biochemical oxygen demand (BOD_5), ammonia nitrogen, nitrate nitrogen, water soluble phosphorus, total alkalinity, total hardness, calcium, magnesium and sulphate. The analyses were carried out according to the standard methods[6,7]. The results (table) indicate that there is a striking removal of various nutrients, which were originally present in the influent water. The permanganate value, BOD_5, ammonia nitrogen, and water soluble phosphorus contents were reduced by 61, 85.3, 95.3 and 85%, respectively. The values for total solids, total alkalinity, total hardness, calcium, magnesium, and sulphate were reduced by 24, 18, 23.5, 19.7, 19.9, and 29.4%, respectively.

The removal of nutrients by aquatic plants is not uncommon. Wolverton and McDonald[2] reported that the water hyacinth *(Eichhornia crassipes)* in a facultative lagoon treating sewage was able to reduce the BOD by 95%, total Kjeldahl nitrogen by 71.7% and total phosphorus by 56.8%. In the treatment of waste water and in water pollution control in general, a variety of microorganisms[8–10] are known to have the ability to remove pollutants. But the involvement of higher plants or weeds is of considerable importance, and *Pistia stratiotes* L. appears to be yet another in the list which may prove beneficial in the removal of nutrients from water. In the lake, at the point of entry of the pollutants, the plants grew vigorously, with an average density of 42 plants per m^2, and removed the nutrients to a great extent. As the concentration of nutrients decreased due to their removal, the plants became less vigorous and grew smaller in size with a density of 223 per m^2 at a distance of 250 m from the entry of pollutants. The plants contained about 94% water and it is commonly believed that the high water content may be uneconomical for possible use as an animal feed. While the experiments on the nutritional quality of the plant were in progress, laboratory experiments using grasshoppers as test animals showed that this plant may prove useful as a fodder or as a feed supplement in a fresh state.

1 A.H. Pieterse, Bull. Dept. Agric. R. Trop. Inst. Amsterdam, 1–20 (1977).
2 B.C. Wolverton and R.C. McDonald, Ambio *8*, 2 (1979).
3 C.D.K. Cook, Weed Abstr. 15, 529 (1966).
4 L.G. Holms, D.L. Plucknett, J.R. Pancho and J.P. Herberger, The World's Worst Weeds. East West Center, Honolulu 1977.
5 C. Sculthorpe, The Biology of Vascular Plants. Arnold, London 1967.
6 Standard Methods for the Examination of Water and Wastewater, 14th edn. American Public Health Association, 1975.
7 Methods of Chemical Analysis as Applied to Sewage and Sewage Effluents, 2nd edn. HMSO. Ministry of Housing and Local Government, London 1956.
8 M.K.C. Sridhar and S.C. Pillai, Envir. Pollut. *6*, 195 (1974).
9 K. Wuhrmann, Adv. appl. Microbiol. *6*, 119 (1964).
10 W.O. Pipes, Adv. appl. Microbiol. *8*, 77 (1966).

Spectroscopic study of the disulfide bond in oxidized glutathione

Maria Anna Rosei

Istituto di Chimica Biologica, Università di Roma, I-00185 Rome (Italy), 23 November 1979

Summary. Raman and circular dichroism spectra are used for obtaining structural information on the disulfide bridge in oxidized glutathione. Quantitative estimates of dihedral angles and bond angles are proposed.

Several investigations have shown that stereochemical parameters of the disulfide bond can be obtained by electronic and vibrational spectroscopy, in particular circular dichroism (CD)[1–4] and Raman scattering[5–9]. In order to extend spectra-structure correlations to disulfide bridges in proteins further work with simple peptides is necessary. On this subject we present a study of oxidized glutathione (GSSG) in solid phase and in aqueous solution by Raman and CD spectroscopy.

Raman spectra were recorded on a 25-300 Jarrel-Ash Laser Raman spectrometer. Characteristic Raman frequencies of GSSG and related disulfides are shown in the table.

CD spectra were obtained with a Cary 60 and they were in full agreement with those reported in the literature[2].

