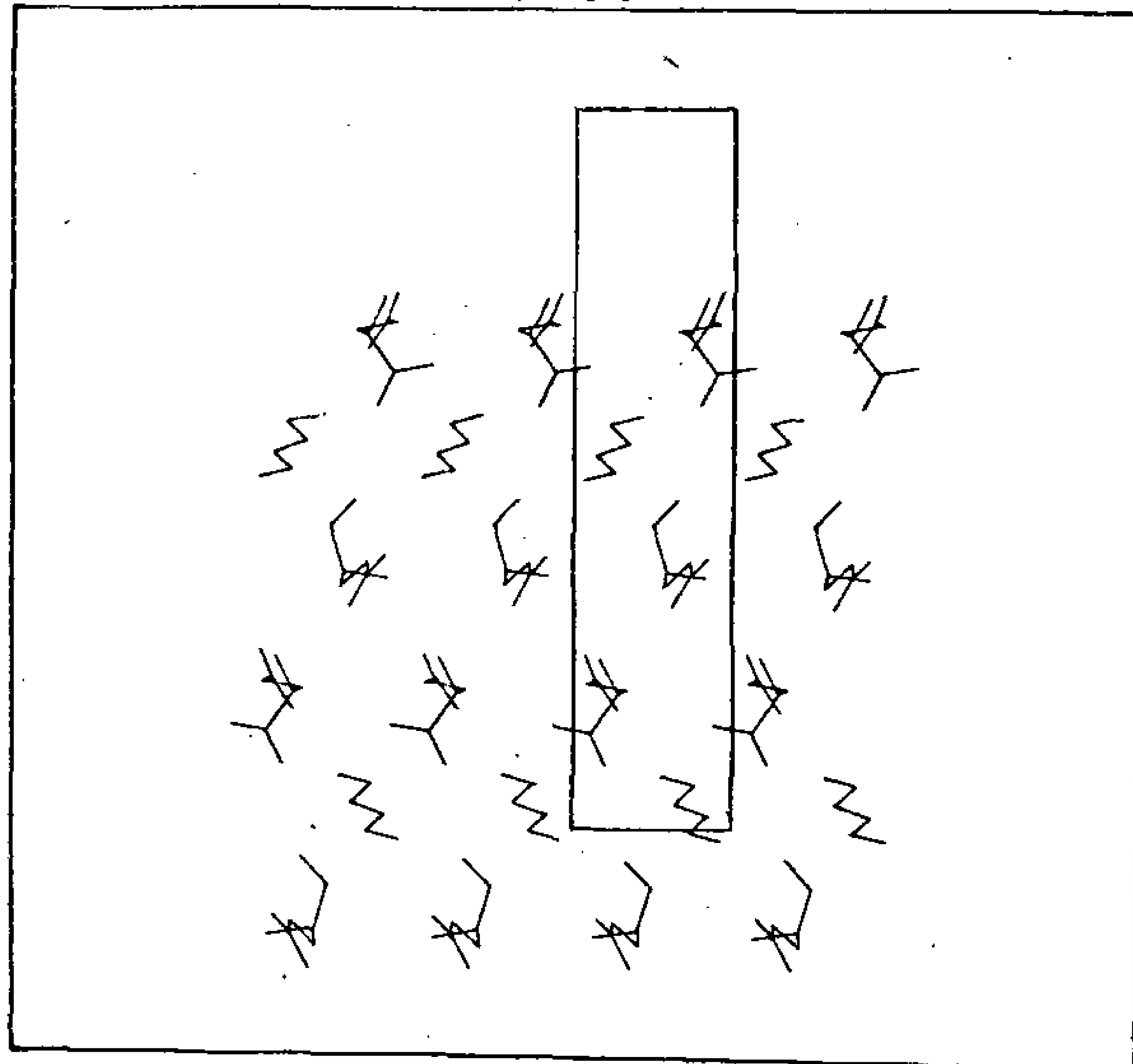

Figure 2. Packing diagram of putrescine–L-glutamic acid complex.

molecules along the *a* axis. Also, there is a continuous chain of hydrogen bonds between the main-chain carboxyl and main-chain amino groups along *a*. The layers are additionally stabilized by hydrogen bonding between main- and side-chain carboxylates and putrescine amino groups. The two layers of D and L glutamic acid molecules sandwiched between putrescine layers are held together by hydrogen bonding between side-chain carboxyl groups and main-chain amino groups. A comparison of the two complexes suggests that the conformation of putrescine and the pattern of packing and hydrogen bonding are conserved, although the chemical environments surrounding the putrescine are different. The putrescine has retained its most-favourable *trans* conformation in both structures. The conformational flexibility of the glutamic acid side chains accounts for the similarities of packing environment and conservation of polyamine conformation in the structures.

