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The Complex Formation of Biuret with Alkali and Alkaline Earth Metal Ions. NMR and Solubility Studies

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The formation of complexes between biuret and the ions of Li, Na, K, Rb and Be, Mg, Ca, Sr and Ba in DMSO solutions has been studied by means of ^1H NMR spectroscopy. Equilibrium constants have been evaluated from the concentration dependence of NMR shifts. The results of these investigations have been supplemented by studies on changes of salt solubilities in DMSO upon addition of biuret. The complex formation tendency is compared with stabilization energies for the 1:1 and 1:2 complexes obtained from HF–SCF–MO calculations.

Introduction

The tendency of complex formation between the biuret molecule and transition metals has been observed long ago and many of these complexes have been studied extensively [1–9]. The formation of main group metal complexes, however, was not reported until recently, when a solid complex of strontium perchlorate with biuret was reported [10]. In a previous paper we have reported some predictions for the complex formation of biuret with alkali and alkaline earth metal ions, as derived from quantum chemical calculations within the LCGO–MO–SCF framework [11]. The interest for this peculiar ions arises from the possibility of a relatively easy combined theoretical and experimental treatment and from their importance in some bioinorganic systems for which biuret can be regarded – within certain limitations – as a model compound, namely for large molecules containing one or more peptide or amido groups as coordinative center.

Further, the binding between biuret and these ions is not too strong and enables therefore studies on preferential complex formation within a series of metals of similar electronic stucture under well accessible experimental conditions and the comparison of these data with the bond theoretical predictions of the quantum chemical calculations on 1:1 and 1:2 complexes.

Two experimental methods were used for the investigations presented in this paper: NMR spectroscopy for the determination of equilibrium constants, and solubility measurements. The latter should give some more information about the ion specifity of the ligand and thus serve as a control for the overall complex formation constants derived from the NMR measurements.

Experimental

^1H NMR Investigations

For these and all further investigations DMSO was chosen as solvent, as it does not interact very strongly with biuret, represents a good solvent for both salts and ligand, and guarantees a rather slow proton exchange rate leading thereby to NMR spectra with fairly sharp peaks for both the NH- and the NH_2-signals of biuret. Reagent grade biuret and salts (MERCK, FLUKA, ALFA-VENTRON alkali and alkaline earth chlorides) were used, all experiments being carried out in DMSO under nitrogen atmosphere.

Free and metal bonded biuret molecules seem to exchange rapidly and do not display, therefore, distinct NMR signals. The influence of complex formation can be observed, however, in shifts of both the amido and imido group signal, the latter one being the more strongly affected one. We therefore decided to take the imido proton signal in reference to the internal standard TMS for the determination of the equilbrium constants. The evaluation of these constants from the NMR data will be discussed later.

All NMR measurements were performed using a C-60-HL JEOL spectrometer with temperature control at 25 °C.

Solubility Studies

Saturated solutions of the chlorides were prepared at 25 °C by stirring several days with dry DMSO. The content of salt was determined by argentometric titration and gravimetric analysis, respectively, of the Cl^- ion. The same procedure was performed with DMSO containing 0.2524 mol biuret in order to study the improvement of the salt solubility due to complex formation. All experiments were carried out under nitrogen atmosphere.

Results and Discussion

NMR Studies

Evaluation of equilibrium constants

NMR shifts have been used already in other systems for the determination of equilibrium constants [12, 13]. For the system being investigated here we had to take into account the fact that more types of complexes may be formed, contributing in a different way to the shift of the resulting signal. It could be expected, further, that within the concentration range of 0–2 *M* the shift of the resulting signal is related linearly to the concentration of the complex species being formed [14]. Thus we had to choose a system of equations describing the equilibria in the solution and to relate the concentration of the complexes – having specific chemical shifts – to the total shift of the resulting signal being observed.

Generally speaking, we have to consider the n equations resulting from the equilibria $MB_{n-1} + B = MB_n$; in practice, however, the probability of formation of MB_n complexes with n being larger than 2 is rather small. This follows from statistical reasons as well as from the rather low binding energies for the ions to biuret and sterical factors. Even the existence of a solid strontium complex with four biuret ligands [10] does not give much reason for the assumption that such complexes might be realized also in dilute solutions. Finally, the statistical treatment discussed below did also not give any evidence for a significant contribution of complexes with more than two ligands.

