THE EFFECT OF CATION BINDING ON THE ROTATION BARRIER OF THE PEPTIDE BOND

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1. Introduction

The effect of neutral salts on conformational changes in polypeptides has been known for a long time [1] and there has been growing evidence that direct binding of the peptide groups [2–6] is involved in the initiating process. The mechanism by which this binding may induce such transformations is, however, not entirely understood. A lowering of the barrier to internal rotation about the peptide linkage has first been suggested as a general mechanism [7,8]. Although the hypothesis has known varying degrees of favor [9] and is not the sole possibility [10], it seems reasonable [11] in the particular case of the salt induced reversible transition between polyproline I and polyproline II which contain only cis and only trans residues respectively.

However, the argumentation in support of such an induced cis–trans isomerization is far from being demonstrative. All the evidence invoked for the existence of cation binding to peptides points to the carbonyl oxygen as the site of binding [6,12–14] and this, on the basis of very simple valence-bond intuition should lead to an *increase* in the double-bond character of the NC bond and thus to an increase of the rotational barrier.

Table 1
Geometry and energy characteristics of the most stable proton and lithium adducts of formamide[a].

	Formamide	H^+-formamide		Li^+-formamide		
		on O	on N	normal	bridged	Li^+ over N[b]
CO	1.221	1.310	1.203	1.261	1.221	1.221
CN	1.406	1.320	1.545	1.349	1.448	1.459
$\tau H_5 NCO$	−12.0	−0.1	−28.9	−3.0	−90.8	−23.5
$\tau H_6 NCO$	−158.0	180.0	−151.7	−178.1	137.2	−155.0
OM^+		0.98		1.570	1.958	2.833
NM^+		–	1.063	–	2.002	1.947
COM^+		110	–	177.4	80.8	
CNM^+		–	105.0	–	77.6	80.7
τM^+NCO		–	89.6	–	12.5	90.0
Energy[c]	−166.68919	−167.10474	−167.08446	−173.97844	−173.96292	−173.91668

[a] bond lengths in Å, angles in degrees, energies in atomic units (1.a.u. = 627.5396 kcal/mol). H_5 and H_6 are respectively *cis* and *trans* to the carbonyl oxygen in formamide; τ_{ABCD} is the angle between the planes ABC and ABD; $\tau = O$ corresponds to a planer *cis* arrangement of the bonds AB and CD; Viewed from the side of A, τ is positive for clockwise rotation of CD with respect to AB. M = H or Li.

[b] Li^+ constrained to remain in a plane perpendicular to NCO.

[c] E (Li^+) = −7.13545 a.u.

This situation is in fact confirmed by CNDO computations [15].

The present study was undertaken in view of a broader exploration of the possibilities of cation binding and its effect on the rotational barrier of the peptide bond. The method used is the self-consistent field (SCF) ab initio procedure. (The CNDO method is known to yield artefacts both in cation binding studies [16,17] and in the evaluation of rotational barriers [18,19].

2. Methods

The SCF LCAO MO procedure [20] was implemented in a gaussian basis set. The STO 3G program [21] has been used throughout. This relatively small basis is known to yield numerical values of the cation-binding energies which are too large and distances of approach somewhat too small, but to reproduce correctly their relative values [16,22]. Keeping this restriction in mind, this is an acceptable basis set for the present evaluation particularly since a thorough geometry optimization was carried out in all cases studied.

Protonation being the simplest example of cation binding we started by assessing its effects on the geometry and lability of the peptide bond. Two recent ab initio studies of formamide protonation [23,24] have included only partial geometry optimization and we shall report here complementary features obtained relevant to the present problem.

The same computations were then done for the binding of Li^+, with a full search of the most stable position(s) of the cation, and geometry optimization of the adducts(s).

Formamide was used as a model for the peptide bond. It was necessary to start from a fully optimized geometry of the isolated molecule (in the appropriate basis set). Such an optimization performed recently [25] was made available to us. The corresponding bond lengths and angles relevant to the present study are reproduced in table 1*.

3. Results and discussion

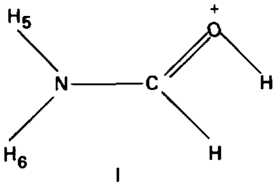
3.1. *Proton binding*

The afore-mentioned ab initio studies [23,24,29] on formamide have shown that among the two possible sites of protonation, oxygen binding is favored over nitrogen binding in agreement with the general experimental contention [30] and with CNDO computations [31]. The present fully optimized results confirm once again this conclusion, the most stable oxygen-protonated form being here 12.7 kcal/mol more stable than the nitrogen-protonated species.

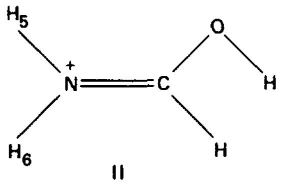
More interesting are the details of the geometrical changes undergone by the molecule upon protonation (table 1).

