Monatshefte für Chemie 109, 871—881 (1978)

Monatshefte für Chemie

The Effect of N-Substitution on the Metal Binding Properties of Peptide Binding Sites

Bernd M. Rode* and **Thomas Pontani**

Institut für Anorganische und Analytische Chemie, Universität Innsbruck, A-6020 Innsbruck, Austria

(Eingegangen 9. September 1977. Angenommen 20. September 1977)

The influence of methyl- and ethylsubstitution at the nitrogen atom of peptide groups on their interaction with alkali and alkaline earth metal ions has been studied by means of quantum chemical calculations on the complexes of lithium, sodium and beryllium with the different substituted amides, and by means of difference energy surfaces obtained from *ab initio* calculations employing minimal *Gauss*ian basis sets. Characteristic ion specific differences are found to occur in the interaction according to the respective substitutions, which will influence the potential for the metal ion in the field of the peptide groups quite strongly. Binding energies and electron density distribution in the complexes are discussed with respect to recent experimental data obtained by metal nmr spectroscopy. The results of the calculations give some indications to possible ways of influencing the ion specifity and reactivity of peptide and protein metal binding sites in biological systems.

Der Effekt der N-Substitution auf die metallbindenden Eigenschaften von Peptiden

Der Einfluß von Methyl- und Ethyl-Substitution am Stickstoff der Peptidbindung auf die Wechselwirkung mit Alkali- und Erdalkali-Metallionen wurde mittels quantenchemischer Berechnungen an Komplexen mit Lithium, Natrium und Beryllium mit verschieden substituierten Amiden, und mittels der Differenz-Energie-Flächen von *ab initio* Berechnungen mit minimalem *Gauß*-Basis-Set, studiert. Es wurden charakteristische ionenspezifische Differenzen in den Wechselwirkungen — entsprechend den verschiedenen Substituenten — gefunden, die das Potential der Metallionen im Feld der Peptidbindungsgruppe sehr stark beeinflussen. Es werden Bindungsenergien und Elektronendichten in den Komplexen, bezogen auf neuere experimentelle Daten der Metall-NMR-Spektroskopie, diskutiert. Die Ergebnisse der Berechnungen zeigen mögliche Wege auf, die Ionenspezifität und die Reaktivität von Peptid- und Protein—Metall-Bindungszentren in biologischen Systemen zu beeinflussen.

0026-9247/78/0109/0871/$ 02.20

Introduction

The interaction of the peptide group deserves the interest of the chemist and the biologist for several reasons, among which we want to mention the solvating properties of amides as widely used nonaqueous solvents[1], the formation of ion specific metal complexes by oligopeptides, which can mimick quite accurately some important biosystems[2–4], and the bonding of metal ions to carrier proteins and protein membrane layers playing an essential part in numerous biological processes.

The metal binding properties of the peptide group can be influenced by several factors, as for example the substitution at the nitrogen and carbon atom, at neighbouring coordination centres or by the steric properties of the ligand carrying the peptide group as the functional group for complex formation.

Several theoretical studies on the interaction of metal ions with the peptide group have been carried out already[5–11], leading to the result, that quantum chemical methods are able to supply basic information about this subject. Calculations on the relative affinity of ether, ester and peptide groups[12] have shown the latter to have a very high affinity to alkali and alkaline earth metal ions.

In our work we want to extend the quantum theoretical studies to a systematic investigation of the above mentioned influence of substitution on the metal binding properties of the peptide group. In the first part of this work, presented in this paper, we have studied the influence of substitution at the nitrogen atom, namely by methyl and ethyl groups, being the most frequently occuring substituents. Experimental data about the influence of these groups allowing a comparison with the results of the theoretical work have been obtained recently[13] and will be discussed briefly in relation to the predictions of quantum chemistry.

