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Disulphide bond formation by glutathione via the glutathione–trimethylamine-*N*-oxide complex

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Abstract

Glutathione and its diethyl ester complexes (1 : 1) with trimethylamine *N*-oxide (TMAO) were studied by FTIR and ^{1}H NMR spectrocopy. Immediately after mixing, complexes with strong SH···ON⇌S$^-$···H$^+$ON hydrogen bonds are formed. They show large proton polarizability due to the fluctuation of the proton within these bonds. These complexes are, however, not stable since disulphide bonds are formed. Thus, TMAO regulates the disulphide bond formation in glutathione systems.

1. Introduction

Glutathione is a very important substance for many biological reactions such as detoxification, disulphide exchange processes, amino acid transport and removal of free radicals [1]. Recently, we have demonstrated that the aliphatic trimethylamine *N*-oxide (TMAO) forms SH···ON⇌S$^-$···H$^+$ON bonds with large proton polarizability. These systems are, however, not stable. They react with disulphide bond formation [2]. This is particularly true in the case of cysteine and homocysteine [3]. This result could be of great significance for biological processes. In this paper we studied this reaction with the well-known tripeptide, glutathione, which contains an SH group in the reduced form.

2. Experimental

The reduced and oxidized forms of glutathione were purchased from Aldrich. The diethyl esters of the two forms of glutathione were prepared following the procedure described in [4].

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 [5].

The complexes of the reduced form of glutathione and of its diethyl ester with TMAO were prepared by mixing respective amounts of the corresponding compound and TMAO in D_2O and acetonitrile, respectively. The concentration of the solutions prepared for the FTIR measurements was 0.1 mol dm^{-3} in acetonitrile.

The FTIR spectra were recorded at 293 K with a Bruker IFS 113 v spectrophotometer, using a cell with Si windows (sample thickness 0.176 mm, detector DTGS, resolution 2 cm^{-1}).

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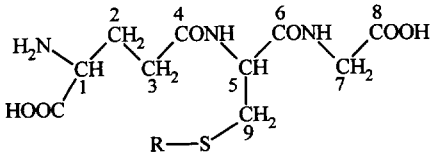
The ^{1}H NMR spectra were recorded from 0.2 mol dm^{-3} solutions in D_2O at 293 K on a Varian Gemini VT 300 spectrometer using dioxane as internal standard.

All preparations and transfers of the solutions were done in a carefully dried glove box under a nitrogen atmosphere.

3. Results and discussion

The reaction of the reduced form of glutathione (GSH) to the oxidized form (GSSG) was studied by FTIR and ^{1}H NMR spectroscopy.



R = H GSH
R = SG GSSG

The FTIR measurements were performed in acetonitrile solutions with the diethyl ester of GSH. The estrification of both carboxylic acid groups was necessary to improve the solubility of GSH in organic solvents. The study of the reaction in acetonitrile was necessary to obtain information about the formation of water and trimethylamine during the reaction.

Fig. 1 shows the FTIR spectra of the diethyl ester of GSH (solid line) and GSSG (dashed line). The comparison of these spectra shows that only ν(SH) at 2389 cm^{-1} vanishes with the reaction.

Fig. 2 shows the FTIR spectra of a 1 : 1 mixture of the diethyl ester of GSH with TMAO as a function of the time after mixing. Immediately after mixing (solid line) a continuum is observed beginning at about 2700 cm^{-1}. It extends towards the lower wavenumbers. This continuum is particularly intense in the region 1500–700 cm^{-1}. It demonstrates that SH···ON $\rightleftharpoons$ S$^-$···H$^+$ON hydrogen bonds are formed showing large proton polarizability due to fluctuation of the proton within these bonds. Furthermore, the wavenumber-dependent intensity distribution shows that these hydrogen bonds are relatively strong [6–10].

This IR continuum has vanished after 1.5 h (dotted line in Fig. 2), demonstrating that the SH···ON $\rightleftharpoons$ S$^-$···H$^+$ON hydrogen bonds are destroyed. During this process new bands arise in the regions 3700–3600 cm^{-1} and 2850–2750 cm^{-1}; this is more clearly illustrated in Figs. 3 and 4, respectively. The band in the region 3700–3600 cm^{-1} demonstrates that non-hydrogen-bonded water molecules are formed during the reaction, whereas the bands arising in the region 2850–2750 cm^{-1} are the so-called Bohlmann bands [11] demonstrating that trimethylamine is formed.