3.1.1. Oxygen-protonated species

In the oxygen-protonated species where the proton is attached to the oxygen in a *trans* position relative to the CN bond, the CO bond has undergone a lengthening and the CN bond an appreciable shortening. At the same time, the NH bonds which were bent out of the NCO plane in the isolated molecule, have become practically coplanar with it, with the CNH angles close to 120°. The hybridization on the nitrogen is now close to a pure trigonal state and the CN bond is more double than before. The Mulliken populations of the π electrons are 0.690, 1.733 and 1.576 on the carbon, oxygen, and nitrogen respectively, as opposed to 0.902, 1.234, 1.803, in the isolated molecule, showing that the valence-bond picture of the protonated species is closer to II than to I (otherwise speaking, in formamide a π lone-pair on the nitrogen is conjugated with a CO double bond, whereas the protonated form on the oxygen resembles more a π lone-pair on the oxygen conjugated with a CN double bond).



I



II

Accordingly the barrier to internal rotation has become 52.4 kcal/mol, much higher than in the isolated species [19]. A second stable proton equilibrium position exists on the cis side of the CN bond. (The rest

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* This STO 3G optimized geometry is in very satisfactory qualitative agreement with the known gas phase structure of formamide [26,27] which shows an out-of-plane non-equivalent bending of the two NH bonds and an elongated CN bond with respect to the value observed in the planar crystal structure [28]. Thus, it is likely that the trends that we obtain in the present computation are meaningful.

of the geometry remains practically the same as in the trans form.) The barrier for the in-plane transition of the proton between the two positions is 4 kcal/mol, much smaller than for an out-of-plane rotation of the OH bond (17 kcal/mol).

3.1.2. Nitrogen-protonated species

In the nitrogen-protonated species the changes in geometry upon protonation involve a small shortening of the CO bond and a large lengthening of the CN bond, together with a pyramidalization of the NH_3^+ group which makes the valence angles around the nitrogen close to tetrahedral. The barrier for a 60° rotation of this NH_3^+ group is computed to be 0.1 kcal/mol, thus negligible.

3.2. *Li^+-binding*

Using as a guide the results obtained for protonation the approach of this cation to the formamide molecule was studied from two different directions: one towards the oxygen atom starting in the NCO plane and the other towards the amino nitrogen, starting from above the NCO plane (opposite to the bent NH_5, NH_6 bonds). In both cases a complete deformation of the geometry of the adduct was allowed for.

The final results yield two stable positions of cation fixation with the geometrical characteristics given in table 1. Examination of these results leads to the following conclusions:

i) The most stable compound is a *'classical' oxygen-bound Li^+-adduct* with the cation practically in the NCO plane at a 'normal' distance of the oxygen atom, in a quasi-axial position with respect to the CO bond (the fully optimized distance 1.57 Å is somewhat too small as commented above but in a reasonable range. When the approach is made without relaxing the geometry of the formamide framework, the O-Li^+ distance is 1.64 Å, for a quasi-linear arrangement also).

The influence of the cation binding on the molecular framework appears thus in this case qualitatively similar to that observed for protonation, although smaller in magnitude as seen in the lengthening of the CO bond and the shortening in the CN bond. The dihedral angles at the nitrogen also show a tendancy towards flatening of the NH_2 group like that observed in protonation.

ii) There exists also however another stable form, 9.7 kcal/mol above the previous one, in which the Li^+ ion appears as bound at the same time to the oxygen and to the nitrogen atom in a *bridged conformation* and in which the distances O-Li^+ (1.96 Å) and N-Li^+ (2.00 Å) are similar to each other and longer than in the previous case. But the most interesting feature of this conformation is the fact that the NH_2 group is now rotated from its initial position so that H_5 lies nearly perpendicularly to the NCO plane with the Li^+ ion slightly above the plane (see the Newman projection of fig. 1) Simultaneously the CN bond has been lengthened with respect to its initial value and the valence angles at the amino group have come closer to the tetrahedral values. Here again the qualitative trend is the same as in the case of nitrogen protonation but the conformation in which the amino group has rotated about the CN bond is now *favored,* owing to the fact that the cation can form a bridged structure by favorable interaction with the oxygen atom when lying nearly in the molecular plane. When the Li^+ ion is constrained to remain in a plane perpendicular to the molecule, in a situation

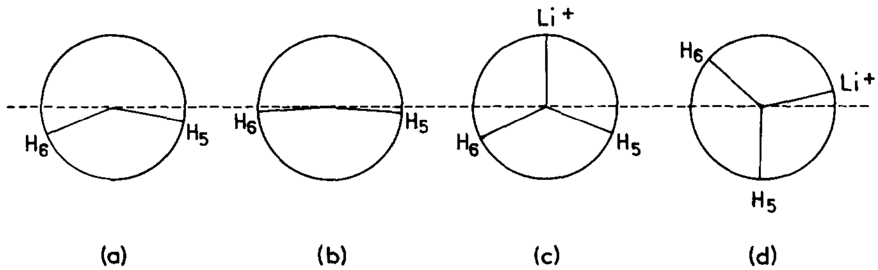

Fig. 1. Projection along the NC axis (the horizontal line is the trace of the NCO plane) H_5 and H_6 are respectively cis and trans with respect to the carbonyl oxygen. a: formamide b: Li^+-normal adduct c: Li^+-over N-adduct d: Li^+-bridged adduct (see definitions in text).

analogous to that of the best nitrogen-protonated form, the structure is less stable by 29 kcal/mol than the rotated bridged form.

