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DETERMINATION OF THERMODYNAMIC PARAMETERS OF BIOMOLECULES BY SPECTROPHOTOMETRIC TITRATIONS

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ABSTRACT

An automatic spectrophotometric titration system is described, which enables a fast and precise determination of thermodynamic parameters of reversible reactions in solution, requiring small amounts of material ($<10^{-7}$ mole). The set-up is applied for the determination of K_c^{app}, ΔH_{VH}^{app} and ΔS^{app} of the protolysis of membrane-active antibiotics.

INTRODUCTION

Thermodynamic parameters of reversible chemical reactions in solution can be determined by spectrophotometric titrations as an alternative to the widely used calorimetric methods, provided that the reaction is accompanied by a change of the spectral properties (absorption or fluorescence) of the system. Here, a new experimental set-up for automatic spectrophotometric titrations is described, which enabled the investigation of the binding of cations to ligands of biological interest.

MATERIALS and METHODS

The system for automatic spectrophotometric titrations (1) is based on commercially available components. The photometer (Hewlett Packard 8450) uses a diode array for the photometric detection. This allows the registration of a complete spectrum from 200 to 800 nm within one second. The titration system consists of one or two modified motor driven burettes (Hamilton, Microlab M, 0.05 ml syringes), which are controlled by a programmable calculator (Hewlett Packard 9815). The titrating solution is added to the solution in the cuvette via Teflon tubing. Effective mixing of the solutions is achieved by means of a micro-motor driven stirrer, which is attached directly to the cuvette through a Teflon adapter (Fig.1a). pH-titrations are usually carried out in 2 cm cuvettes, into which is inserted a specially manufactured glass electrode (Möller, Zürich) (Fig.1b). Furthermore, the temperature can be measured in the cuvette by a Pt 100 thermo resistor. The photometer, the titrator unit, the pH-meter and the thermometer are

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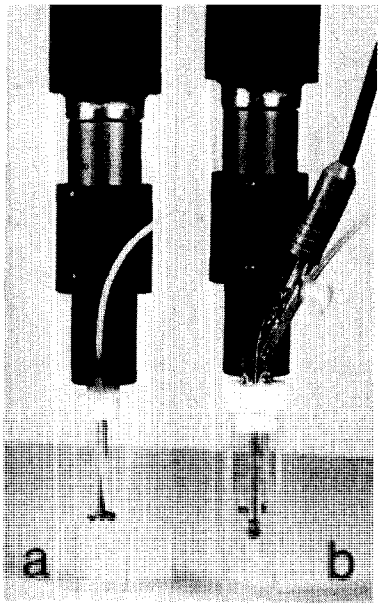

Fig. 1. a) cuvette (1 cm) with stirrer and supply tube
b) cuvette (2 cm) with stirrer, supply tube and pH-electrode.

interfaced to a mini computer (Hewlett Packard 1000). The automatic titration is controlled by a special program running on this computer. The time required for one cycle, including the addition of the titrating solution, a period allowing for complete mixing, the measurement of the spectrum, the pH and the temperature, and the storage of the data, usually takes one minute. Thus, a complete titration with 40 to 50 titration steps needs about one hour. The reliability of the method has been tested by determining the apparent equilibrium constant K_c^{app}, and the apparent enthalpy ΔH_{VH}^{app} and entropy ΔS^{app} of the complex formation of the cryptand 222 with Rb^+ ions in the presence of 60 mM tetramethylammonium chloride (TMACl) at pH 12 (LiOH). The values obtained from this method (2) are in very good agreement with the data published elsewhere (3,4).

Plauracin was provided by Pfizer (Groton, USA), virginiamycin by Smith Kline (Brussels, Belgium) and viridogrisein by Parke, Davies (Detroit, USA), as well as by Bristol Labs. (Syracuse, USA). Components have been isolated according to (5). Model compounds were prepared by chemical synthesis (6).