The amides being investigated in this work can be regarded as model compounds for the following types of peptide bonds:

a) monosubstituted amides:

R—CO—NH—	corresponding to natural occuring peptides and
CH_2—X	proteins,

b) disubstituted amides:

R—CO—N—CH_2—X	corresponding to peptide groups, which are
|	mainly found in natural or synthetic pharma-
CH_2—Y	cologically active compounds as penicillines and other antibiotics, barbiturates, cholinergics as oxotremorine, and also in analgetics as indomelacine, anaesthetics as epontol^R or antipyretics.

Information, whether a change of the X and Y rest would still influence the metal binding activity of the carbonyl group (this is over a range of five atoms) seemed to be of some interest, since it would indicate, if and how far a side chain influence has to be considered in the metal binding activity of a peptide group. Such basic information could be expected from the results of the calculations on either methyl—or ethyl—substituted amides, where $X = H$ or CH_3, respectively, since this is the smallest possible change, and all other substitutions of X and Y should give still more pronounced effects.

Method

As one of the most reliable descriptions of the interaction of two closed shell systems, as a neutral molecule and an alkali or alkaline earth metal ion, we chose the energy surface for the ion in the field of the substituted peptide group, represented in our examples by the N-substituted formamide molecules. So we carried out calculations on the energy surfaces of the systems Li^+/formamide (*FA*), Li^+/*N*-methylformamide (*NMF*), Li^+/*N*,*N*-dimethylformamide (*DMF*), Li^+/*N*-ethylformamide (*NEF*), Na^+/*FA*, Na^+/*NMF*, Be^{2+}/*NMF* and Be^{2+}/*NEF*. For each of these systems, we calculated fifty points of the energy surface in the molecular plane.

For a direct presentation of the substituent's influence, "difference energy surfaces" were constructed, subtracting the energy difference between a surface point and the surface minimum for the ion/*FA*-system from the corresponding values obtained for the surfaces of this ion in the field of the substituted amides. The surface minima were found to be located at the same point for all systems under investigation.

The large number of necessary calculations and the relatively huge molecules involved in these calculations bring about some inevitable restrictions in the accuracy of the quantum chemical procedure, in order to maintain a reasonable extent of computing time. Semiempirical procedures were, for methodical reasons[14], not suitable for our purposes, since they predict completely wrong energy surfaces. Thus we had to choose an *ab initio* procedure and to restrict ourselves to a minimal basis set. For the systems under investigation, a 2/1 GLO basis set had already proved to give quite satisfactory results[11, 14]. Thus we used this well tested basis set in all surface calculations reported here. It can be expected, that the obtained results are reliable at a semiquantitative level, which would be sufficient for the purpose of our work.

All calculations have been performed at the CDC 3300 computer of the University of Innsbruck.

Results and Discussion

1. Energy Surfaces

The calculated difference energy surfaces are presented in Figs. 1–6. Positive numbers indicate an increase in energy compared to the unsubstituted amide, i.e. a destabilizing effect, negative numbers a stabilization arising from the substituent.

As a common feature, an increased probability for the ions to be located nearer the binding center, is found for all substituents being considered, whereas a destabilization takes place within the reaction coordinate beyond the minimum position. This effect increases with the introduction of a second methyl group or upon exchange of the methyl substituent by an ethyl group.

Regarding the NCO region of the peptide group, one finds a rather limited, short range stabilization in the area closed to the ligand molecule for both methyl and ethyl substitution, concerning the alkali ions. Dimethylation has a destabilizing effect, due to the repulsion by the second substituent. In the case of the beryllium ion, however, we find a much pronounced stabilization for the ion in this area, which is extended over a considerably larger space. This specific difference between the monovalent and divalent ions results probably from the better chelate complex forming properties[15] of the alkaline earth metal ion. Generally, the ethyl group causes quantitatively larger effects than the methyl group, but the nature of the effects remains the same.

In the HCO region, which, of course, could be influenced more strongly by carbon substitution, the substituent induced changes in the interaction of Li^+ and Na^+ with the monosubstituted ligands are very similar, independantly, whether the substituent is CH_3 or C_2H_5. The positions near the reaction coordinate are favoured, and a displacement of the ion to the outer HCO region is more difficult than for unsubstituted formamide.

