Volume 333, number 3, 331–333 FEBS 13192 November 1993

Formation of disulphide bonds in the reaction of SH group-containing amino acids with trimethylamine N-oxide

A regulatory mechanism in proteins

Bogumil Brzezinski[a,*], Georg Zundel[b]

[a]*Faculty of Chemistry, A. Mickiewicz University, Grunwaldzka 6, PL-60780 Poznan, Poland*
[b]*Physikalisch-Chemisches Institut, Universität München, Theresienstr. 41, D-80333 Munich, Germany*

Received 27 August 1993; revised version received 15 September 1993

Two amino acids containing SH group (cysteine and homocysteine)+trimethylamine N-oxide systems were studied by FTIR and ^{1}H NMR spectroscopy. This study demonstrates that cysteine and homocysteine ethylesters react with trimethylamine N-oxide. Immediately after mixing, SH···ON⇌S$^-$···H$^+$ ON hydrogen bonds with large proton polarizability are formed. Then a reaction proceeds resulting in the formation of corresponding disulphides. Trimethylamine N-oxide is present in biological systems. Thus, our results suggest that trimethylamine N-oxide may play a regulatory role in S–S bond formation in enzymes and other proteins.

Disulphide bond; Cysteine ethyl ester; Homocysteine ethyl ester; SH-trimethylamine N-oxide; Hydrogen bonded complex

1. INTRODUCTION

The structure and behavior of enzymes and other proteins is strongly determined by disulphide bonds. The importance and formation of such bonds has been described by Toohey [1]. On the other hand, it is well known that trimethylamine N-oxide (TMAO) is present in many biological systems [2].

Recently we studied complexes of thiophenols with TMAO with the results that first SH···ON⇌S···H$^+$ ON bonds with large proton polarizability are formed [3–6]. These complexes have, however, a short lifetime. A dimerization of the thiophenols via disulphide bonds occurs connected with a formation of trimethylamine and water. On the basis of these results we assumed that TMAO may induce the formation of disulphide bonds in biology [3]. Recently, a hypothesis was published suggesting that such a reaction is important in biology for the formation of disulphide bonds [7].

In this paper we studied the formation reaction of disulphide bonds by TMAO with cysteine ethylester (CYS) and homocysteine ethylester (HCYS), to show that TMAO may also induce disulphide bond formation in real biological systems.

2. EXPERIMENTAL

The cysteine and homocysteine ethylesters were purchased from Aldrich and were purified by sublimation under nitrogen atmosphere.

TMAO is commercially available only as the dihydrate $(CH_3)_3NO{\cdot}2H_2O$. The dehydration of this substance was performed following the procedure described in [8].

All solvents were stored over 3 Å molecular sieves. All preparations and transfers of the solutions were done in a carefully dried glovebox under a nitrogen atmosphere.

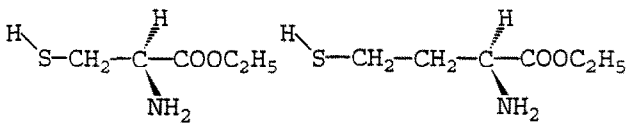
The complexes of the CYS or HCYS with TMAO were prepared by mixing respective amounts of the corresponding CYS or HCYS and TMAO in chloroform-d_1 or chloroform. The concentration of the solutions prepared for the FTIR measurements was 0.2 mol·dm^{-3} in chloroform.

The FTIR spectra were recorded at 293 K with a Bruker IFS 113v spectrophotometer, using a cell with Si windows (sample thickness 0.260 mm, detector DTGS, resolution 2 cm^{-1}).

The ^{1}H NMR spectra were recorded from 0.2 mol dm^{-3} chloroform-d_1 solutions at 293 K on a Varian Gemini VT 300 spectrometer using TMS as internal standard.

3. RESULTS AND DISCUSSION

The reactions of cysteine and homocysteine ethylesters were studied by FTIR as well as by ^{1}H NMR spectroscopy.



CYS HCYS

Fig. 1 shows the IR spectrum of a 1:1 mixture of CYS with TMAO. The spectrum (solid line) demonstrates that immediately after mixing CYS with TMAO an infrared continuum is observed beginning at about 2,700 cm^{-1} and extending towards smaller wave numbers, which is particularly intense in the region 1,300–800

*Corresponding author.