We therefore take into account the equations

$$M + B = MB \qquad K_1 = MB/M{*}B \tag{1}$$

$$MB + B = MB_2 \qquad K_2 = MB_2/MB{*}B \tag{2}$$

$$M + 2B = MB_2 \qquad K_{total} = MB_2/M{*}B^2 \tag{3}$$

M = equilibrium concentration of the metal
B = equilibrium concentration of biuret
MB_n = equilibrium concentration of the metal complex with n ligands

and connect them with the chemical shift, related to the uninfluenced biuret signal as reference value zero. Besides the linear concentration of the shifts, we have to make one more assumption, concerning the ion influence on either the 1:1 or 1:2 complex. Since the shift of the imido proton signal due to ion influence is mainly due to electrostatic and polarization effects, we assume this influence to have the same effect on each ligand in the complex, irrespective of the coordination number. Thus, the contribution of a 1:2 complex to the resulting shift should be just twice as large as that of the 1:1 complex. This assumption seems to be confirmed further by quantum chemical calculations on the ion influence in both types of complexes [11].

With these assumptions, the shift of the signal can be expressed as

$$dv = (MB + 2MB_2)/k'$$

k′ being a proportionality constant. If we introduce still the total concentrations of metal and biuret (M′ and B′) by

$$M' = M + MB + MB_2 \tag{4}$$

$$B' = B + MB + 2MB_2 \tag{5}$$

we can derive from (1)–(5) an equation which allows an iterative optimization of the unknown constants K_1, K_2 and k′ by means of the Newton method, using the measured chemical shifts at various concentrations.

The procedure was programmed in BASIC for a Hewlett Packard 9830 A computer.

Results

The shift of the imido proton signal under the influence of the metal chlorides with increasing concentration is illustrated in Figure 1. Difficulties were encountered only in the case of beryllium, for which the signals are split. For this reason Be was not included in the further investigations. The peculiar behaviour of this ion could have several reasons, the most important ones being the very strong binding to the ligand and the preference of a non-chelate structure (*trans*-complex) as indicated by quantum chemical calculations [11].

For some of the ions the solubility limited the concentration range of our investigations, as indicated in Figure 1, but sufficient data for the evaluation of equilibrium constants could be collected. In Table I the equilibrium constants K_1, K_2 and K_{total} (according to the equations of the previous section) are given, together with the proportionality constant k′.

TABLE I. Formation Constants for Metal–Biuret Complexes (see eq. 1–3).

Cation	K_1	K_2	K_{total}	k′
Li	4.24	0.72	3.07	0.26
Na	1.80	4.50	8.10	0.33
K	0.55	7.00	3.90	0.40
Rb	0.50	7.80	3.90	0.35
Mg	354.80	0.81	287.40	0.23
Ca	3.00	2.61	7.83	0.25
Sr	4.06	3.05	12.40	0.22
Ba	8.70	1.34	11.70	0.26

Apparently the stability of the 1:1 complex decreases with increasing atomic number of the alkali ions, remaining rather constant after potassium. For the alkaline earth metals we observe a slight increase

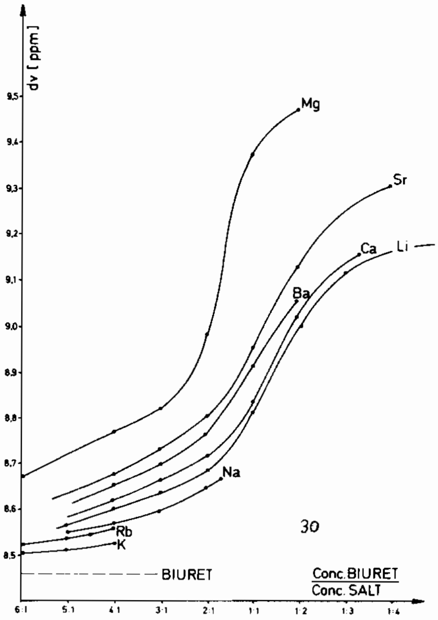

Figure 1. Shift of the imido proton signal *vs.* concentration ratio biuret: salt, related to internal standard TMS.

of the stabilization again after passing through a minimum at calcium. It can be concluded qualitatively from the Be spectra that the corresponding value should be even higher than that for Mg. A numerical evaluation was not possible, however, for reasons mentioned above.

For the stabilization of a second biuret ligand being added to the 1:1 complex, a quite opposite tendency is observed. The stability constants become larger with increasing atomic numbers. Only for Ba we find a slight decrease again.

For the overall reaction we obtain therefore maxima for the complex formation tendency for Na and Mg, respectively. In addition, the minimum for Ca within the alkaline earth metal series seems to deserve some interest.

Although a direct comparison of stability constant of the complexes in solution with stabilization energies from quantum chemical calculations of isolated species is somewhat problematic, it seems to be quite instructive in the case of the biuret complexes. Within the series of ions, solvent influence and entropy factors can be assumed to be quite constant, making the stabilization energy therefore the main factor determining the stability constant. In Table II, the calculated stabilization energies for the formation of the 1:1 and 1:2 complexes are listed, as far as they are available from *ab initio* Hartree–Fock LCAO MO SCF calculations with minimal basis set [11].