In the case of $Be^{2+}$the situation is quite different. The ion can move far from the ligand molecule, at a comparable loss of stabilization energy, after a methyl or ethyl substituent has been introduced at nitrogen. Dimethylation leads to the latter effect also for the monovalent Li^+ ion.

As a general result one can conclude, than N-substitution of the peptide group influences remarkably the energy surface for metal ions situated in the neighbourhood of the binding site. These differences will influence the exchange processes (i.e. binding and dislocation of the metal ions to and from the peptide group). The results of our calculations indicate, that the type of substitution will determine this influence in a qualitative and/or quantitative way, and that this

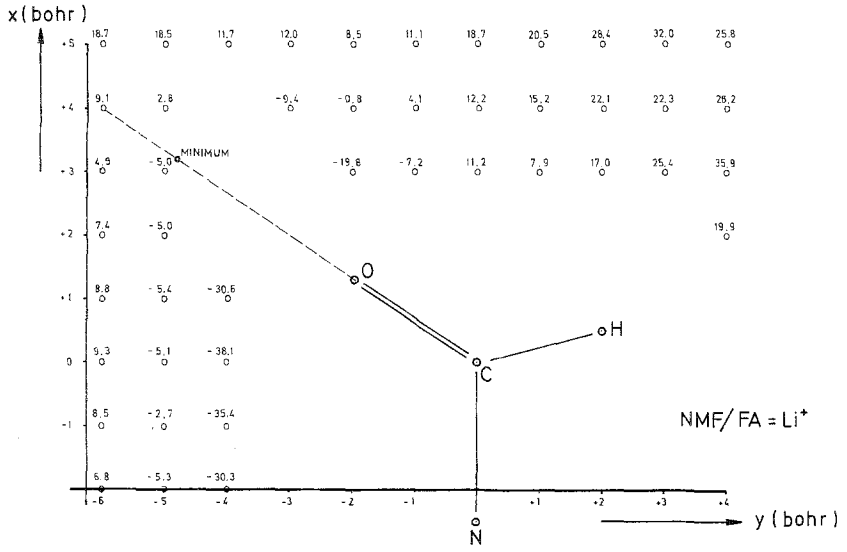

Fig. 1. Difference energy surface for monomethylsubstitution, for lithium; the numbers in the diagrams (energy differences) are given in 10^{-4} atomic energy units

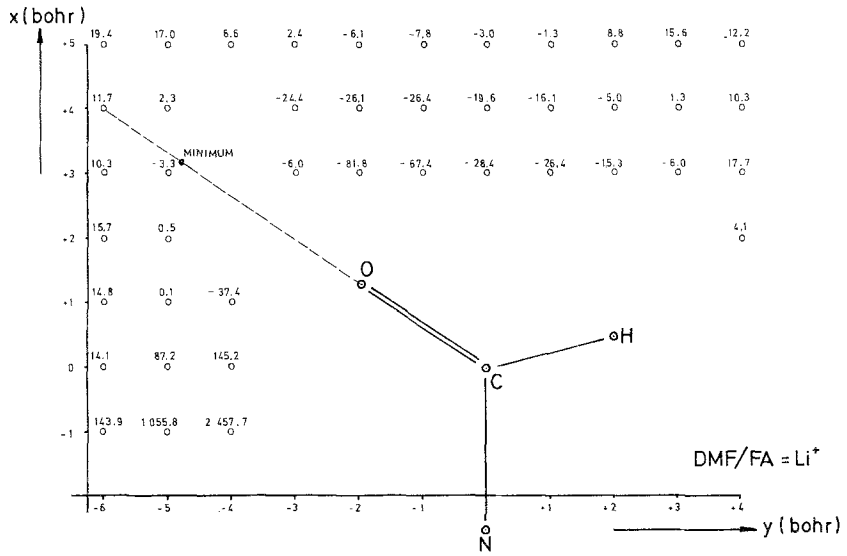

Fig. 2. Difference energy surface for dimethylsubstitution, for lithium; ΔE in 10^{-4} a.e.u.

