turn of the B-amylose helix, and the equivalent maltose molecule in the adjacent unit cell (in the *c* direction) may represent the residues in the next turn of the helix. The similarity is complete down to the interturn hydrogen bonding through the water molecule, with the only exception that in maltose this bonding is O(6)–O(W)–O(3), while in B-amylose it is O(6)–O(W)–O(2). This difference is minor in contrast to the support which the present result lends to the idea that in the B-amylose structure the hydrogen bonding between successive turns of the helix is *via* water of hydration.

Ion-Electrode Study of Alkali Metal Adenosine Triphosphate Complexes

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Received March 18, 1970

Abstract: Association constants of the ion pairs $KATP^{3-}$ and $NaATP^{3-}$ have been measured by direct potentiometry using K^+-sensitive electrodes of the valinomycin and glass membrane type. Formation constants taken at 25° are substantially greater than those previously reported from indirect measurements.

The adenine nucleotides, in particular adenosine 5′-triphosphate (adenosine 5′-(tetrahydrogen triphosphate)), take part in many important biochemical reactions. The hydrolysis of adenosine 5′-triphosphate is the chief source of energy for the active transport of K^+ and Na^+ ions in nerve, muscle,[1] and blood cells.[2] It is now generally recognized that adenosine 5′-triphosphate and its analogs exist in various metal-complexed forms in biological fluids and it is important for obvious reasons to know the stabilities of these complexes.[3] The alkali metals form complexes of weak but measurable stability. Melchior[4] measured the difference in the pK_a value for the reaction

$$HATP^{3-} \rightleftharpoons ATP^{4-} + H^+$$

obtained by replacing $(CH_3)_4N^+$ with Na^+ or K^+, and obtained a value of 9.8 ± 0.2 for the stability constant of the complex $MATP^{3-}$, where M is the alkali metal. In this study the ionic strength was maintained around 0.2 *M* and the assumption was made that the tetraalkylammonium ions did not associate with the ligand. By a similar technique of potentiometric titrations, using the same assumptions, Smith and Alberty[5] obtained values of 11.5 ± 1.0 and 14.3 ± 0.4 for the $KATP^{3-}$ and $NaATP^{3-}$ species, respectively, at an ionic strength of 0.2 maintained with tetra-*n*-propylammonium bromide. The difference in stabilities between the sodium and potassium complexes was considered significant by the authors. From the apparent stability constants of $CaATP^{2-}$ and $MgATP^{2-}$ complexes in the presence of sodium chloride, O'Sullivan and Perrin[6] estimated the stability constant of $NaATP^{3-}$ species to be 15 ± 2 M^{-1}. The value for the $KATP^{3-}$ complex was estimated to be 14 ± 2 M^{-1} by a similar method.

Although there is apparent agreement among the results of these investigations, they have all been, in essence, indirect methods. Furthermore, the assumption that the tetraalkylammonium ions do not associate with a polyvalent anion like ATP^{4-} is open to question. Since the stability constants have been determined at constant ionic strength, they are applicable only under the conditions specified, and the interactions of the ions of interest with the solvent or medium ions cannot be distinguished. A more direct determination of the thermodynamic stability constants of alkali metal complexes of adenosine triphosphate therefore seems appropriate and desirable.

In recent years ion-selective electrodes have been successfully used as concentration and activity probes in several thermodynamic and kinetic studies.[7-10] The introduction of a new valinomycin[11,12] electrode of the liquid exchanger type with a very high selectivity for potassium over sodium of 5000:1 promises to be an excellent tool for investigating biological phenomena involving changes in potassium activity in the presence of a larger concentration of sodium ions. In the work reported here the thermodynamic stability constant for the $KATP^{3-}$ complex has been measured using a valinomycin electrode and a Corning monovalent cationic glass electrode. The stability constant for the $NaATP^{3-}$ complex measured with the latter electrode is also reported.

Experimental Section

Reagents. The Σ-grade disodium salt of adenosine 5′-triphosphate ($Na_2H_2ATP \cdot 3.5H_2O$) manufactured by Sigma Chemicals

(1) P. C. Caldwell, A. L. Hodgkin, R. D. Keynes, and T. I. Shaw, *J. Physiol. (London)*, **152**, 561 (1960).
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(12) L. A. R. Pioda, V. Stankova, and W. Simon, *Anal. Lett.*, **2** (12), 665 (1969).