1. Tabor, C. W. and Tabor, H., *Annu. Rev. Biochem.*, 1984, **53**, 749.
2. Frederick, C. A., Williams, D. L., Giovanni, U., Van der Marel, G. A., Van Boom, J. H., Rich, A. and Wang, A. H. J., *Biochemistry*, 1990, **29**, 2538.
3. Gary, J. Q., Martha, M. T. and Rich, A., *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 64.
4. Frederick, G. W., Jr. and Kitareewan, S., *J. Biol. Chem.* 1990, **265**, 7127.
5. Smith, T. A., *Annu. Rev. Plant Physiol.*, 1985, **36**, 117.
6. Ramaswamy, S., Nethaji, M. and Murthy, M. R. N., *Curr. Sci.*, 1989, **58**, 1160.
7. Ramaswamy, S. and Murthy, M. R. N., *Curr. Sci.*, 1990, **59**, 379.
8. Ramaswamy, S. and Murthy, M. R. N., *Curr. Sci.*, 1991, **60**, 173.

ACKNOWLEDGEMENTS. M.R.N.M thanks CSIR for financial assistance. S.R. is a UGC senior research fellow.

Received 21 February 1991; accepted 3 April 1991

Uptake, binding and photodynamic action of haematoporphyrin derivative in brain tumour cells

Kunju Joshi, Preeti G. Joshi and Nanda B. Joshi

Department of Biophysics, National Institute of Mental Health and Neuro Sciences, Bangalore 560 029, India

The photosensitizer haematoporphyrin derivative (Hpd) is a mixture of various monomeric forms of porphyrins such as haematoporphyrin (HP), protoporphyrin (PP), hydroxyvinyldeuteroporphyrin and a covalently linked aggregated form known as dihaematoporphyrin ester/ether (DHE). Hpd, *in combination with light*, is used *in* the diagnosis and treatment of cancer. We have studied uptake, binding and photodynamic action of Hpd in brain tumour cells. Hpd uptake by cells increased with increase in Hpd concentration in medium as well as with increase *in incubation time*; the cells were more photosensitive in the latter case. Fluorescence intensity of cell-bound Hpd increased with increased uptake by the cells. Upon prolonged incubation, the fluorescence emission spectrum of cell-bound Hpd showed changes in the position of one peak and relative intensities of the peaks. Our data suggest that the increase in photodynamic cellular damage on prolonged incubation may be due to increased accumulation of aggregated ester/ether component of Hpd and/or binding of Hpd to specific sites in the cell.

PHOTODYNAMIC therapy (PDT) for cancer[1-3] is based on bringing about selective accumulation of Hpd by tumour tissue followed by irradiation with light. The fluorescence emitted by tumour-bound Hpd serves for detection while laser irradiation of Hpd-containing tumour is used for the treatment. It has been proposed that, in photodynamic action, the excited photosensitizer may transfer electrons to the surrounding biomolecules with the formation of free radicals, or transfer energy to oxygen leading to the formation of highly reactive singlet oxygen[4,5]. It has been shown that the photodynamic action of Hpd on cells in culture is higher when cells are incubated for longer times before irradiation but the exact cause of this is not yet known[6-8]. In an attempt to understand the cause of this enhanced photodamage, we have studied uptake, binding of Hpd by brain tumour cells and photosensitization of the cells.

Hpd was prepared from haematoporphyrin dihydrochloride (Sigma) by the method of Lipson *et al.*[9] BMG-1 cells were grown in Nunc plastic tissue culture flasks using Dulbecco's minimum essential medium (DMEM; Hi-Media) supplemented with 5% bovine serum and

antibiotics. After a growth period of 48 h, the growth medium was replaced by DMEM containing 1% bovine serum and 25 or 100 $\mu g\,ml^{-1}$ of Hpd. After incubation, cells were washed, released with trypsin, and finally suspended in PBS (pH 7.4). Aliquots were taken for Hpd uptake measurements, protein estimation and fluorescence studies. All procedures subsequent to Hpd addition were performed in the dark. Light irradiation was performed using two 40-W cool daylight fluorescent tubes (Philips) covered with a Perspex diffuser sheet. The fluence rate at the position of the cells was about 1 $W\,m^{-2}$, measured by a Kyoritsu Model 5200 illuminometer. Hpd uptake by cells was measured by the method of Christensen *et al.*[7] Fluorescence emission spectra of cell suspensions were recorded on an SLM 8000C spectrofluorimeter using 395-nm excitation. The ability of cells to form macroscopic colonies served as a measure of cell survival[8]. Protein was estimated by the method of Lowry *et al.*[10]