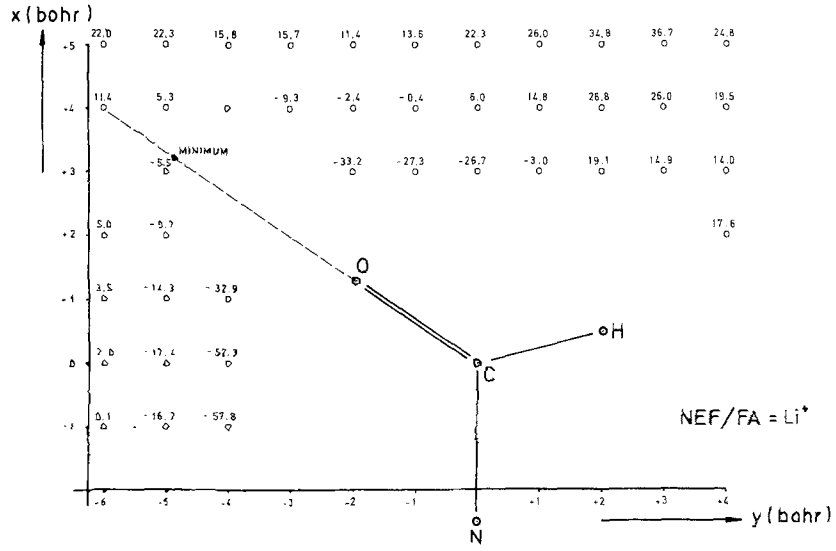

Fig. 3. Difference energy surface for monoethylsubstitution, for lithium; ΔE in 10^{-4} a.e.u.

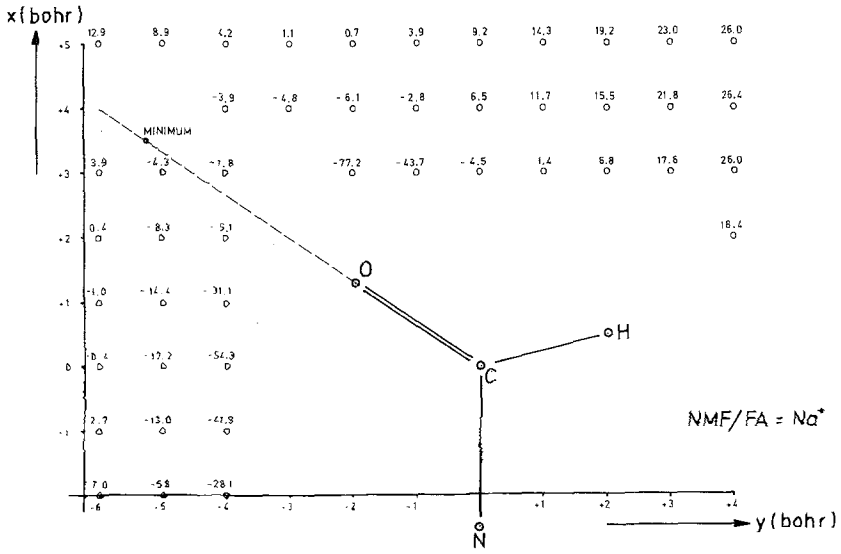

Fig. 4. Difference energy surface for monomethylsubstitution, for sodium; ΔE in 10^{-4} a.e.u.