(Lot A-3127), and the dipotassium salt ($K_2H_2ATP \cdot 1.5H_2O$), A grade, manufactured by Calbiochem (Lot 901122), were used without further purification. The sodium and potassium content in the respective nucleotides was determined with the help of a Perkin-Elmer Model 303 atomic absorption spectrophotometer. The manufacturer's assay for the disodium salt was 98%, while the average of three determinations with the atomic absorption spectrophotometer gave an assay of 97.3%. The corresponding values for the dipotassium salt were 97 and 95.6%, and these corrections were introduced into the calculations. The nucleotides were stored in a desiccator at $-10°$.

Distilled water, deionized by passing through a mixed-bed resin column, was used for preparation of all stock solutions. Carbonate-free stock solutions of potassium hydroxide and sodium hydroxide were prepared by dilution of Acculute reagent concentrates (manufactured by Anachemia Ltd.) and restandardized against potassium biphthalate.

Apparatus. Potentiometric measurements were carried out in a double-walled Pyrex vessel maintained at 25 ± 0.1°. Carbon dioxide was excluded from the experimental solution by nitrogen bubbling. Potassium ion activities were monitored with an Orion Model 92-19 valinomycin potassium and a Corning monovalent cationic electrode (Catalog No. 476220), while sodium ion activities were measured using the latter electrode. An Orion Model 90-02 double-junction electrode was used as reference with 1 *M* lithium trichloroacetate[13] in the outer chamber for K^+ ion activity measurements. Potassium nitrate (1 *M*) was used in its place when making Na^+ ion activity measurements. A Beckman glass electrode (Catalog No. 39000) was used to monitor pH. Potentials were measured with a Beckman research pH meter in conjunction with an Orion Model 855 automatic electrode switch.

Results

The electrodes were calibrated before and after each "titration" by adding increments of 0.025 *M* KCl solution to a known volume of water. A sufficient quantity of 0.05 *M* KOH was added to maintain the pH at 9.1 ± 0.1. In order to minimize the leakage from the reference electrode it was immersed in the experimental solution for the least time necessary for taking the reading, even though a 15-min period of immersion did not change the potential readings by more than 0.2 mV. The activity coefficients were calculated using the Davies equation[14]

$$-\log f_z = AZ^2\left(\frac{I^{0.5}}{1 + I^{0.5}} - 0.3I\right) \quad (1)$$

where f_z is the activity coefficient of the z-valent ion, I the ionic strength, and A the Debye–Hückel parameter. Equation 1 has been similarly employed in the calculation of the thermodynamic stability constant for the $NaP_4O_{12}^{3-}$ ion pair.[10] Though no detailed study of the response time of the two electrodes was made, it was observed that less than 30 sec was required to obtain steady potentials (±0.1 mV) for K^+ ion concentrations $>2 \times 10^{-3}$ *M*. Below this concentration level, an interval of 5–10 min was allowed for equilibration. The slope and $E°$ values were obtained by fitting the data to a linear least-squares program using a Hewlett-Packard 9100A calculator. An $E°$ value of 59.5 ± 0.5 mV for the Corning electrode could be reproduced over a period of weeks, whereas the $E°$ value for the valinomycin electrode tended to drift by about 2 mV over a 48-hr period. The slope for either electrode was 56.5 ± 0.3 mV. A calibration curve for the valinomycin electrode showed a Nernstian response from 10^{-1} to 10^{-5} *M* and a slightly lower response from 10^{-5} to 10^{-6} *M*.

