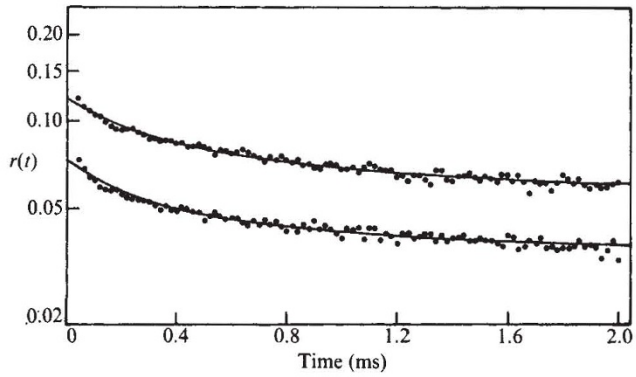

Fig. 2 Time dependence of the absorption anisotropy for untreated and cross-linked erythrocyte membranes. Flash-induced transient dichroism of the eosin probe was measured using a flash photolysis apparatus which is described in detail elsewhere[13]. The sample was excited at 540 nm by a linearly polarised flash of duration 1–2 μs. Absorbance changes arising from ground state depletion were measured at 520 nm. Upper curve, untreated control; lower curve, cross-linked as described in the legend to Fig. 1. Experimental points were obtained by averaging 32 signals. The solid lines were obtained by fitting the data to equation (2). For illustrative purposes the two curves have been artificially separated by a vertical displacement of the lower curve by 20%. Otherwise the data almost exactly superimpose. Measurements were made at 37 °C in 5 mM phosphate buffer, *p*H 7.4, under argon.

the data to equation (2). In this way we obtain $D_{\parallel} = 1{,}296 \pm 99\ s^{-1}$ and $1{,}492 \pm 202\ s^{-1}$ for the control and cross-linked membranes, respectively.

If we regard band 3 as a cylinder of radius a immersed to a depth h in the membrane, then the coefficient for rotational diffusion about the membrane normal is given by[15]

$$D_{\parallel} = (kT)/(4\pi a^2 h\eta) \quad (3)$$

where η is the membrane viscosity. Thus, the rotational diffusion coefficient depends on the square of the radius of the rotating species. Assuming that dimerisation increases the radius of the particle by a factor of 1.5–2, we thus expect a two- to fourfold difference in $D_{\parallel}$ between the monomer and the dimer. As we fail to observe any decrease in $D_{\parallel}$ following cross-linking, we conclude that the band 3 dimer exists as a stable complex in the erythrocyte membrane independent of chemical cross-linking.

A possible objection to this conclusion is that the cytoplasmic portion of band 3 on which the reactive SH-group is located[16], is attached by a flexible linkage to the major part of the protein whose rotation we observe. Hence, cross-linking in this position might not change the rotation of band 3 monomers. However, Ross and McConnell have reported[17] that a spin label bound to the same SH-group is 'immobilised' (on the conventional electron spin resonance time scale). This strongly suggests that the cytoplasmic fragment is rigidly attached to the bulk of the protein and is not an independently rotating entity.

It should also be pointed out that cross-sectional shapes of band 3 could in principle be devised which would considerably reduce the expected change in $D_{\parallel}$ on association of monomers into dimers. Because we could detect a change in radius with cross-linking as small as 15%, we consider this a rather improbable explanation of our data. Taken together with the earlier chemical studies, there can be little doubt that band 3 does exist as a stable dimer within the human erythrocyte ghost membrane. However, further association of band 3 dimers among themselves or with other integral membrane proteins such as the major sialoglycoprotein are not ruled out.

The functional significance of the band 3 dimer is unclear. As the major part of band 3 is the anion transport system[1,2,18], it is tempting to speculate that the anion channel is formed at the interface between the two subunits of the dimer. However, inhibition studies with disulphonic acid stilbene inhibitors[18] and eosin maleimide[19] show that about one inhibitor bound per band 3 monomer is required for total inhibition of anion transport. This suggests that there are two anion binding sites per channel, if there is only one channel per band 3 dimer. Alternatively, one half of the inhibitor molecules could be bound to nonspecific sites on the protein.

Thus, the experiments reported here demonstrate how rotational diffusion measurements can be used to detect stable oligomeric complexes of proteins within biological membranes. We anticipate that the approach may be extended to other membrane proteins, for example, the (Mg^{2+}, Ca^{2+})ATPase of the sarcoplasmic reticulum, where the possibility of self-association is now under consideration[20,21].

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Errata

In the review article 'Multiple receptors for dopamine' by J. W. Kebabian and D. B. Calne, *Nature* **277**, 93–96, line 27 in the right-hand column of page 95 should read 'activity, have been used to . . .' and line 34 should read 'studies are only just beginning to be . . .'

In the letter 'MHC matching shows that at least two T-cell subsets determine resistance to HSV', by E. L. Howes *et al.*, *Nature* **277**, 67–68, in line 10 in paragraph 6, for 'in the thymus' read 'in the remnant thymus'.

In the letter 'Loss of immunoreactivity in long-term bone marrow cultures' by T. M. Dexter and E. Spooncer, *Nature* **275**, 135–136 (1978), on page 136, lines 5 and 8 for H-v-G read G-v-H.