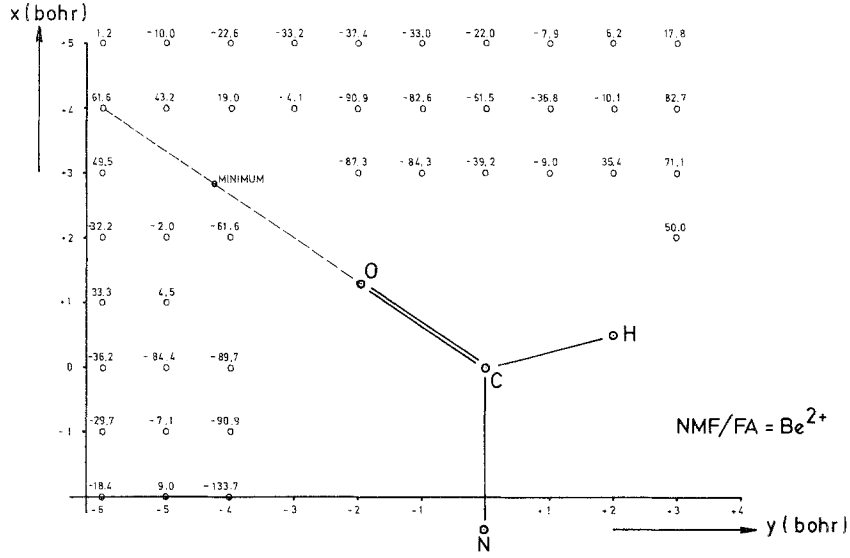

Fig. 5. Difference energy surface for monomethylsubstitution, for beryllium; ΔE in 10^{-4} a.e.u.

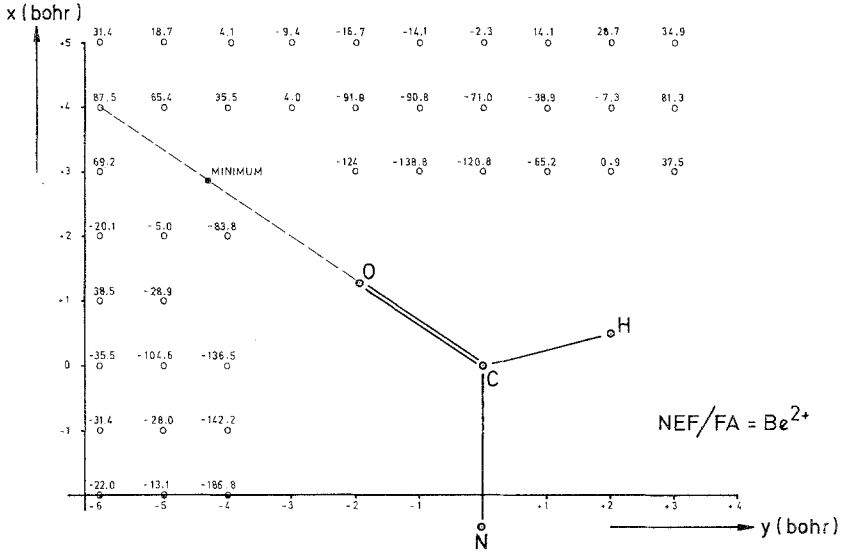

Fig. 6. Difference energy surface for monomethylsubstitution, for beryllium; ΔE in 10^{-4} a.e.u.

influence shows also quite a high specifity according to the nature of the cation, i.e. whether this ion belongs to the alkali or alkaline earth metal ions.

We further find, that the metal binding activity of any peptide binding site will be influenced remarkably even by changes in parts of the molecule, which are considerably "far" from the oxygen of the carbonyl group. This means, that a side chain influence has to be considered, whenever one intends to discuss metal/peptide or metal/protein interactions.

Finally, the calculations indicate, that it is possible, even without constructing sterically specific binding sites, to build up quite ion specific potential surfaces and potential walls by means of differently substituted sequences of peptide groups, e.g. in a polypeptide chain. The substituents can facilitate the "pass-by" of some of the ions or retain some of them preferentially. This effect should be still more pronounced, if such a sequence of peptide groups is sterically fixed in a way that the ions are forced to pass within a limited space, where the energetic substituent influence cannot be compensated by a change in the ion's movement along this sequence.

2. *Substituent Specific Changes of the Complexes in Their Stablest Configuration*

We will deal now with some characteristic properties of the ligand molecule with respect to its metal binding ability, and the influence of the substituent at nitrogen on these properties.