Within the framework of the SCF procedure, due to the size of the systems (which are "large" in the sense of a quantum chemist) a minimal basis set had been used for the MO calculations. This basis set requires some corrections for an artificial transfer of electron density from the ligand to the ion in the case of the higher atomic numbers (K and Ca). The estimation of this basis set effect was performed by the usual procedure, calculating the influence of the ions basis set on the ligand in the absence of the ion nucleus. In Table II both corrected and non corrected values are given, therefore, for both K and Ca complexes. The computational details, program, geometries and basis set exponents are given in reference [11].

TABLE II. Stabilization Energies for 1:1 and 1:2 Metal–Biuret Complexes from HF–MO–SCF Calculations (in kcal/mol).

Cation	1:1 Complex	1:2 Complex
Li	64.3	72.2
Na	59.0	99.7
K	59.4	–
	52.4[a]	–
Be	190.0	256.2
Mg	159.0	248.0
Ca	133.4	–
	129.4[a]	–

[a] Including the basis set correction for artificial basis improvement of the ligand as a function of large cations.

The trends observed in the stability constants are reflected quite well in the stabilization energies, showing a decrease from Li to K and Be to Ca in the case of the 1:1 complexes and an increase from Li to Na for the 1:2 complexes. The values for Be agree with the expected highest stability constant assumed from the NMR spectra.

The experimentally determined stability constants seem to be, therefore, also a good confirmation of the theoretical predictions about the binding and the role of the chelate effect in the biuret complexes [11].

Solubility Studies

Some solubilities of salts in DMSO have been determined already earlier [15, 16], but the values in the literature disagree remarkably. This may be due mainly to the strong hygroscopic properties of the pure solvent making an inert gas atmosphere inevitable for all experiments and to the strong tendency of DMSO to form oversaturated solutions. We have carried out, therefore, all operations under nitrogen atmosphere and prepared the solutions by stirring cold DMSO with the salts for several days.

In Table III the measured solubilities of the alkali and alkaline earth chlorides are compared with the solubilities obtained after addition of 0.2524 mol biuret. Figure 2 illustrates the increase of solubility

TABLE III. Solubilities of Metal Chlorides in DMSO and Biuret Containing DMSO (DMSO–bi) in Mol per Liter.

Cation	L_{DMSO}	$L_{DMSO-bi}$
Li	2.672	2.868
Na	0.094	0.141
K	0.045	0.062
Rb	0.042	0.061
Cs	0.052	0.076
Be	0.191	0.550
Mg	0.146	0.277
Ca	0.877	0.941
Sr	0.960	1.023
Ba	0.223	0.290

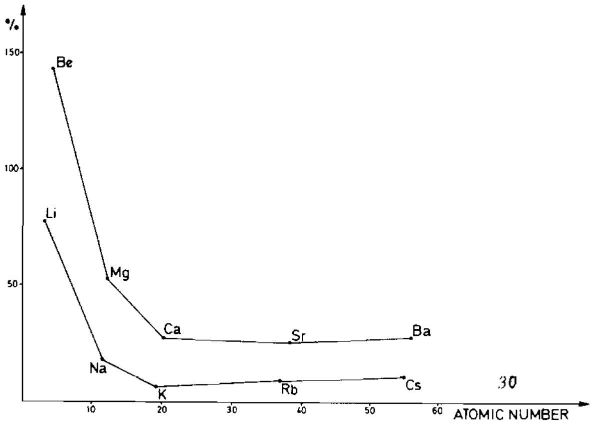

Figure 2. Percent increase of metal chloride solubility in DMSO upon addition of biuret (0.2524 mol/liter).

due to complex formation with biuret in percent related to the molarity of the ligand. These curves are to be compared with the complex concentrations calculated from the equilibrium constants (*cf.* Table IV). The comparison shows a very good agreement of the values, confirming thus the reliability of the equilibrium constants obtained by the NMR measurements.

TABLE IV. Complex Concentrations in Saturated Metal Chloride Solutions in DMSO Containing 0.2524 Mol Biuret.

Cation	MB	MB_2	$MB + MB_2$
Li	0.226	0.003	0.229
Na	0.028	0.023	0.051
K	0.006	0.009	0.015
Rb	0.005	0.010	0.015
Mg	0.230	0.003	0.233
Ca	0.140	0.020	0.160
Sr	0.170	0.018	0.188
Ba	0.130	0.016	0.146

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