Conclusion

It is thus seen that the present ab initio computations shows the possibility of the existence, beside the classical cation-oxygen adduct, of a second relatively stable product in which the cation assumes a bridged position between the two heteroatoms of the peptide bond. While the first adduct rigidifies the conformation of the peptide linkage, the second one labilizes it through intermediate favouring of a perpendicular arrangement of the NH_2 and COH groupings. It seems tempting to postulate that it is this second arrangement which intervenes in the cases in which the effect of cations is to produce a cis-trans isomerization of the peptide bond as in the polyprolines.

The reasons for which the bridged arrangement plays an enhanced role in this last case (with respect to the case of the common polyaminoacids) is not yet clear. The problem is under investigation presently in our laboratory.

References

[1] For an extensive review, see Von Hippel, P. H. and Schleich, T. (1969) in: Structure and Stability of Biological Macromolecules, Vol. 2, pp. 417, (S. N. Timasheff and G. D. Fasman, eds.), M. Dekker, N.Y.
[2] Bello, J. and Bello, H. R. (1961) Nature 190, 440.
[3] Bello, J. and Bello, H. R. (1962) Nature 194, 681.
[4] Franzen, J. S., Bobik, C. and Harry, J. B. (1966) Biopolymers 4, 637.
[5] Kurtz, J. and Harrington, W. F. (1966) J. Mol Biol. 17, 440.
[6] Baddiel, C. B., Chandhuri, D. and Stace, B. C. (1971) Biopol. 10, 1169.
[7] Harrington, W. F. and Sela, M. (1958) Biochim. Biophys. Acta, 27, 24.
[8] Mandelkern, L., Halpin, J. C., Diorio, A. F. and Posner, A. S. (1962) J. Am. Chem. Soc. 84, 1383.
[9] Mandelkern, L. (1967) in: Poly-α-amino acids, Ch. XIII, (Fasman ed.), M. Dekker, N.Y.
[10] Steinberg, J. Z., Harrington, W. F., Berger, A., Sela, M. and Katchalski, E. (1960) J. Am. Chem. Soc. 82, 5263.
[11] Hopfinger, A. J. (1973) Conformational Properties of Macromolecules, Ch. IV, Acad. Press. N.Y.
[12] Baron, M. H. and de Lozé, C. (1972) J. Chim. Phys. 69, 1084.
[13] Wuepper, J. L. and Popov, A. I. (1970) J. Amer. Chem. Soc. 92, 1493.
[14] Balasubramanian, D. and Schaikh, R. (1973) Biopolymers, 12, 1639.
[15] Rao, C. N. R. (1973) in: Conformation of Biological Molecules and Polymers, Fifth Jerusalem Symposium, (E. D. Bergmann and B. Pullman eds.) The Israel Acad. of Sciences and Humanities, p. 108. (Distributed by Academic Press).
[16] Perricaudet, M. and Pullman, A. (1973) FEBS Lett. 34, 222.
[17] Russegger, P. and Schuster, P. (1973) Chem. Phys. Lett. 19, 254.
[18] Perahia, D. and Pullman, A. (1973) Chem. Phys. Lett. 19, 73.
[19] Perricaudet, M. and Pullman, A. (1973) Int. J. of Pept. Prot. Res. 5, 99.
[20] Roothaan, C. C. J. (1951) Review Mod. Phys. 23, 69.
[21] Gaussian 70, Hehre, W. J., Lathan, W. A., Ditchfield, R., Newton, M. D. and Pople, J. A., Q.C.P.E. Program 236.
[22] Pullman, A. (1973) Int. J. of Quantum Chemistry; 1st Symposium on Molecular Biology, in press.
[23] Pullman, A. (1973) Chem. Phys. Lett. 20, 29.
[24] Hopkinson, A. C. and Csizmadia, I. G. (1973) Canadian J. of Chemistry, 51, 1432.
[25] Daudey, J. P. , in preparation.
[26] Costain, C. C. and Dowling, J. M. (1957) J. Chem. Phys. 27, 585.
[27] Kitano, M. and Kuchitsu, K. (1974) Bull. Chem. Soc. Jap. 47, 67.
[28] Ladell, J. and Post, B. (1954) Acta Crystallogr. 7, 559.
[29] Bonaccorsi, R., Pullman, A., Scrocco, E. and Tomasi, J. (1972) Chem. Phys. Lett., 12, 622.
[30] Homer, R. B. and Johnson, C. D. (1970) in: Chemistry of Amides, (J. Kabicky, ed.) Ch. III Wiley Interscience, New York.
[31] Mély, B. and Pullman, A. (1973) C. Rend. Acad. Sc. Paris, 274 C, 1371.