First, we have studied the differences in the ion binding energies caused by the substituents. In Table 1 the differences, related to unsubstituted formamide, are collected.

Table 1. *Binding energy differences by N-substitution (related to formamide), in kcal/mol*

Ion	*NMF*	*DMF*	*NEF*
Li^+	+ 1.4	+ 1.2	+ 1.4
Na^+	+ 1.6	+ 3.0	+ 4.0
Be^{2+}	+ 11.6	+ 14.5	+ 13.6

For all ions, substitution leads to a gain in binding energy. The ethyl group has, with exception the Li^+ ion, a slightly stronger effect than the methyl group. Disubstitution also seems to strengthen the binding, again with exception the lithium ion. As far as this can be predicted

from the few ions considered, this stabilizing tendency seems to become the more pronounced, the larger the ion is (at constant charge), and the higher its charge is. This assumption agrees with general considerations concerning the stabilization of ion/molecule complexes by electrostatic forces, mutual polarization and charge transfer, which also depend on size and charge of the ions. The calculated data also agree with experimental results concerning solvation energies, as far as they are available[16–19].

Table 2. *Substituent induced changes in charge distribution of the peptide group (related to formamide), and chemical shifts of the metal nuclei*[13] *(related to their signals in formamide),* $S^{Me}m$ *(in Hz)*

Ion	Ligand	H(CH)	N	C	O	S^{Me}
Li^+	*NMF*	−0.010	−0.013	0.041	−0.007	−8.1
	DMF	−0.001	−0.028	0.065	−0.013	−10.4
	NEF	0.003	0.022	0.026	+0.002	+0.0
	DEF	0.033	0.039	0.044	0.011	+3.9
Na^+	*NMF*	−0.010	−0.012	0.042	−0.006	+5
	DMF	0.000	−0.015	0.062	−0.008	+13
	NEF	0.002	0.031	0.022	0.002	−2
	DEF	—	—	—		−19
Be^{2+}	*NMF*	−0.021	−0.018	0.021	0.034	+4
	DMF	−0.015	−0.038	0.042	0.048	+23
	NEF	0.011	0.016	0.002	0.049	+5
	DEF	0.012	0.026	0.017	0.063	+20

Second, we have performed a *Mulliken* population analysis of the ion/ligand complexes and the substituent induced changes, respectively. The values, related again to unsubstituted formamide, are listed in Table 2. The population of the metal ion itself has not been listed, since it is not affected by the change of the N-substituents.

As a result of this population analysis, one finds characteristic differences in the net charges of the atoms of the peptide group. It is clearly to be seen, that the substituent has to compensate for some of the charge transfer occuring at the peptide group. Regarding first the N atom we find its negative net charge to be increased by methyl groups, whereas ethyl groups show an opposite effect. This trend is also observed for the H atom. The C atom of the carbonyl group becomes more positive in all kinds of substituted complexes. The O atom, which is nearest to the metal ions and the actual binding site, deserves special

interest. In the case of Be^{2+}, it is more positive in all substituted complexes, than in the complex with formamide. In the case of the alkali ions, it gains electron density upon methyl substitution at N and looses upon ethylation.

^{7}Li, ^{9}Be and ^{23}Na nmr measurements have shown a similar behaviour of the metal chemical shifts[13]. It was found, that methylation induces a ^{7}Li resonance shift to lower field, ethyl substitution a shift to higher field strengths. For the ^{23}Na resonance lines, the opposite behaviour was observed. The inverse effect for either Li or Na shifts can be explained by the different mechanism responsible for the shift of these metal nuclei, especially by the dominance of the paramagnetic term in the contribution to ^{23}Na shifts[20]. So one can conclude, that the reason for the downfield shifts in the case of Li^{+}/NMF and DMF-complexes and the Na^{+}/NEF and DEF-complexes, respectively, is a common physical effect in their interaction with the ligand[13].

In the case of the strong interaction of the amides with Be^{2+}, a different influence of either methyl or ethyl groups was not observed any longer, but all kinds of substituents led to a shift to higher field.