These results taken together with the ^{1}H NMR data discussed below, prove that the GSH + TMAO complexes are destroyed and disulphide bonds are

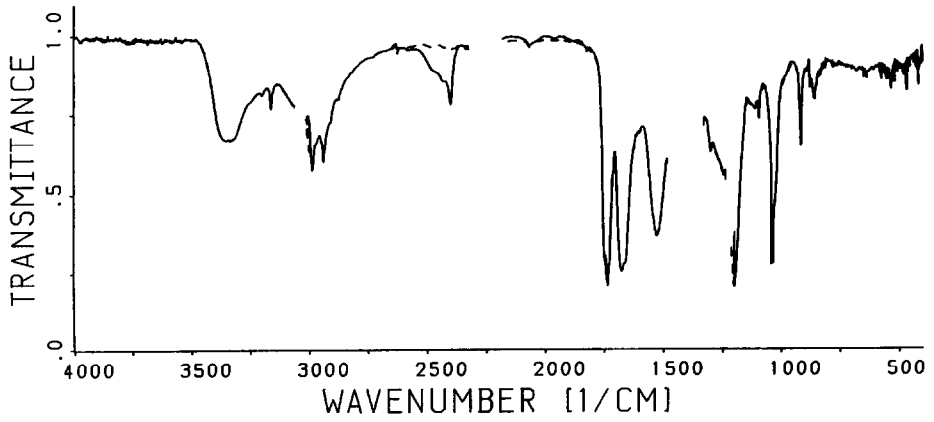


Fig. 1. FTIR spectra of acetonitrile solution of glutathione diethyl ester: (——) GSH; (– – –) GSSG.

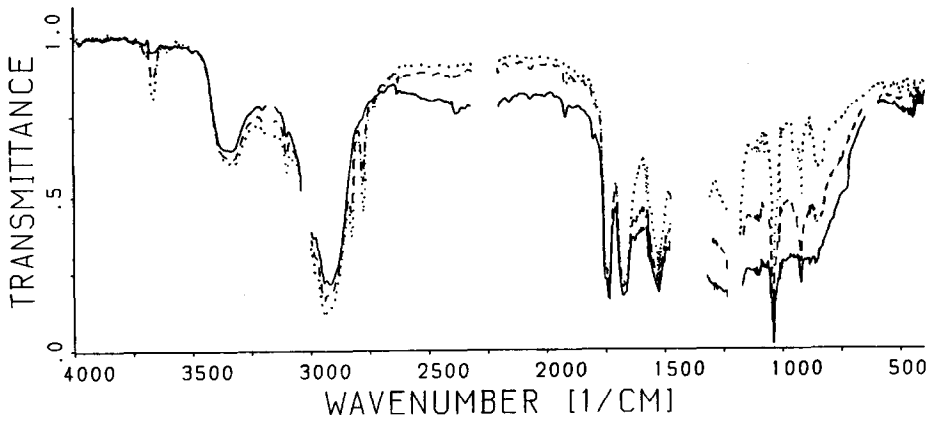


Fig. 2. FTIR spectra of 1 : 1 mixture of GSH diethyl ester with TMAO: (——) immediately after mixing, (– – –) after 40 min, and (· · · · ·) 90 min after mixing.

formed by the following reaction:

$$2\,GSH + ON(CH_3)_3 \rightarrow GSSG + N(CH_3)_3 + H_2O$$

The ^1H NMR chemical shifts of GSH and of its complex with TMAO are summarized in Table 1. For comparison, the respective data for GSSG are given. All these spectra were taken in D_2O.

Immediately after mixing, the ^1H signals of all the protons except those in position 9, i.e. those of the CH_2S group, remain unchanged. In the case of the CH_2S protons instead of one doublet two doublets are observed. During the reaction, the ^1H signals of the protons in positions 5 and 9 are strongly broadened and are multiplets. After 24 h the signals of the CH_2S protons are observed as two quartets. These results correspond to the observations obtained with cysteine and homocysteine [3]. After the reaction all the ^1H signals are identical with the signals observed with GSSG.

Immediately after mixing GSH with TMAO (1 : 1) only one singlet of the TMAO protons at 3.85 ppm is observed. During the reaction the integrated intensity of this signal decreases and a new singlet of $(CH_3)_3N$ protons arises at 2.41 ppm. After 24 h the integrated intensity of the TMAO protons has decreased by exactly 50% and the intensities of both signals are equal. These results demonstrate the stoichiometry given in the reaction scheme.

