Complex Formation between the Uncoupler Carbonyl Cyanide p- Trifluoromethoxyphenylhydrazone (FCCP) and Valinomycin in the Presence of Potassium*

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THOMAS A. O'BRIEN, DAVID NIEVA-GOMEZ,§ AND ROBERT B. Gennies

From the Department of Chemistry, University of Illinois, Urbana, Illinois 61801

SUMMARY

Spectroscopic evidence is presented which indicates that the uncoupler carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) and the peptide antibiotic valinomycin form a complex in the presence of potassium. Complex formation has been observed both in aqueous and nonaqueous media. Several techniques have been used to indicate the existence of a complex in aqueous solution. In the presence of valinomycin and K⁺, the absorption spectrum of FCCP is significantly perturbed, and there is also a large induced circular dichroism signal. In addition, the previously characterized complex which forms between valinomycin and K⁺, and the fluorescent probe 8-anilino-1-naphthalenesulfonate (ANS) in aqueous solution is apparently disrupted by the addition of FCCP. The result is an effective quenching of the fluorescence due to the bound probe as it is displaced from the valinomycin·K⁺ by the uncoupler. In a nonpolar solvent, the absorption spectrum of FCCP is also perturbed by valinomycin in the presence of K⁺, again indicating the formation of a complex.

These data point to the importance of considering the role of a valinomycin·K⁺·uncoupler complex in interpreting physiological or ion transport data in which these substances have been used together.

The use of protonophoretic and ionophoretic substances has become widespread in recent years for the purpose of manipulating or measuring electrochemical potential gradients across biomembranes or model membranes (1). It is common for several of these substances to be used in combination to achieve a specific purpose. Recent work by Green and co-workers (2, 3) has pointed out the synergistic effect of uncouplers and ionophores in mediating cation transport through an organic phase. It was inferred that a 1:1:1 complex of uncoupler, monovalent cation, and ionophore was responsible for the observations. In this work, spectroscopic perturbations are used to directly demonstrate an interaction between an uncoupler in the carbonyl cyanide phenylhydrazone class (FCCP) and the ionophore valinomycin in the presence of K⁺. Complex formation between these substances occurs in aqueous solution even at the low concentrations at which they are commonly utilized. In organic media, a K⁺-dependent complex between valinomycin and FCCP is also evident, although it is different from the complex which forms in water.

EXPERIMENTAL PROCEDURES

Materials—The carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), which was used in these studies, was supplied through the generosity of Dr. P. G. Heytler (DuPont). Other reagents were obtained from the indicated sources and used without further purification: valinomycin (Sigma); 8-anilino-1-naphthalenesulfonate (ANS) and N-phenyl-1-naphthylamine (NPN) (Eastman). The purity of the dyes was confirmed by thin layer chromatography and comparison between observed and reported fluorescence spectra. All other reagents were of the highest purity commercially available.

Absorbance Measurements—Absorbance spectra were obtained with a Cary Varian model 635 and a Beckman Acta III spectrophotometer. All spectra were recorded at room temperature. Spectra of the samples containing valinomycin were obtained with the ionophore present in both the reference and sample cuvettes.

Circular Dichroism Measurements—Circular dichroism spectra were obtained with a Jasco J-40A spectropolarimeter. All samples were scanned at room temperature. FCCP alone in aqueous solution exhibits no circular dichroism, and the presence of either 2 M NaCl or 2 M KCl does not result in any significant changes in the circular dichroism.

Fluorescence Measurements—ANS fluorescence emission spectra were obtained with a Perkin-Elmer MFP-44A fluorometer at room temperature. The excitation wavelength for all samples was 340 nm. An excitation band-pass of 4 nm and an emission band-pass of 2 nm were used in conjunction with a 350 nm cut-off filter. With these settings, the Raman scattering intensity was negligible. Also, the large quenching of ANS fluorescence intensity observed upon the addition of FCCP was not due to an inner filter effect. At the concentrations of FCCP used (4 μM), there was less than 5% inner filter quenching due to the FCCP absorbance.

RESULTS

The uncoupler FCCP in aqueous solution has an absorption maximum at 380 nm. Fig. 1 demonstrates that perturbations of the FCCP spectrum in this region can be used to detect complex formation with valinomycin. In the absence of salt, the addition of valinomycin (4 μM) has no effect on the FCCP (4 μM) absorption spectrum. There is only a slight effect if the experiment is performed in the presence of 2 M NaCl. However, a large decrease in intensity and a nm red shift is noted when valinomycin is added in the presence of 2 M KCl. This is consistent with the much higher association constant for valinomycin with K⁺ compared with Na⁺. About half of the observed decrease in intensity is apparent immediately after adding the valinomycin to the cuvette. There is a further slow decrease in the absorbance to a constant level which usually takes about 30 min to complete. This may represent aggregation or disaggregation rearrangements of the valinomycin·K⁺·FCCP complex.

1 The abbreviations used are: FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; ANS, 8-anilino-1-naphthalenesulfonate; NPN, N-phenyl-1-naphthylamine.

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Valinomycin-K⁺·FCCP Complex

The circular dichroism spectra of FCCP under various conditions are shown in Fig. 2. The uncoupler has no CD spectrum when present alone in aqueous solution. The addition of valinomycin in the absence of salt or in the presence of 2 M NaCl has little effect on the FCCP CD spectrum. However, in the presence of valinomycin plus 2 M KCl, a large induced CD signal is observed. A positive band centered around 416 nm increases in intensity with time over a period of about 30 min to a constant level. This time dependence is similar to that observed for the changes in the absorption spectrum.

The organic anion ANS combines with the valinomycin-K⁺ cation to form a fluorescent complex which has been previously characterized (4). As a further indication of complex formation, the effect of FCCP on the fluorescence of the valinomycin-K⁺·ANS complex was examined. It is likely that the anionic form of FCCP forms a similar complex with valinomycin-K⁺. As seen in Fig. 3, the addition of FCCP to an aqueous solution containing the complex with ANS results in the quenching of the fluorescence. The simplest explanation is that the FCCP is displacing the ANS from the complex with valinomycin-K⁺, and the fluorescence decrease results from the fact that the quantum yield of free ANS is considerably smaller than that for bound ANS. The effect of FCCP on the ANS fluorescence does not show the same time dependence of the FCCP spectral perturbation experiments shown in Figs. 1 and 2.
the nature of the complex is undoubtedly complicated, since valinomycin in aqueous solution probably exists as polydisperse aggregates. The dissociation constant of valinomycin for K+ has been estimated to be 0.43 mM (4) in water and high concentrations of this cation are necessary for any spectroscopic evidence of an interaction between valinomycin and FCCP. Sodium, which binds much more weakly to the ionophore (7) has virtually no influence on the valinomycin-FCCP interaction. The slow time dependence of the spectroscopic changes induced in FCCP by valinomycin-K+ attests to the complexity of the solution properties of these species in water. For this reason, no attempt was made to determine the stoichiometry or the binding constants for this complex. Previous work has indicated that when ANS interacts with valinomycin-K+, it is likely that a number of ANS molecules bind to an aggregate of valinomycin (4). The binding of FCCP to valinomycin-K+ is probably similar. It is also likely that the form which is binding is the dissociated anionic form of the uncoupler, although this has not been proven. It has been previously demonstrated that an organic anion such as laurate will enhance the partitioning of the metal-valinomycin complex from water into an organic solvent (8). In any event, it is clear that in the presence of K+, valinomycin and FCCP will perturb each other’s solution behavior, and that in experiments involving the simultaneous presence of both, this must be taken into account.

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