RAPID COMMUNICATIONS IN MASS SPECTROMETRY, VOL. 5, 316–318 (1991)

Collision-induced Liquid Secondary-ion Mass Spectra of Oxytocin Derivatives. A Study of the Influence of Modifications in the Disulfide Bond on the Adjacent Peptide Bond Cleavage

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The influence of the inductive effect of modifications in the disulfide bridge in oxytocin analogs on the cleavage of the neighboring peptidic bond was studied. A correlation between the inductive constant of the group in the disulfide bridge and the particular fragment ion abundance was observed.

Substituent effects on the mass spectrometric behaviour of substituted benzenes and various other compounds have been the subject of a number of studies in the fifties, sixties, and seventies. The ratio of the abundance, or the logarithm of the abundance, of a specified fragment ion to that of the molecular ion, was used for the quantification of the substituent effect. A good correlation between δ constants of substituents and the values of these logarithms was observed in many cases.[1]

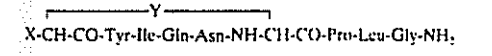
We studied the influence of disulfide bridge modifications on the adjacent peptide bond cleavage. For this purpose we used a series of eight oxytocin derivatives: oxytocin (1), deaminooxytocin (2), deamino-1-carbaoxytocin (3), deamino-6-carbaoxytocin (4), deamino-1,6-dicarbaoxytocin (5), the sulfoxide of deamino-1-carbaoxytocin (6), the sulfone of deamino-1-carbaoxytocin (7), and [6,1-aminopimelic acid] oxytocin (8) (Table 1).

These compounds are suitable for this study because of the rigidity of their skeletons and the short chain outside the ring, on which the influence of the modified disulfide bridge on the fragmentation should be observable.

EXPERIMENTAL

Oxytocin derivatives were synthesized in the Department of Peptide Chemistry of the Institute of Organic Chemistry and Biochemistry, Prague.[2] Spectra were acquired on a ZAB-EQ mass spectrometer (VG Analytical Ltd, Manchester, UK), equipped with a 15 kV caesium-ion gun and a VG11-250J data system.

Table 1. Oxytocin analogs with modified disulfide group



1: X = $-NH_2$	Y = $-S-S-$	2: X = $-H$	Y = $-S-S-$
3: X = $-H$	Y = $-CH_2-S-$	4: X = $-H$	Y = $-S-CH_2-$
5: X = $-H$	Y = $-CH_2-CH_2-$	6: X = $-H$	Y = $-CH_2-SO-$
7: X = $-H$	Y = $-CH_2-SO_2-$	8: X = $-H$	Y = $-CH_2-$

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0951–4198/91/070316–03 $05.00

Daughter-ion spectra were acquired using the linked scan technique at constant B/E, with collisional activation in the first field-free region. The collision gas was helium; the pressure in the collision cell corresponds to 50% transmission of the molecular ion of oxytocin. The primary beam energy was 35 kV, the accelerating voltage 10 kV and the scan time was 100 s. Five spectra for each measurement were accumulated using the multichannel analysis method.

The sample (10–20 μg) was placed directly into the matrix on a probe tip. Glycerol with 5% heptafluorobutyric acid (modified after Ref. 3) was used as a standard matrix. For taking the spectrum of reduced oxytocin, a mixture of dithiothreitol and dithioerythritol (DTT/DTE) 5:1 was used as a matrix and the spectrum was acquired after 30 min reduction of the sample in the matrix at room temperature.

RESULTS AND DISCUSSION

In all spectra, the same fragments—B_n, B_7, B_6, and B_5 (See Ref. 4)—are predominant. Other fragments are present in the spectra, but they are not so abundant and they were not studied further. We can suppose that the inductive effect of a modified S-S group has a greater influence on the nearer bonds than on the more distant ones. Therefore, the modification of a disulfide bond in oxytocin analogs should have a greater influence on B_n fragment formation than on B_9; the ratio of these two fragments should reflect the inductive effect.

The daughter-ion spectra of the oxytocin analogs are given in Fig. 1. The correlation with δ constants of the value $\log([B_n]/[B_9])$ is shown in Fig. 2. The correlation is only qualitative, because δ constants for the modified disulfide bridges are not available and had to be substituted by δ_1 (for the liquid phase) constants of similar groups (Table 2 (Ref. 5)).

The influence of the inductive effect on B_n fragment formation is evident. The high inductive effect of alkylsulfonyl and alkylsulfinyl groups (great attraction of electrons) causes lowering abundance of B_n fragments in the spectra of corresponding peptides (for peptide (6), the sum of the abundances of B_n and $(B_n\text{-}O)$ fragments was used for $\log([B_n]/[B_9])$ calculations). Higher abundance of B_8 fragments in the spectra of peptides (1, 1red, 2, 3, 4, 5) corresponds to smaller