Table 1 shows uptake of Hpd by cells incubated with two different concentrations of Hpd for different durations. Cell survival measured under identical conditions is also given for comparison. The surviving fraction of cells incubated with 25 $\mu g\,ml^{-1}$ Hpd for 24 h and irradiated for 2 min was approximately 40 times less than that obtained after 1 h incubation. The data also show that longer exposure to Hpd results in increased accumulation of Hpd by the cells. This increase in cell-bound Hpd could be considered as a plausible cause of the increased photosensitivity of the cells. Hpd concentration in cells could also be increased by increasing Hpd concentration in the medium (43% more uptake with 100 $\mu g\,ml^{-1}$ Hpd for 1 h than with 25 $\mu g\,ml^{-1}$ for 24 h). However, the surviving fraction of cells irradiated after incubation with 100 $\mu g\,ml^{-1}$ of Hpd for 1 h was only about 2.9 times less than that of cells incubated for 1 h with 25 $\mu g\,ml^{-1}$ of Hpd. These data clearly show that the increase in photosensitivity of cells upon prolonged incubation with Hpd is not only due to more accumulation of Hpd by the cells. Figure 1 shows the fluorescence spectra of cell-bound Hpd after incubation of cells with 25 or 100 $\mu g\,ml^{-1}$ Hpd for 1 or 24 h. The fluorescence spectrum of Hpd in cells after 1 h incubation with 25 $\mu g\,ml^{-1}$ of Hpd shows peaks at 616, 636 and 678 nm, with the highest intensity at 616 nm. When Hpd concentration in the incubation medium was increased to 100 $\mu g\,ml^{-1}$ and cells were incubated for the same duration, i.e. 1 hour, a total increase in fluorescence intensity was observed without any change in the position or relative intensities of the fluorescence peaks. However, remarkable changes were observed in the spectrum with increase in incubation time. After 24 h of incubation with 25 $\mu g\,ml^{-1}$ Hpd the 616-nm peak shows a shift to 620 nm and decrease in intensity, whereas the 636-nm peak shows significant increase.

Table 1. Hpd uptake by BMG-1 cells and surviving fraction after irradiation.

Hpd ($\mu g\,ml^{-1}$)	Incubation time (h)	Hpd uptake (μg Hpd/mg protein)	Cell survival*
25	1	1.50 ± 0.25	0.87 ± 0.06
25	24	2.80 ± 0.25	0.022 ± 0.008
100	1	4.00 ± 0.30	0.30 ± 0.01

*Surviving fraction was measured after irradiating the cells for 2 min (12 $mJ\,cm^{-2}$).