RESULTS and DISCUSSION

This spectrophotometric titration method has been applied to the study of the interaction between streptogramine group B antibiotics and various cations. These compounds, which are produced by microorganisms, interfere with the protein biosynthesis at the bacterial ribosomes. The macrocyclic structure of these com-

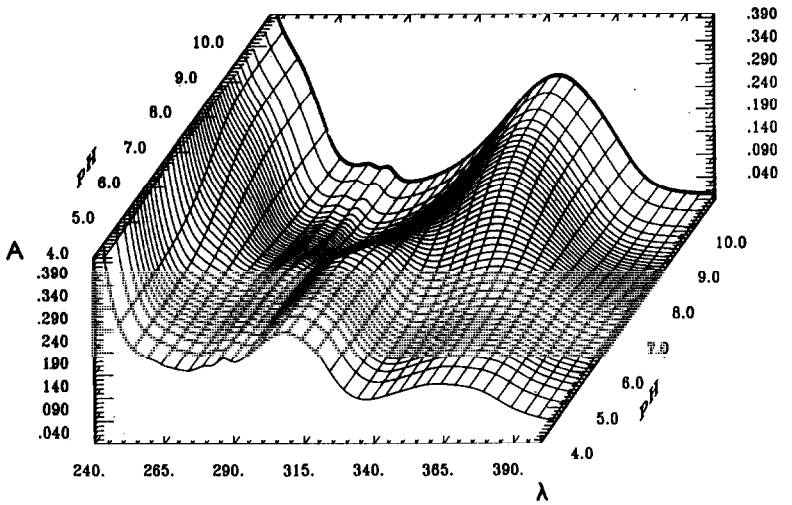

Fig. 2. Spectrophotometric pH-titration of Plauracin ($1.93\ 10^{-5}\ M^{-1}$) in 30% water in methanol containing 0.1 M TMACl and 8 mM MES, TES and CHES as buffers.

pounds, however , suggests that they may also act as ion carriers in the bacterial membrane. Such a carrier activity for protons, alkali and alkaline earth ions, has indeed been found in model membranes (7). The streptogramine antibiotics of group B exhibit two common structural features. These are a peptide lactone ring, which for most members of this group is formed of 6 amino acid residues and an exocyclic 3-hydroxypicolinic acid residue. The latter is the main chromophoric group and the binding site for protons and divalent cations (2,8). The monovalent alkali ions do not form a complex with this chromophoric group. This assignment of the binding sites has been concluded from the characteristics of the spectral changes. As an example, some of the results related to binding of protons to the 3-hydroxypicolinic acid residue are presented here. The absorption spectra obtained from a titration of the antibiotic plauracin with OH^- ions are shown in fig.2. The third coordinate, which represents the titration coordinate, is the pH-value of the solution. The curves at constant wavelengths are the titration curves. The binding constant is obtained by fitting of the theoretical binding function to these curves.

In order to determine the enthalpy and entropy of formation, the titrations have been carried out at different temperatures. The results for some of these antibiotics and for some model compounds are summarized in table 1. The latter have been used in order to investigate the effect of the ring structure on these parameters. All the titrations have been performed in 30% water in methanol (w/w) containing 0.1 M tetramethylammonium chloride.

TABLE 1
Thermodynamic parameters of the protolysis of streptogramine group B antibiotics and some selected model compounds.

	$\log K_c^{app}$ (298.2 K)	ΔG^{app} kcal/mole	ΔH_{HV}^{app} kcal/mole	ΔS^{app} cal/(mole K)
Viridogrisein I	8.00 ± 0.05	-10.9	-2.3 ± 0.3	-29 ± 1
Virginiamycin S_1	7.96 ± 0.05	-11.0	-4.5 ± 0.3	-22 ± 1
[a]HO-Pic-$NHCH_3$	9.20 ± 0.08	-12.4	-5.6 ± 0.5	-23 ± 2
[a]HO-Pic-Gly-Gly-$N(CH_3)$	8.82 ± 0.05	-12.3	-4.8 ± 0.3	-24 ± 1
[b]Sal-Gly-Gly-$N(CH_3)$	9.01 ± 0.05	-12.2	-6.6 ± 0.3	-19 ± 1

[a]HO-Pic- 3-Hydroxypicolinic acid-, [b]Sal- Salicylic acid residue

The differences of the log K^{app} values between the antibiotics and the model compounds reflect the influence of the peptide lactone ring and its side chains. The reaction enthalpies are in the range of -4 to -6 Kcal per mole and the formation entropies are in the range of - 20 to -23 cal per mole and K, which is typical for phenolic OH groups (9). This agreement, as well as the spectral characteristics suggest that in the neutral form of the hydroxypicolinic acid residue the proton is preferentially bound to the phenolic oxygen.

CONCLUSIONS

The automated experimental set-up allows a titration to be performed in a fast and precise manner. The small amount of substance needed for one titration, being sometimes only a few nano moles, is one of the main advantages of this method, especially since for most studies with biological material only small amounts are available. The requirement, however, that the compounds have suitable spectral properties, is limiting for the application of these method. The characteristics of the spectral changes, on the other hand, provide additional information, which may be of great help for the molecular interpretation of the reaction system under investigation.

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