Received 3 May 1991
Accepted 24 May 1991

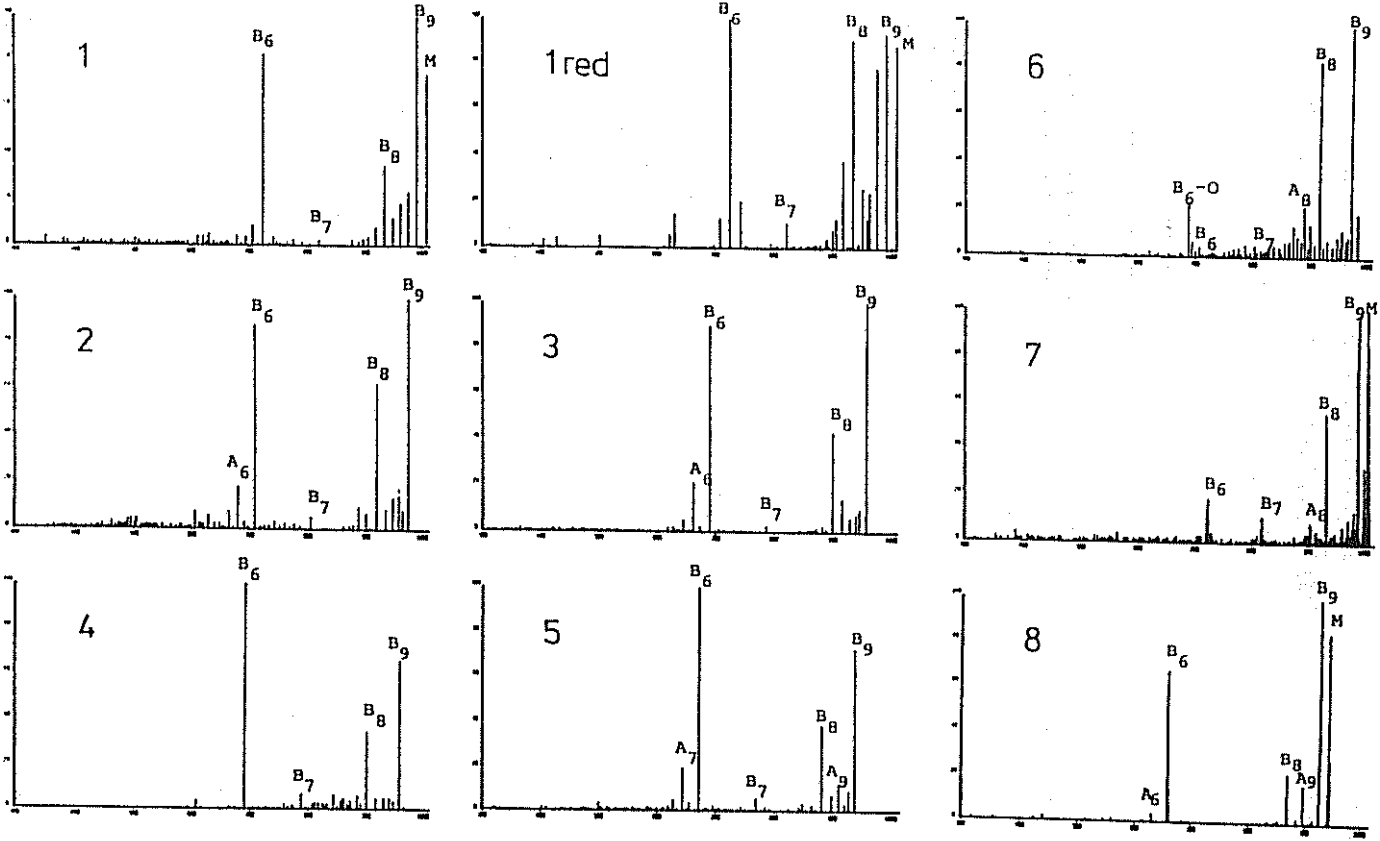

Figure 1. Daughter-ion mass spectra of oxytocin analogs.

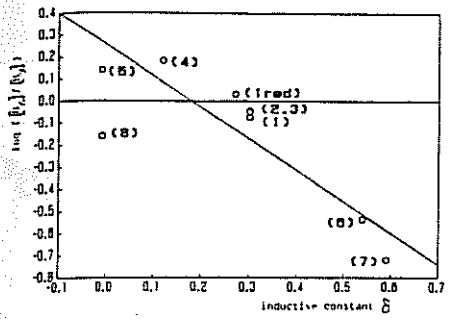

Figure 2. The correlation with δ constants of the value log ($[B_n]/[B_6]$).

Table 2. Values used in the diagram (Fig. 2). Tabulated inductive constants δ_I[5] of groups similar to the parts of modified disulfide bridges of the oxytocin analogs; values log ($[B_6]/[B_9]$)

Oxytocin analog	Similar group with known σ_I	σ_I	log($[B_n]/[B_6]$)
1	– SMe	0.30	– 0.076
1 red	– SH	0.27	0.029
2	– SMe	0.30	– 0.051
3	– SMe	0.30	– 0.046
4	– CH_2SMe	0.12	0.179
5	– Et	– 0.01	0.137
6	– SOMe	0.54	– 0.538
7	– SO_2Me	0.59	– 0.721
8	– Et	– 0.01	– 0.174

inductive effect of the sulphur and methylene groups. Following the theory, the spectrum of peptide (8) should show similar features to the spectra of peptides (4, 5) but it does not: the B_n/B_6 value corresponds to higher value than the δ of the methylene group. This can be caused by conformational changes, because the ring is smaller by one unit than in other derivatives. The values of this peptide were not included for the linear regression calculation.

CONCLUSION

The results in this work demonstrate the correlation between the inductive constant of groups in the modified disulfide bridge and the ease of the cleavage of the adjacent peptidic bond. It can be concluded that the inductive effect of amino-acid side chain substitution determines the abundance of the particular fragment ion. The side chain withdrawing electrons from the peptide bond prevents its cleavage and the side chain donating electrons promotes it.

Acknowledgement

The authors are grateful to Professor Otto Exner for valuable consultations.

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