The potentiometric measurements were made by adding increments of a freshly prepared stock solution of K_2H_2ATP, $\sim 10^{-2}$ *M*, and 0.05 *M* KOH to 50 ml of water so that the pH of the resultant solution was 9.2 ± 0.1. The potassium activity of the solution was measured after each addition of the ligand and base and was employed to obtain an independent value of the stability constant. The slight amount of hydrolysis in the ligand solution was negligible in the duration of the experiment.[15]

The following equations were employed to calculate the concentrations of the ionic species in solution.

$$E_{cell} = E_0 + S \log \{K^+\} \quad (2)$$

$$T_M = 2T_{ATP} + T_{base} \quad (3)$$

$$[KATP^{3-}] = T_M - [K^+] \quad (4)$$

$$[ATP^{4-}] = T_{ATP} - [KATP^{3-}] \quad (5)$$

T_{ATP} and T_{base} are the added total ligand and total base, respectively, and T_M is the total potassium. Since the pH at which the measurements were made was sufficiently higher than the pK_a value for the reaction

$$ATPH^{3-} \underset{}{\overset{pK_a = 6.95}{\rightleftharpoons}} ATP^{4-} + H^+ \quad (6)$$

it was reasonable to assume that the total ligand was present as ATP^{4-} or $KATP^{3-}$ (the error is less than 0.6%). The K^+ concentrations were calculated from the activities by eq 1. The thermodynamic association constant for the reaction

$$K^+ + ATP^{4-} \rightleftharpoons KATP^{3-} \quad (7)$$

$$K = \frac{[KATP^{3-}]f_3}{[ATP^{4-}][K^+]f_1 f_4} \quad (8)$$

was obtained by successive approximation of the ionic strength[16]

$$I = 0.5\{16.0[ATP^{4-}] + 9.0[KATP^{3-}] + [K^+] + [OH^-]\} \quad (9)$$

with the aid of a CDC 6400 computer.

The results given in Tables I and II were obtained with the Corning electrode and the valinomycin electrode, respectively. The values for the $NaATP^{3-}$ species are given in Table III and were obtained by a similar procedure.

Discussion

Since the literature values[4-6] for the formation constants of the $KATP^{3-}$ and $NaATP^{3-}$ ion pairs have been obtained at an ionic strength of 0.2, there is no reliable method of comparing them with the values obtained in the present study. The activity coefficients of electrolytes tend to become highly individual at such high ionic strengths, and the empirical extensions of the Debye–Hückel equation are no longer functional. However, a rough calculation indicates that increasing the ionic strength from 0.01 to 0.2 *M* would decrease the K values by a factor of 2. The much greater discrepancy observed here points either to the fact

(13) Preliminary instruction manual, Model 92-19 potassium electrode, Orion Research Inc., Cambridge, Mass., p 1.

(14) C. W. Davies, "Ion Association," Butterworths, London, 1962, p 41.

(15) M. Tetas and J. M. Lowenstein, *Biochemistry*, **2**, 350 (1963).

(16) G. H. Nancollas, "Interactions in Electrolyte Solutions," Elsevier, Amsterdam, 1966, p 88.

Table I. Potassium Adenosine Triphosphate Ion Pair Formation at 25° Measured with the Corning Cationic Glass Electrode[a]

pH	$T_M \times 10^3\ M$	$T_{ATP} \times 10^3\ M$	$I \times 10^2$	$\{K^+\} \times 10^3$	$[KATP^{3-}] \times 10^4\ M$	K, l. mol^{-1}
9.276	3.611	0.723	0.679	3.114	1.984	191.3
9.270	4.932	1.138	1.028	4.150	3.258	200.6
9.285	4.504	1.039	0.945	3.823	2.764	191.9
9.257	5.756	1.329	1.181	4.771	4.265	215.2
9.240	7.272	1.681	1.468	5.905	6.030	221.9
9.281	5.355	1.235	1.109	4.477	3.688	202.4
9.252	7.630	1.763	1.526	6.152	6.681	235.5
9.242	6.154	1.420	1.263	5.084	4.559	206.6
9.238	6.534	1.510	1.338	5.373	4.943	205.3
9.235	6.911	1.596	1.392	5.621	5.800	233.4
9.250	5.709	1.332	1.189	4.745	4.063	201.4
9.270	5.270	1.228	1.103	4.410	3.583	198.6
9.272	6.568	1.531	1.343	5.372	5.281	222.4
9.250	7.367	1.720	1.493	5.960	6.304	228.8
9.270	6.152	1.433	1.265	5.064	4.751	217.8
9.272	6.658	1.531	1.343	5.372	5.281	222.4
7.678[b]	5.867	1.456	1.235	4.786	5.083	262.9
7.530	6.111	1.544	1.297	4.965	5.388	263.6
7.442	6.510	1.662	1.382	5.258	5.904	266.5
7.301	6.614	1.723	1.431	5.366	5.622	241.1
7.191	6.788	1.799	1.488	5.522	5.481	223.0
7.254	7.448	1.951	1.608	5.992	6.489	235.3
Mean K = 222.2 l. mol^{-1}; standard deviation ±22.4 (49 determinations)						

