11 Hillman, P., Hochstein, S., and Minke, B., *Science*, 175, 1486–1488 (1972).
12 Hochstein, S., Minke, B., and Hillman, P., *J. gen. Physiol.*, 62, 105–128 (1973).
13 Minke, B., Hochstein, S., and Hillman, P., *J. gen. Physiol.*, 62, 787–791 (1973).
14 Muijser, H., Leutscher-Hazelhoff, J. T., Stavenga, D. G., and Kuiper, J. E., *Nature*, 254, 520–522 (1975).
15 Pak, W. L., and Lidington, K. J., *J. gen. Physiol.*, 63, 740–756 (1974).

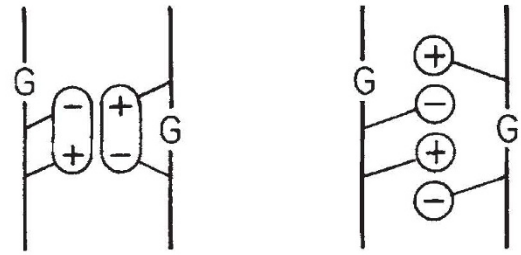
## Possible contribution of ionic clustering to molecular packing of collagen

An analysis of the primary structure of the α1 chain of a collagen demonstrated that electrostatic and hydrophobic interactions between collagen molecules were maximal when the rod-like molecules were staggered by 234*n* amino acid residues, where *n* is 0, 1, 2, 3 or 4 (ref. 1). It was not possible, however, to determine what value of *n* was most likely. This information would help to select from among possible molecular packing models[2,3]. Doyle *et al.*[4] re-examined the electrostatic interactions in an attempt to determine *n*. Since amino acid side chains of opposite charge frequently occur close to each other in the sequence[1], they considered that these potential intrachain ion pairs might function as dipoles with approximately the same direction in the molecule as in the sequence. If antiparallel dipoles on adjacent molecules were attractive (Fig. 1*a*) and parallel dipoles were repulsive, they found that the net number of attractions was maximal at $n = 1$ and negative at other values of *n*. They concluded that dipole–dipole interactions were important in stabilising the native fibril and that the Smith microfibril[5] was favoured over other packing models since it is essentially a helix of collagen molecules staggered by 234 residues between nearest neighbours.

Although we find the assumptions made by Doyle *et al.*[4] difficult to accept, they were apparently able to discriminate from among possible values of *n*. This suggests that the reason should be more carefully examined. We discuss here other aspects that may be important and propose as an alternative concept that ionic clustering may contribute significantly to the stabilisation of collagen fibril structure.

First, interactions between dipoles cannot be estimated when the location of the charges is not well defined. This point is of particular importance in the case of collagen. The charges are at the ends of side chains, which may assume many configurations, and there are generally three sets of charges at any level since there are three similar or identical chains in the molecule. Therefore, neighbouring charges of opposite sign in a single chain will not necessarily form a dipole in an actual structure, and even if dipoles are formed, the contributing ions and their orientation would be difficult to predict. A related conclusion is that the ions would be able to arrange themselves so as to minimise electrostatic repulsion, which then would not be a major factor, contrary to the assumption of Doyle *et al.*[4]

Second, there is information in the sequence, not explicitly used by Doyle *et al.*[4], that explains their result without recourse to a consideration of dipole–dipole interactions. Not only are amino acid side chains of opposite charge found close to each other in the sequence much more often than would occur by chance, but glutamic acid and arginine are not equally distributed between position X and Y in the collagen triplet Gly–X–Y, with the result that about three out of four positive charges precede glycine and about three out of four negative charges follow glycine in the sequence. Therefore triplets of the form Y–Gly–X and Gly–X–Y, where Y has a positively charged side chain and X has a negatively charged side chain, are common. Of the fifty 'dipoles' considered by Doyle *et al.*[4], fifteen are of the former type and thirteen are of the latter type. This geometry suggests an arrangement (Fig. 1*b*) of four alternating unit charges approximately in a line that could result in cooperative electrostatic interactions with a lower potential energy than two separated ion pairs and a more favourable stereochemical relationship than the two 'dipoles' in Fig. 1*a*. An examination of molecular models shows that such an arrangement is possible




*a* *b*

**Fig. 1** *a*, Antiparallel dipoles between collagen molecules considered to be attractive by Doyle *et al.*[4]. *b*, An alternative arrangement of the ions to form a line of alternating charges. G is glycine. The positive and negative ions show two characteristic orders of charged amino acids in the triplet sequence of collagen chains.

because of the separation of the one pair by a glycine residue. Furthermore, the arrangement provides a reason for the unusual distribution of glutamic acid and arginine. Similar arrangements of other opposing antiparallel charge pairs can be devised. This approach to the location of ionic groups leads to the conclusion reached by Doyle *et al.*[4] that $n = 1$, if the same scoring system is used. If, however, the possibility of repulsive interactions is de-emphasised and other arrangements of charges are allowed, as we argue, a most probable value of *n* can no longer be selected with confidence by this analysis.

The considerations discussed here suggest a concept that may help in understanding how collagen fibrils are stabilised. The argument is as follows. Since the collagen molecule has three similar or identical chains as already noted, and since more than one chain per molecule may contribute groups to interacting edges between molecules, an arrangement of the type shown in Fig. 1*b*, if viewed in three dimensions, could involve additional ionic groups. Furthermore, there are often more than two ionic groups near each other in the sequence, and it is possible that an interesting region may involve more than two molecules. Therefore, clusters of ions may exist. Schematic representations of the Smith microfibril suggest that as many as 16–20 ions could cluster in ordered arrangement. These would be expected to approximate the properties of an ionic crystal and contribute some measure of stability to the collagen fibril structure analagous to crystal field energy. This could be an especially important contribution if the clusters were in the interior of the Smith microfibril, or one of the similar alternative structures, since the movement of water, other small molecules, and amino acid side chains would be restricted, increasing the likelihood of cooperative ordering and depolarisation. A more complete analysis may settle some of the details of the molecular packing of collagen, such as the value of *n*, but this has not yet been done.

The principle of ordered ionic clustering proposed here may apply generally to supramolecular structures, which create extended regions where movement is restricted. Guss *et al.*[6] have found an example by X-ray diffraction of a helical form of hyaluronic acid which has a line of alternating charges of opposite sign down the centre of the helix.

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1 Hulmes, D. J. S., Miller, A., Parry, D. A. D., Piez, K. A., and Woodhead-Galloway, J., *J. molec. Biol.*, 79, 137–148 (1973).
2 Doyle, B. B., *et. al., Proc. R. Soc.*, **B186**, 67–74 (1974).
3 Piez, K. A., and Miller, A., *J. Supramolec. Struct.*, 2, 121–137 (1974).
4 Doyle, B. B., *et al., Biochem. biophys. Res. Commun.*, 60, 858–864 (1974).
5 Smith, J. W., *Nature*, 219, 157 (1968).
6 Guss, J. W., *et al., J. molec. Biol.*, 95, 359–384 (1975).