

are strongly hydrophobic as the trimer dissociates only in the presence of denaturants and its subunits are not covalently joined¹².

Trimeric haemerythrin is a compact structure maintained by strong helix-helix interactions between subunits (Fig. 2). The long axes of the subunits are tilted about 40° from the molecular 3-fold axis in a right-twisted manner, such that subunits cross at an angle of approximately 68°. The principal interactions occur where the B helix of one subunit crosses the A' (93°) and B' (-84°) helices of another subunit. These interactions are rather tight; crossed B and A' helix axes are separated by < 8 Å, in a manner reminiscent of the B-E helix cross observed in myoglobin, where two glycine residues are close to each other²⁶. A less extensive perpendicular contact also exists between C and B' helices (94°). There may be 3-fold axial interactions between residues 20 of the A helix and between residues 49 of the B helix. In addition, there may be interactions between the B helix and residues 9-11 of a neighbouring N-terminal arm. Otherwise, non-helical regions of the molecule are not involved in maintaining the trimeric aggregate.

Structures are known for other helix-bundle proteins which aggregate primarily through helix contacts. In these cases the associations continue the antiparallel helix-bundle motif with extensive helix contacts between each pair of interacting subunits²⁷. This antiparallel mode of association is observed in the 2-fold interaction of cytochrome *c'* (ref. 7), in the principal interaction of the helix-bundle of apoferritin⁵, and in the equatorial interaction of the tobacco mosaic virus helix²⁸ or disk⁴. While one of the subunit interfaces in octameric haemerythrin does involve helix-helix contacts (these in the perpendicular mode of association, contrary to statements in ref. 27), the non-helical N-terminal arm also contributes extensively to this interface and we do not consider this to be an association of helix bundles. The exclusively perpendicular mode of association in trimeric haemerythrin is fundamentally different from the other helix-bundle associations, and can be viewed schematically as the crossing of two helix hairpins (B-C and A'-B' faces of neighbouring subunits). The marked twist of the helix-bundle determines that only three intersubunit helix contacts can occur at each hairpin cross, and these are observed between the B and A', B and B', and C and B' helices of trimeric haemerythrin (if the helix bundles were of opposite twist, four such intersubunit contacts could form). The perpendicular mode of association thus involves inherently less extensive contact between each pair of subunits than the antiparallel mode. However, while it is well suited to dimer formation, the antiparallel mode of association is geometrically unfavourable for 3-fold aggregation. Instead, three mutually perpendicular B helices form the nucleus of a tight trimeric association in which two faces of each subunit interact in a cyclical manner with the two neighbouring subunits. In view of this diversity in mode of association adapted to molecular symmetry, we await with interest the further determination of structures of multimeric helix-bundle proteins²⁹.

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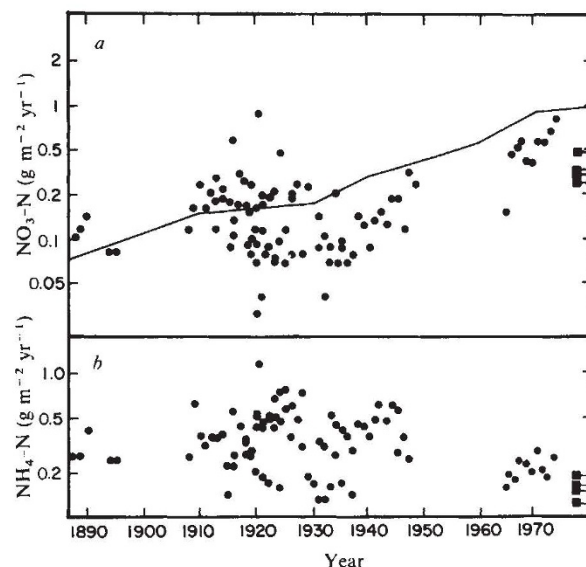
Corrigendum

Historical evidence for a dramatic increase in the nitrate component of acid rain

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The increase in nitrate deposition in the northeastern USA was somewhat exaggerated in Fig. 2 of our recent paper¹. A replotted version is shown below. The data from 1964-74 are total deposition data (wet and dry) for Hubbard Brook². The 1976-79 data shown as squares are wet deposition only from Wilson *et al.*³. It now seems that both European and US nitrate deposition have risen by about a factor of 5 over the past century. The nitrate flux alone appears to exceed the neutralizing ability of the ammonium flux, independent of any SO₂⁻ contribution. This conclusion is reinforced for the USA by Miller⁴ who states that "ambient levels of NO₂ increased a total of 20% between 1970 and 1980".



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