[a] Values at each ionic strength represent single results. [b] K values obtained at pH values lower than 9.0 were corrected for the presence of $HATP^{3-}$ species.

Table II. Potassium Adenosine Triphosphate Ion Pair Formation at 25° Measured with the Orion Valinomycin Electrode[a]

pH	$T_M \times 10^3\ M$	$T_{ATP} \times 10^3\ M$	$I \times 10^2$	$\{K^+\} \times 10^3$	$[KATP^{3-}] \times 10^4\ M$	K, l. mol^{-1}
9.314	1.973	0.446	0.415	1.744	1.036	282.0
9.225	5.581	1.284	1.160	4.672	3.664	184.6
9.238	6.132	1.411	1.257	5.071	4.491	204.3
9.270	6.660	1.532	1.343	5.443	5.415	228.0
9.260	4.568	1.048	0.966	3.899	2.526	166.0
9.253	5.136	1.180	1.057	4.296	3.612	215.0
9.243	5.410	1.244	1.104	4.492	4.080	230.8
9.244	5.678	1.307	1.155	4.696	4.368	230.6
9.260	5.939	1.368	1.203	4.890	4.713	235.1
Mean K = 219.6 l. mol^{-1}; standard deviation ±24.1 (21 determinations)						

[a] Values at each ionic strength represent single results.

Table III. Sodium Adenosine Triphosphate Ion Pair Formation at 25°[a]

pH	$T_M \times 10^3\ M$	$T_{ATP} \times 10^3\ M$	$I \times 10^2$	$\{Na^+\} \times 10^3$	$[NaATP^{3-}] \times 10^4$	K, l. mol^{-1}
9.292	2.024	0.490	0.458	1.796	0.919	213.7
9.168	2.928	0.715	0.647	2.524	1.803	242.5
9.262	3.807	0.926	0.824	3.217	2.703	248.6
9.285	4.226	1.028	0.927	3.581	2.696	199.6
9.305	4.634	1.126	1.006	3.891	3.198	210.1
9.257	5.016	1.223	1.082	4.176	3.698	219.1
9.301	5.411	1.316	1.153	4.465	4.288	233.2
9.276	5.781	1.408	1.228	4.744	4.707	233.0
9.310	6.155	1.496	1.298	5.020	5.206	238.3
9.313	6.506	1.583	1.373	5.292	5.506	230.5
9.257	6.842	1.668	1.426	5.510	6.281	254.1
Mean K = 229.3 l. mol^{-1}; standard deviation ±19.6 (23 determinations)						

[a] Values at each ionic strength represent single results.

that indirect methods employing the pH glass electrode are not particularly suited to the measurement of small ion pair formation constants, or that tetraalkylammonium ions associate with the ligand.

An examination of the formation constants of alkali metal ion pairs with other ligands of the same charge type as ATP^{4-} indicates the order of K values obtained in the present study to be entirely reasonable. In the particularly analogous case of $P_2O_7^{4-}$, Monk,[17] in a conductometric study, obtained a value of 228 l. mol^{-1} for the $NaP_2O_7^{3-}$ ion pair. This compares very favorably with the value of 229 l. mol^{-1} for the $NaATP^{4-}$ species obtained in the present study. Under similar conditions of ionic strength, Wolhoff and Overbeek[18] obtained values of ~200 l. mol^{-1} for the $KP_2O_7^{3-}$ and $NaP_2O_7^{3-}$ species. Stability constants of the same order have been obtained for $K[Fe(CN)_6]^{3-}$ [19]

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(18) J. A. Wolhoff and J. T. G. Overbeek, *Recl. Trav. Chim. Pays-Bas*, **78**, 759 (1959).

and the sodium tetrametaphosphate ion pair, $NaP_4O_{12}^{3-}$.[10]

The close agreement between the stability constant values for the $KATP^{3-}$ species obtained with the cationic glass electrode and the valinomycin electrode indicates that the latter might prove to be an excellent tool for thermodynamic studies involving changes in K^+ activity. While the present study was conducted with pure solutions, the excellent selectivity properties[11] of this electrode permit its use in the presence of a large excess of sodium ions as found in many biological systems.