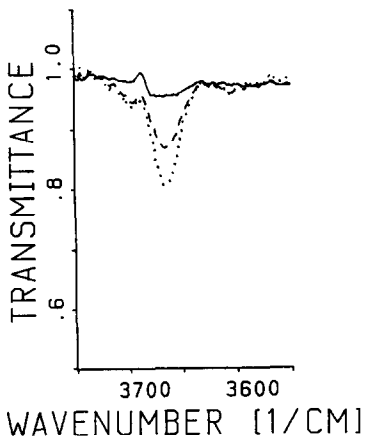


Fig. 3. FTIR spectra of 1 : 1 mixture of GSH diethyl ester with TMAO in the region 3750–3550 cm^{-1}: (——) immediately after mixing, (– – –) after 40 min, and (· · · · ·) 90 min after mixing.

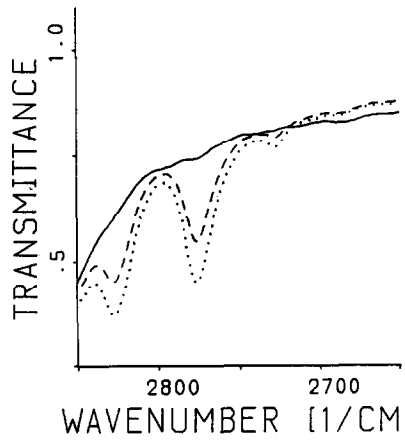


Fig. 4. FTIR spectra of 1 : 1 mixture of GSH diethyl ester with TMAO in the region 2850–2650 cm^{-1}: (——) immediately after mixing, (– – –) after 40 min, and (· · · · ·) 90 min after mixing.

Table 1
^{1}H chemical shifts (ppm) of glutathione (GSH) and its oxidized form (GSSG) in D_2O

Compound	Stoichiometry	Time (h)	Protons						$(CH_3)_3NO$	$(CH_3)_3N$
			1	2	3	5	7	9		
GSH	–	–	3.62t	1.97q	2.35t	4.36t	3.78s	2.74d	–	–
GSH + TMAO	1 : 1	0.2	3.61t	1.96q	2.33t	4.46t	3.78s	2.74d 2.96d	3.85s	–
GSH + TMAO	1 : 1	1.0	3.40m	1.96q	2.33t	4.40m	3.78s	2.74–3.10m	3.84s	2.41s
GSH + TMAO	1 : 1	24	3.62t	1.97q	2.34t	4.55t	3.77s	2.76q 3.07q	3.85s[a]	2.40s[a]
GSSG	–	–	3.62t	1.97q	2.34t	4.55t	3.77s	2.76q 3.07q	–	

s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.
[a] The stoichiometry of protons 1 : 1.

TMAO is present in biological systems [12] and therefore it can play a regulatory role in the disulphide bond formation by GSH.

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References

[1] A. Meister and S.S. Tate, Annu. Rev. Biochem., 45 (1976) 559.
[2] B. Brzezinski and G. Zundel, J. Mol. Struct. (Theochem), 303 (1994) 141.
[3] B. Brzezinski and G. Zundel, FEBS Lett., 333 (1993) 331.
[4] B. Brycki, B. Brzezinski and J. Olejnik, J. Chem. Soc., Faraday Trans. 2, (1989) 2109.
[5] B. Brycki, B. Brzezinski, G. Zundel and Th. Keil, Magn. Reson. Chem., 30 (1992) 507.
[6] R. Janoscheck, E.G. Weidemann and G. Zundel, J. Chem. Soc., Faraday Trans. 2, 69 (1973) 505.
[7] G. Zundel, in P. Schuster, G. Zundel and C. Sandorfy (Eds.), The Hydrogen Bond: Recent Developments in Theory and Experiments, Vol. 2, Elsevier, Amsterdam, 1976, Chapter 15, 683.
[8] G. Zundel, in A Müller, H. Ratajczek, W. Junge and E. Diemann (Eds.), Electron and Proton Transfer in Chemistry and Biology, Elsevier, Amsterdam, 1992, p. 313.
[9] M. Eckert and G. Zundel, J. Phys. Chem., 91 (1987) 5170.
[10] G. Zundel, in J. Menon (Ed.), Trends in Physical Chemistry, Publ. Research Trends, Trivandrum, 3 (1992) 129.
[11] F. Bohlmann, Chem. Ber., 91 (1958) 2157.
[12] D. Schlee, Oekologische Biochemie, VEB, Fischer Verlag, Jena, 1986.