A main parameter describing the geometry of disulfide bonding is the dihedral angle χ (CS-SC), which is related both to the frequency of the sulphur-sulphur stretching vibration[6–9] and to the frequencies of electronic transitions of the disulfide chromophore[6,10]. In the Raman spectra of GSSG sulphur-sulphur stretching ν (SS) is observed at 512 cm^{-1} in the crystal and 514 cm^{-1} in aqueous solution (pH 3), suggesting that no major conformational change occurs on solution. According to an early proposal of Van Wart et al.[6], the value of ν (SS) can be related to the dihedral angle χ (CS-SC) by a linear relationship

$$\chi \text{ (CS-SC)} \simeq [2.2\, \nu \text{ (SS)} - 1060] \text{ degrees}$$

(with ν (SS) in cm^{-1}) which gives a value $\chi \simeq 70°$ for GSSG. Yet, recently Van Wart and Sheraga[9] revised their original relationship, restricting its validity from 0° to 65° and accepting the view that in the range 65–90° ν (SS) is practically invariant to χ (CS-SC). On the other hand a further evaluation of the disulfide dihedral angle can be obtained by electronic spectra, since a quasilinear correlation also exists between χ (CS-SC) and the energy of the lowest electronic transition of the disulfide chromophore–at least in simple molecules[6,10]. In GSSG the wavelength of the first electronic absorption, as seen in CD spectra (near UV absorption, spectra do not show any resolved peak), is about 260 nm, corresponding to a dihedral angle of 75°. This rather good agreement between Raman and CD results suggests that for the χ (CS-SC) angle of GSSG a value of about 70–75° can be confidently accepted; this

Characteristic Raman frequencies in GSSG and model disulfides

		(S-S) cm^{-1}	(C-S) cm^{-1}
Oxidized glutathione	(solid)	512	670
	(solution)	514	670
L-cystine	(solid)	500	680
	(solution)	507	666
L-homocystine	(solid)	510	640
	(solution)	509	640
Cystamine-2 HCl	(solid)	511	643
	(solution)	510	640

compares well with the value of 74° measured by X-ray in crystalline L-cystine[8]. A more difficult task is to gain pieces of information on the sign of the dihedral angle χ (CS-SC), i.e. on the chirality of disulfide bond. Indeed, when χ (CS-SC) is nearly 90°, 2 skewed conformations, named M (minus) and P (plus), are expected for a disulfide molecule[3,4]. Unfortunately, Raman bands seem to be insensitive to disulfide chirality, and on the other hand CD data are not totally unambiguous[2]. However, the ellipticity of GSSG ($[\Theta]_{260} = -1300$ deg/cm^2 decimole^{-1}) compares quite well with that of cystine ($[\Theta]_{253} = -1600$), suggesting that there is probably some predominance of the M conformation in solution at room temperature[3].

At this point a short comment is also necessary on the conformation about the C-S bond. A study on the methyl ethyl disulfide[11] and an investigation of several proteins[8] have indeed shown the existence of 3 rotational isomers, with similar dihedral angles χ (CS-SC) - about 90° - but with the different dihedral angles χ (SS-CC). These rotamers - designated A (with χ ((SS-CC) $\simeq$ 20-30°), B(90°) and T (180°) - can be partially distinguished by their ν (SS) frequency, since for the A conformation SS stretching appears at about 525 cm^{-1} and for the B and T conformations ν (SS) is observed in the range 510-520 cm^{-1} [8]. As we mentioned above, in GSSG ν (SS) is found at about 513 cm^{-1} and no Raman peak appears at 525 cm^{-1}, so we conclude that A rotamers are absent both in the solid and in solution, while neither B nor T conformations can be excluded. Finally we mention another correlation, proposed by Lord and Yu[6], between the Raman intensities of ν (CS) and ν (SS) bands and the bond angle CŜS. According to these authors an intensity ratio $I_{CS}/I_{SS} \lesssim 0.1$ indicates an angle CŜS $\simeq 115°$ while a ratio $\gtrsim 0.5$ corresponds to a bond angle of about 105°. For GSSG we observed $I_{CS}/I_{SS} \simeq 0.4$, which suggests that CŜS $\simeq 105°$. As the correlation is based only on a small number of model disulfides, this value has to be taken with caution but, on the other hand, it is characteristic of cystine-related compounds[8].