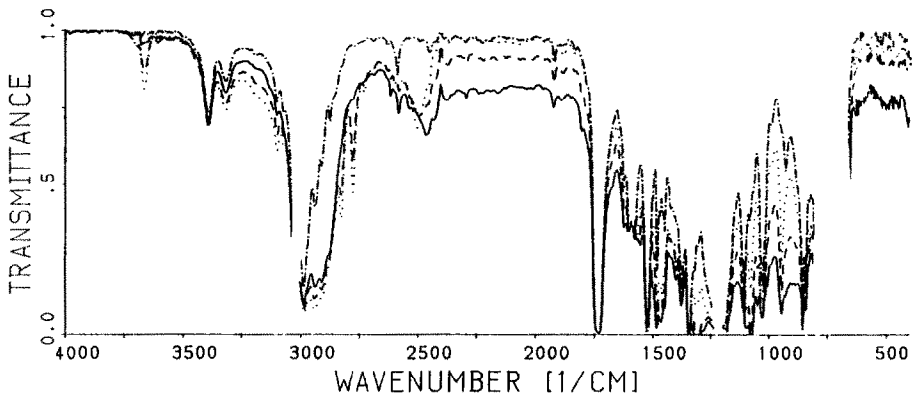


Fig. 1. FTIR spectra of (-·-·-) cysteine ethylester and its 1:1 mixture with TMAO: (—) immediately after mixing, (---) after 0.5 h and (.....) after 1 h.

cm^{-1}. This infrared continuum indicates the formation of SH···ON⇌S^-···H^+ ON hydrogen bonds with large proton polarizability [3–6]. Furthermore, at 2,580 cm^{-1} the ν(SH) vibration is still observed, showing that the formation of the CYS–TMAO complex is not complete.

The comparison of the dashed and dotted spectra with the spectrum drawn with a solid line demonstrates that with increasing time after mixing this hydrogen-bonded complex is increasingly destroyed, as shown by the following observations: (i) the continuum vanished; (ii) a band of ν(OH) vibration of free water arises at about 3600 cm^{-1} and the ν(OH) vibration of bonded water is seen in the region 3,400–3,250 cm^{-1}. (iii) the so-called Bohlmann bands arise at 2,825 and 2,775 cm^{-1} (Fig. 2) [9,10]. They show that during the reaction free trimethylamine (TMA) is formed.

Analogous spectra are obtained with the homocysteine ethylester+TMAO system. These results, together with the NMR data discussed below, show that the CYS–TMAO complex is destroyed and disulphide is formed by the following reaction:

$$2\text{-A-S-H}\cdots\text{ON(CH}_3)_3 \rightarrow \text{A-S-S-A} + \text{ON(CH}_3)_3 + \text{N(CH}_3)_3 + \text{H}_2\text{O}$$

where A-S-H=CYS or HCYS

The ^{1}H NMR chemical shifts are given in Table I.

With a solution of pure CYS only one signal for the SH and NH_2 protons is observed at 2.46 ppm, indicating that these protons exchange rapidly.

In the 1:1 mixture of CYS with TMAO, immediately after mixing two signals of the SH and NH_2 protons are observed. The signal of the NH_2 protons remains at 2.26 ppm, whereas the signal of the SH group shifts to 10.51 ppm. This result demonstrates that the S–H proton is strongly bonded in the SH···ON⇌S^-···HH^+ ON hydrogen bonds. Furthermore, one singlet for the CH_3 protons of TMAO is observed at 3.37 ppm.

After 0.5 h the signal of the SH proton is shifted towards higher fields, and after 24 h it has vanished completely, demonstrating that these SH···ON⇌S^-···H^+ ON hydrogen bonds are destroyed. Furthermore, two singlets of CH_3 from TMAO and TMA are found at 3.38 ppm and 2.22 ppm, respectively. The integrated intensity of these signals is 1:1, demonstrating that the stoichiometry of TMAO and TMA is now 1:1.

In the 2:1 mixture of CYS–TMAO, after the reaction only one signal for the CH_3 protons of TMA is found at 2.20 ppm. This result shows clearly that two CYS molecules react with one TMAO molecule.

Table I also includes the ^{1}H chemical shifts of the corresponding disulphides. The chemical shifts of the CH and CH_2S protons of the disulphide are the same as with the reaction product, indicating that disulphide bonds are formed in the reactions of CYS or HCYS with TMAO. This result was also confirmed by the isolation of this reaction product and by elementary analysis.

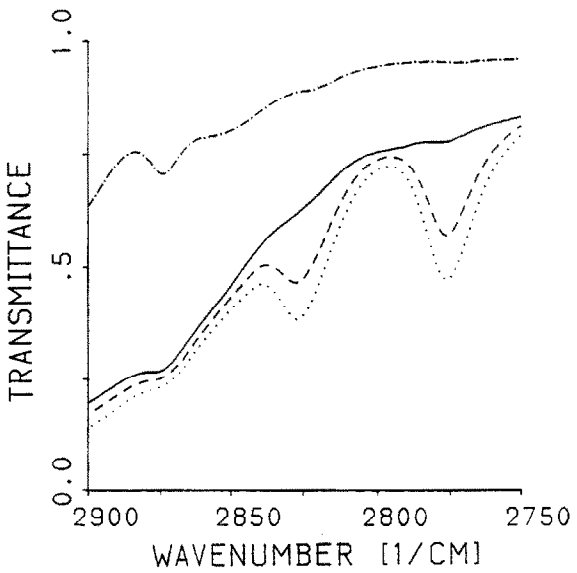


Fig. 2. FTIR spectra in the region of the Bohlmann bands of (-·-·-), cysteine ethylester and its 1:1 mixture with TMAO: (—) immediately after mixing, (---) after 0.5 h, and (.....) after 1 h.