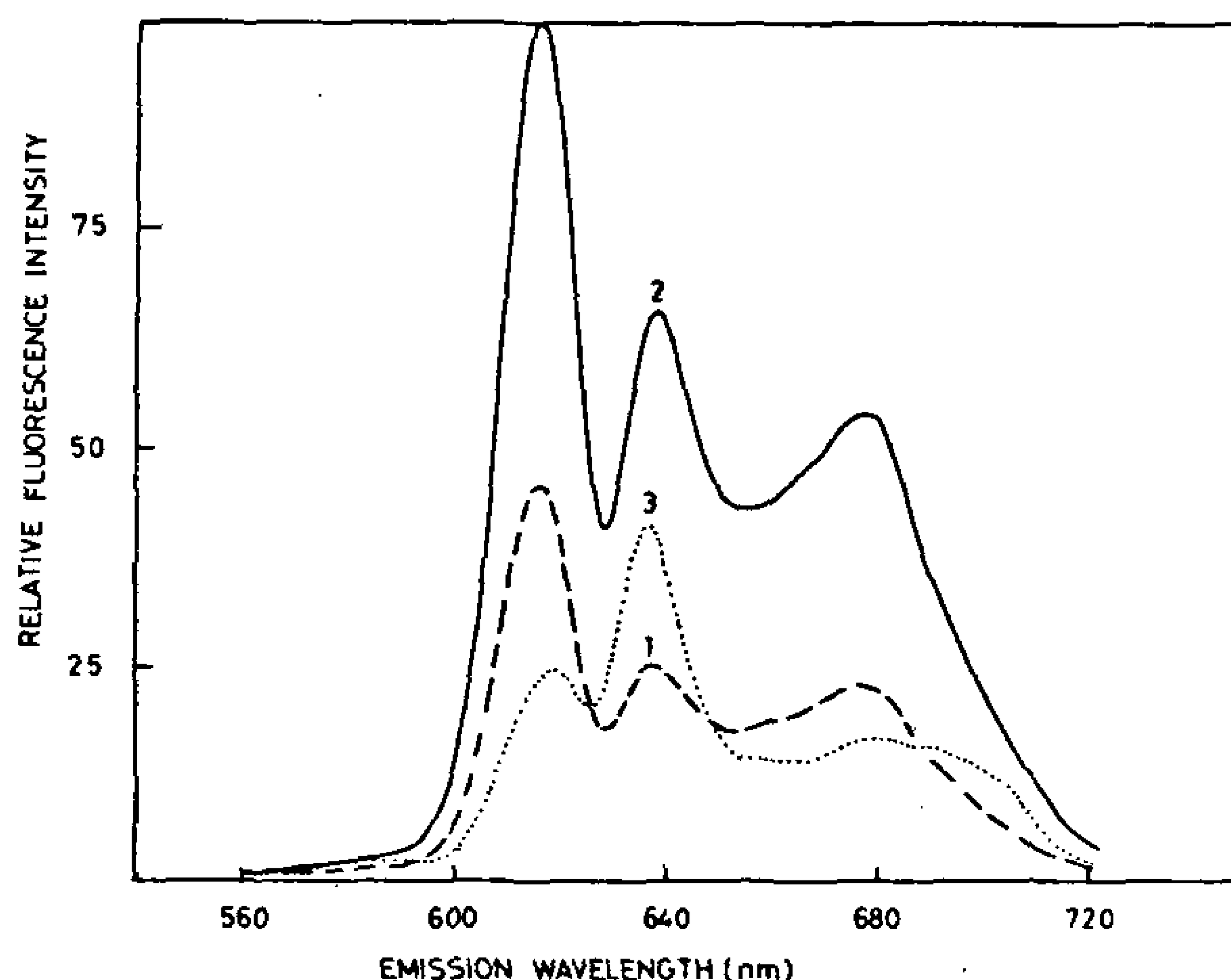

Figure 1. Fluorescence emission spectra of cell-bound Hpd: BMG-1 cells were incubated with (1) 25 $\mu g\,ml^{-1}$ Hpd for 1 h, (2) 100 $\mu g\,ml^{-1}$ Hpd for 1 h, (3) 25 $\mu g\,ml^{-1}$ Hpd for 24 h.

The fluorescence peak at 636 nm appears to be related to increased cell death after irradiation following prolonged incubation. Although at present we do not know the precise origin of the 636-nm peak, we think the increase in intensity upon prolonged incubation may arise owing to (i) increased accumulation of aggregated ester/ether components of Hpd, and/or (ii) binding of Hpd to specific sites in the cell.

1. Dougherty, T. J., *Photochem. Photobiol.*, 1987, **45**, 879.
2. Spikes, J. D. and Jori, G., *Laser Med. Sci.*, 1987, **2/3**, 3.
3. Jori, G. and Spikes, J. D., in *Topics in Photomedicine* (ed. Smith, K. C.), Plenum, New York, 1984, p. 183.
4. Valenzeno, D. P. and Pooler, J. P., *Bioscience*, 1987, **37**, 270.
5. Grossweiner, L. I., Patel, A. S. and Grossweiner, J. B., *Photochem. Photobiol.*, 1982, **36**, 159.
6. Kessel, D., *Photochem. Photobiol.*, 1986, **44**, 489.
7. Christensen, T., Sandquist, T., Feren, K., Walksvik, H. and Moan, J., *Br. J. Cancer*, 1983, **48**, 35.
8. Gangola, P. and Joshi, N. B., *Indian J. Med. Res.*, 1990, **B92**, 467.
9. Lipson, R. L., Baldes, E. J. and Olsen, A. M., *J. Natl. Cancer Inst.*, 1961, **26**, 1.
10. Lowry, O. H., Rosebrough, H. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.

ACKNOWLEDGEMENT. We thank Prof. D. D. Pant for helpful discussion. This work was supported by DST.

Received 27 December 1990; revised accepted 19 February 1991