1 D.L. Coleman and E.R. Blout, in: Conformation of Biopolymers, vol.I, p.123. Ed. G.N. Ramachandran. Academic Press, London 1967.
2 D.L. Coleman and E.R. Blout, J. Am. chem. Soc. *90*, 2405 (1968).
3 J.P. Casey and R.B. Martin, J. Am. chem. Soc. *94*, 6141 (1972).
4 T. Takagi and N. Ito, Biochim. biophys. Acta *257*, 1 (1972).
5 R.C. Lord and N.T. Yu, J. molec. Biol. *50*, 509 (1970).
6 H.E. Van Wart, A. Lewis, H.A. Sheraga and F.D. Saeva, Proc. natl Acad. Sci. USA *70*, 2619 (1973).
7 H.E. Van Wart, L.L. Shipman and H.A. Sheraga, J. Phys. Chem. *78*, 1848 (1974).
8 H.E. Van Wart and H.A. Sheraga, J. Phys. Chem. *80*, 1812 (1976).
9 H.E. Van Wart and H.A. Sheraga, J. Phys. Chem. *80*, 1823 (1976).
10 D.B. Boyd, J. Am. chem. Soc. *94*, 8799 (1972).
11 H.E. Van Wart, F. Cardinaux and H.A. Sheraga, J. Phys. Chem. *80*, 625 (1976).

Effect of bombesin on glucose transport system in biomembranes

N. Sopranzi and C. Cavallotti

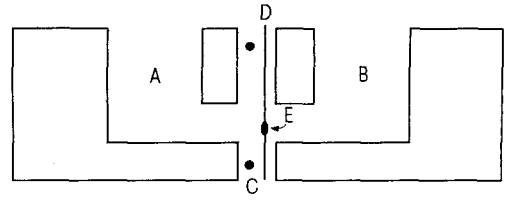
Department of Pharmacology and Department of Human Anatomy, University of Rome, I-00161 Rome (Italy), 5 November 1979

Summary. Bombesin is able to stimulate the glucose transport system supported by a contractile glucose binding protein (GBP) in biomembranes. The increase of glucose transport indicates that bombesin affects GBP.

Previous results indicate that the glucose transport system in biological membranes is supported by an expanding and contracting glucose binding protein (GBP)[1-3] according to the theoretical model of Blumenthal and Katchalsky[4]. The aim of the present communication is to study whether bombesin interacts with GBP in biomembranes.

Bombesin is a tetradecapeptide occurring in the skin of the European discoglossid frogs *Bombina bombina* and *B. variegata*. This polypeptide displays a complex spectrum of effects on gastric and pancreatic secretions, on gallbladder motility, and on gut electrical and mechanical activity. The peptide is also active on other target organs and tissues outside the gastrointestinal tract[5,6]. All these actions suggest a probable effect of bombesin on GBP.

Materials and methods. The experiments were performed with the perspex apparatus shown in the figure, where A = starting compartment, volume 3.0 ml; B = migration compartment, volume 3.0 ml; C = 'O' ring; D = polyethylene disk (PE disk = 1.5 cm diameter with 1 mm central hole). Black films or biomembranes were deposited in the central hole of the PE disk. The formation of black films (bimolecular lipid membranes in aqueous solutions) was obtained, according to the experimental methods first introduced by Mueller et al.[7], from a 0.5% solution of phosphatidyl inositol and a 1.5% solution of phosphatidyl choline in n-decane. Biomembranes (black membrane plus GBP) were obtained by stratifying GBP (aqueous solution, 10 mg protein/ml) on the surface of the black film according to the techniques of Mueller and Rudin[8]. Optical controls were performed previously to verify that the membrane remained black, or black plus GBP, during the course of the experiment[1,3]. The disk was now assembled with the compartments A and B. In compartment A 2.5 ml of phosphate buffer and 50 µl of an aqueous solution of D-C^{14} glucose were introduced. In the compartment B 2.5 ml of phosphate buffer without D-C^{14} glucose was introduced. Samples (0.1 ml) were simultaneously collected from both compartments, at 0, 5, 10, 15, 20, 25, 30 min, dissolved in



Perspex apparatus used for transport experiments. A = Starting compartment; B = migration compartment; C = 'O' ring; D = poliethylene disk; E = central hole.