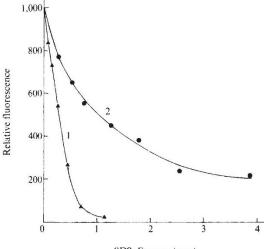


Fig. 3 Quenching of tryptophan fluorescence during the formation of the new fluorophore. Carboxymethylated G3PDH, 3 µM and NAD⁺ 33 µM in 0.1 M phosphate buffer, pH 6.7 was irradiated in the MPF-4 spectrofluorometer with an exciting light of 290 nm and a slit width of 20 nm. The emission spectra with the exciting wavelength also at 290 nm were scanned at a, 0; b, 1.5; c, 3; d, 6; e, 9; f, 12; g, 15 and h, 20 min.

this energy transfer involves probably only two of the four subunits. Calculations based on the Förster equation with the orientation factor K^2 taken as 2/3 give a distance between tryptophan and the new fluorophore of 15.79 Å. As each subunit of the rabbit muscle enzyme has three tryptophan residues, this probably represents a weighted average value of the distances between the three tryptophan residues to the new fluorophore. This value can be compared with the results of X-ray crystallographic analysis of the lobster³ and bacterial⁴ enzymes.

The formation and the fluorescence of the new fluorophore were found to be sensitive to conformational changes of the enzyme protein. Sodium dodecylsulphate (SDS) at a weight ratio of 1:1 with the enzyme led to a 50% decrease in the fuorescence intensity at 410 nm. However, even more marked



SDS: Enzyme (w/w)

Fig. 4 Effect of sodium dodecylsulphate on the formation and intensity of the new fluorophore. Curve 1 shows the effect of SDS present during irradiation on the formation of the 410 nm fluorophore. Curve 2 shows the effect of SDS added after the formation of the new fluorophore on its fluorescence intensity. Relative fluorescence intensity plotted against the weight ratio of SDS to the enzyme. Final concentration of the enzyme was 2 µM with $22 \ \mu M \ NAD^+$ in 0.1 M phosphate buffer, pH 6.7.

was the effect of SDS on the formation of the new fluorophore. As shown in Fig. 4, the presence of SDS at a weight ratio of 0.5 during irradiation was enough to decrease the 410 nm fluorescence formed by 75%. It has been reported⁵ that SDS at a weight ratio to G3PDH of 0.5 loosened its tertiary structure and at a ratio of 1 dissociated this enzyme. Our own studies by difference spectroscopy and protein fluorescence also showed that with increasing SDS to enzyme ratios, the internal tryptophan and tyrosine residues of G3PDH were gradually exposed. The great sensitivity of the formation of the new fluorophore seems to suggest that the photochemical reaction leading to its formation has strict requirements on the spatial relationship of excited tryptophan residue(s), the carboxymethyl group introduced at Cys-149 and the nicotinamide ring.

Further studies are needed to establish the chemical nature of the new fluorophore. At present, we can say that it is tightly, probably covalently, bound to the enzyme protein and its spectral and fluorescence properties closely resemble a series of addition compounds⁶ of NAD⁺ including NADH. In spite of some earlier suggestions^{7,8}, very little is known

about the role of tryptophan residues in G3PDH. Our results demonstrate the close relationship between tryptophan, Cys-149 and the nicotinamide ring and suggests the need for close examination of this problem.

Further details of this work will be published elsewhere⁹.

YEN-SEN HO CHEN-LU TSOU

Institute of Biophysics, Academia Sinica, Peking, China

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Errata

In the letter 'Photographic amplification of faint astronomical images' by D. F. Malin, Nature 276, 591-593, the figures have been transposed. Figure 1 is shown above Fig. 2 legend and Fig. 2 above Fig. 1 legend.

In the article 'Olivine fractionation equations for basaltic and ultrabasic liquids' by T. H. Pearce, Nature 276, 771-774, equation (12) should read:

$$\log\left(\frac{y}{z}\right) = K \log\left(\frac{x}{z}\right) + \log\left(\frac{y_0}{x_0} \times \frac{z_0^{k-1}}{x_0^{k-1}}\right)$$

equation (14) should read:

$$\log\left(\frac{\text{FeO}}{\text{Al}_2\text{O}_3}\right)_i = K \log\left(\frac{\text{MgO}}{\text{Al}_2\text{O}_3}\right)_i + \log\left(\frac{\text{FeO}}{\text{MgO}}\right)_p \times \left(\frac{\text{Al}_2\text{O}_3}{\text{MgO}}\right)_p^{k-1}$$

In the article 'Analysis of ³⁶Cl in environmental water samples using an electrostatic accelerator' by D. Elmore et al., Nature 277, 22-25, the last sentence in the summary should read: 'For each sample less than 70 mg of AgCl were used, requiring 1-5 litres of water.' Line 19 on page 23 should read 'with mass spectrometers lies in the difficulty of separating' In line 41 for 'from' read 'with'. In line 47 for ref. 18 read ref. 17. In Table 1 the units in the last three columns should read: (d.p.m. per kg Cl); $(mg l^{-1})$; $(\times 10^{-6}$, atoms $l^{-1})$. An extra line should be added to the table:

$$\begin{array}{c} \text{Groundwater, deep well near Tucson, AZ} \\ 101 \quad 990 \quad 4 \quad 320 \pm 50 \quad 24 \quad 10 \quad 53 \end{array}$$

In line 31 in the right column for 'neutral' read 'natural' and in line 54, for 100-mg read 20-mg.