaction between silver salts and organometallic chlorides, bromides, or iodides to