These results are of peculiar interest, since both the calculations on the 1:1 complexes and the experiment indicate one more difference in the substituent influence on the peptide/metal interaction concerning alkali and alkaline earth metal ions. Further, they predict a significantly different effect of methyl and ethyl N-substitution on the binding of the alkali metal ion to the coordination site of the peptide group. These differences can be of some importance for the discussion of the reactivity of the metal ion bonded to the peptide group.

In conclusion we can state, that binding energies and the electron density distribution in the ligand are quite sensitive to N-substitution. Chemically "similar" substituents, as are methyl and ethyl groups, show characteristic differences, and also the number of substituents at nitrogen can influence remarkably the metal binding properties and electronic structure of the peptide group. These results could give also some indications to possible reasons of the well known phenomenon, that the replacement of hydrogen, bonded to peptide or amino groups by alkyl groups, can lead to drastic changes in the biological or pharmaceutical activities of molecules.

Acknowledgement

Finanical support of this work by the "Fonds zur Förderung der wissenschaftlichen Forschung", Vienna, Austria (Proj. Nr. 2432/3061), is gratefully acknowledged.

B.M.R. is very indebted to Prof. B. Sarkar, Univ. of Toronto, Canada, for several stimulating discussions on the subject of this work.

References

[1] *J. W. Vaughn*, in: The Chemistry of Nonaqueous Solvents. London-New York: Academic Press. 1967.
[2] *S. J. Lau, T. P. A. Kruck*, and *B. Sarkar*, J. Biol. Chem. **249**, 5878 (1974).
[3] *K. S. N. Iyer* and *B. Sarkar*, Proc. 4th Amer. Peptide Symp., 215—218, Ann Arbor Science Publ. Inc., Ann Arbor, Michigan, 1975.
[4] *B. Sarkar, V. Renugopalakrishnan, T. P. A. Kruck*, and *S.-J. Lau*, in: The Jerusalem Symposia on Quantum Chemistry and Biochemistry VII. Dordrecht: D. Reidel Publ. Co. 1975.
[5] *M. Perricaudet* and *A. Pullman*, Internat. J. Peptide Protein Res. **5**, 99 (1972).
[6] *A. M. Armbruster* and *A. Pullman*, Febs Letters **49**, 369 (1974).
[7] *A. Pullman*, Int. J. Quantum Biology Symp. No. 1, 33 (1974).
[8] *B. M. Rode* and *H. Preuss*, Theoret. Chim. Acta **35**, 369 (1974).
[9] *B. M. Rode* and *R. Fussenegger*, J. Chem. Soc. (Faraday II) **71**, 1958 (1975).
[10] *R. Fussenegger* and *B. M. Rode*, Chem. Phys. Letters **44**, 95 (1976).
[11] *B. M. Rode*, in: The Jerusalem Symposia on Quantum Chemistry and Biochemistry IX. Dordrecht: D. Reidel Publ. Co. 1976.
[12] *A. Pullman*, in: The Jerusalem Symposia on Quantum Chemistry and Biochemistry IX. Dordrecht: D. Reidel Publ. Co. 1976: and references given therein.
[13] *B. M. Rode, T. Pontani*, and *G. Heckmann*, Chem. Phys. Letters, in press.
[14] *B. M. Rode*, Mh. Chemie **106**, 339 (1975).
[15] *B. M. Rode*, Chem. Phys. Letters **26**, 350 (1974).
[16] *J. I. Padova*, Mod. Asp. of Electrochem. **7**, 1 (1972).
[17] *G. Somsen*, Rec. Trav. Chim. **85**, 517 (1966).
[18] *L. Weeda* and *G. Somsen*, Rev. Trav. Chim. **86**, 263 (1967).
[19] *R. P. Held* and *C. M. Criss*, J. Phys. Chem. **69**, 2611 (1965).
[20] *E. G. Bloor* and *R. G. Kidd*, Can. J. Chem. **46**, 3425 (1968).