Acknowledgment. This work was supported by grants from the National Science Foundation and the Office of Saline Water, U. S. Department of the Interior. G. A. Rechnitz is an Alfred P. Sloan Fellow, 1966–1972.

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Fluorescence of Aliphatic Ketones[1]

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Abstract: The fluorescence of a series of aliphatic ketones excited at 313 nm has been studied at room temperature in *n*-hexane, cyclohexane, methanol, and acetonitrile. The results suggest that the α-CH stretching mode is a factor in radiationless processes from the excited singlet state. The fluorescence quantum yields of the carbonyl compounds were observed to increase with alkyl substitution at the α-carbon, and the fluorescence wavelength maxima remained constant at 405 ± 3 nm. The fluorescence yield of di-*tert*-butyl ketone in *n*-hexane at room temperature was determined to be larger by a factor of 4.41 than the value for acetone. Deuterated acetone exhibited enhanced fluorescence relative to ordinary acetone.

Recently we suggested that the 405-nm fluorescence band of acetone was due to an excimer based on concentration effects and its inability to respond to solvent effects.[2] Renkes and Wettack[3a] and Dalton[3b] have, since our initial report, each independently shown that the fluorescence wavelength maximum at 405 nm is normal fluorescence with no unusual behavior attributable to variation in concentration of the ketone. In view of our recent study on triplet deactivation of aliphatic ketones at 77°K[4] this report is presented with the aim of illustrating the effect of structure on the singlet deactivation of aliphatic ketones. The fluorescence of a series of aliphatic ketones, which may be considered derivatives of acetone in which the α-hydrogens are replaced with alkyl groups, have been investigated.

Experimental Section

Materials. The materials used in this study have been characterized elsewhere.[4]

Apparatus and Procedures. Absorption spectra were obtained with either a Bausch and Lomb Model 505 or a Beckman Model DU spectrophotometer. The natural radiative singlet lifetimes, τ_f^0, were estimated from the integrated absorption of the lowest transition of the ketone.

Fluorescence measurements were made with an Aminco grating monochromator equipped with a 1P21 photomultiplier tube and displayed on a Moseley Model 7030A XY recorder. The emission spectra were measured from air-saturated solutions, in view of the reported observations which indicate that the fluorescence of acetone and acetone-d_6 are unaffected by dissolved oxygen.[5,6]

All ketones were excited with 313-nm light which was isolated from an Osram HBO 100 W/2 high-pressure mercury lamp with a Schott interference filter. The monochromator entrance and exit slits were 1 mm and the emission was detected in a direction perpendicular to the exciting beam. Quartz spectrophotometric cells (1-cm path length) were used for absorption and fluorescence measurements, which were made at 25°. A planimeter was utilized to determine the relative fluorescence yield by integration of the emission spectra of all solutions, which were adjusted to have the same optical densities at the wavelength of excitation. The relative fluorescence quantum yield of acetone was determined relative to the value of 0.09 for tryptophan.[7]

Results

The most significant observations are that the spectral distribution of the fluorescence curves was identical for all the ketones studied and that the emission quantum yields increased when the α-hydrogens of acetone were replaced by various alkyl groups. A similar behavior was observed for the phosphorescence of these compounds.[4] The fluorescence wavelength maxima were in the range of 405 ± 3 nm for all the ketones in the four solvents employed, *i.e.*, *n*-hexane, cyclohexane, methanol, and acetonitrile. The relative fluorescence yield spectra of the compounds studied are shown in Figure 1, with the data normalized to acetone, which has the lowest fluorescence yield. Di-*tert*-butyl ketone has the largest fluorescence yield of the series and was found to be greater by a factor of 4.41 than acetone in *n*-hexane. A summary of the solvent effects on the

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