Table I

^{1}H chemical shifts (ppm) of cysteine, homocysteine and their complexes with TMAO, and corresponding disulphides

Compound	Stoichiometry	Time (h)	S-H	NH_2	CH	CH_2-S	C-CH_2-C	$-COOCH_2CH_3$			
								CH_3	CH_2	$(CH_3)_3NO$	$(CH_3)_3N$
CYS	–	–	2.46	2.46	3.74t	2.91t	–	1.30t	4.24q	–	–
CYS+TMAO	1:1	0.2	10.51	2.26*	3.68t	2.91d, 2.96d	–	1.30t	4.24q	3.37s	–
	1:1	0.5	7.20	1.94*	3.69-2.75m	2.90-3.15m	–	1.30t	4.24q	3.36s	2.22s
	1:1	24	–	2.62*	3.79q, 3.74t	2.91d7, 3.15q	–	1.30t	4.24q	3.38s[a]	2.22s[a]
	2:1	24	–	2.95*	3.80q	2.91q, 3.15q	–	1.30t	4.24q	–	2.20s
Disulphide	–	–	–	1.75	3.80q	2.91q, 3.15q	–	1.30t	4.24q	–	–
HCYS	–	–	2.38	2.38	3.72t	2.90t	1.72se	1.30t	4.24q	–	–
HCYS+TMAO	1:1	0.2	10.60	2.20*	3.70t	2.90t, 2.95t	1.71se	1.30t	4.24q	3.38s	–
	1:1	0.5	6.80	1.97*	3.70-3.80m	2.90-3.17m	1.79se	1.30t	4.24q	3.38s	2.22s
	1:1	24	–	2.60*	3.77q, 3.72t	2.95m, 3.17q	1.71se	1.30t	4.24q	3.38s[a]	2.22s[a]
	2:1	24	–	2.91*	3.77q	2.95q, 3.15q	1.71se	1.30t	4.24q	–	2.21s
Disulphide	–	–	–	1.74	3.78q	2.95q, 3.14q	1.71se	1.30t	4.24q	–	–

s, singlet; d, doublet; t, triplet; q, quartet; se, sextet; m, multiplet.
*Very broad
[a]The stochiometry of protons 1:1.

All these results, taken together, demonstrate that disulphide bonds are formed due to TMAO according to the following reaction scheme:

$$2\ A\text{-}S\text{-}H + ON(CH_3)_3 \rightarrow A\text{-}S\text{-}S\text{-}A + N(CH_3)_3 + H_2O$$

The same reaction proceeds with the homocysteine ethylester–TMAO system as demonstrated by FTIR and ^{1}H NMR spectra.

Acknowledgements: Our thanks are due to the Polish Ministry of National Education, the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for providing facilities for this work.

REFERENCES

[1] Toohey, J.I. (1989) Biochem. J. 264, 625–632.
[2] Schlee, D. (1986) Oekologische Biochemie, VEB, Fischer Verlag, Jena.
[3] Brzezinski, B. and Zundel, G. (1993) J. Mol. Struct. (in press).
[4] Janoscheek, R., Weidemann, E.G. and Zundel, G. (1973) J. Chem. Soc. Faraday Trans. II, 69, 505–520.
[5] Zundel, G. (1976) in: The Hydrogen Bond: Recent Developments in Theory and Experiments, Vol II, Ch. 15 (Schuster, P., Zundel, G. and Sandorfy, C. eds.) pp. 683–766, Elsevier, Amsterdam.
[6] Zundel, G. (1992) in: Electron and Proton Transfer in Chemistry and Biology (Müller, A., Ratajczak, H., Junge, W. and Diemann, E. eds.) pp. 313–327, Elsevier, Amsterdam.
[7] Girard, P. and Potier, P. (1993) FEBS Lett. 320, 7.
[8] Brycki, B., Brzezinski, B., Zundel, G. and Keil, Th. (1992) Magn. Reson. Chem. 30, 507–510.
[9] Bohlmann, F. (1958) Chem. Ber. 91, 2157–2167.
[10] Konarski, J. (1971) J. Mol. Struct. 7, 337.