EXPANSION OF THE COLLAGEN LATTICE BY CIS - TRANS - 1, 4 – BIS (AMINOMETHYL) CYCLOHEXAME*

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INTRODUCTION

The work of the author on epitaxis of hydrogen-bonding amides and diols in formalinized collagen (rat tail tendon) from water has been summarized by G. N. Ramachandran (1), with the result that the collagen core diameter was found to be 7.0±0.2A for the aqueous N-H---0 or OH---O bond, with spacing of the repeating C=O interval at 4.8±0.3A along the collagen fiber axis. Work on nonformalinized collagen from propylene glycol by Shaw and Schulte (2) yielded slightly higher parameters, with 7.6±0.2A for the collagen core diameter and spacing of the C=O hydrogen bond accepting unit at 5.5±0.5A along the collagen fiber axis.

EXPERIMENTAL

In the current work, a mixture of the liquid cis-transisomers of 1,4-bis (aminomethyl-) cyclohexane was used for the weighted suspension of non-formalinized rat tendons. The tendon was considerably strengthened, carrying 400 gm as compared to 70 gm for tendon wet with saline. After overnight suspension, the moist tendon was run promptly on X-ray diffraction, using CuKα radiation. The diffractogram showed two discrete spots, both of limited longitudinal spread, as compared to the collagen spot, indicating improvement of the collagen lattice by the amino groups hydrogen-bonded to the carbonyl groups of the collagen spirals, with the collagen equatorial parameter a increased from the collagen control, 12.6A, to 13.2A for the cis- and 20.6A for the trans-. Fig. 1. X-ray diffraction pattern of cistrans 1,4-bis (aminomethyl cyclohexane in collagen. Fig. 2. Model of cis-1,4-bis (aminomethyl) - cyclohexane in collagen. Fig. 3. Model of trans-1,4-bis (aminomethyl)-cyclohexane in collagen. This figure 20.6A for the trans- is the largest expansion obtained so far, and possibly represents the maximum attainable in view of the fact that this fitting in of the molecules undoubtedly takes place at the internodes of the collagen where the structure is more regular than in the nodes where a greater variety of larger amino acids occur, including such polyfunctional amino acids as tyrosine, serine, threonine, lysine, hydroxylysine, aspartic acid, glutamic acid and others. It is possible that the internodes can expand only to the diameter of the nodes, and that in the fully hydrated collagen, the fibrils are smooth cylinders. If this is the

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case, then 20.6A might be considered the maximum equatorial parameter and might measure the nodal dimension of the triple spiral. Subtracting the measured length of the trans-1,4-bis-(aminomethyl)-cyclohexane including the hydrogen bond, on the Fisher-Hirschfelder-Taylor Model Set (13.0A), we reach the dimension 7.6A for the collagen core diameter at the C=O level, which compares favorably with the dimension obtained with other non-aqueous liquids. With the Fisher-Hirschfelder-Taylor models in this stretched position which they are assumed to occupy between the collagen spirals, it is noted that they stack into stable vertical piles at the interval of 4.7A as compared to 4.8A previously measured for the vertical interval along the collagen spirals (3).

DISCUSSION

Disturbance of the normal pattern of body protein is frequently followed by carcinogenesis, as in the case of crosslinking of protein strands and nucleic acids by the sulfur mustards, the nitrogen mustards, and the epoxides. In the case of the sulfur mustards and epoxides, the groupings appear at distances on the chain that are multiples of 3.7A, which corresponds to the interpurine, interpyrimidine and inter-amino acid distances in extended peptide chains, and to the core diameter between the C=O groups in collagen. Hydrogen bonding materials have also been shown (amino-antipyrine, semicarboazide, urea), to cause lathyrism in tadpoles (5). They also stabilize and expand the collagen lattice (6), (3). In lathyrism, the collagen of bone is grossly distorted, forming tumorous growths when calcified. In these cases we are dealing with covalent bonds, whereas in the current work, very much weaker bonds such as the hydrogen bonds and the hydrophobe bonds are being used. With multiple weak bonds, however, the crosslinking can hold well enough to account for the high tensile strength of collagen, and could probably be sufficiently strong to account for crosslinking effects in other proteins.

SUMMARY

When rat tail tendons that have been stretched by a weight of 400 gm in the liquid mixture of cis-and trans-isomers of 1,4-bis (aminomethyl)- cyclohexane are exposed to the CuKα X-ray beam in a G. E. Model 1 X-ray diffractometer, central equatorial spots were produced from each of the isomers at 13.2A for the cis- and 20.6A for the trans-, corresponding to 7.6A for the core diameter of the collagen spirals at the C=O level. It is estimated that the vertical spacing of the C=O hydrogen bond acceptors is 4.7A.

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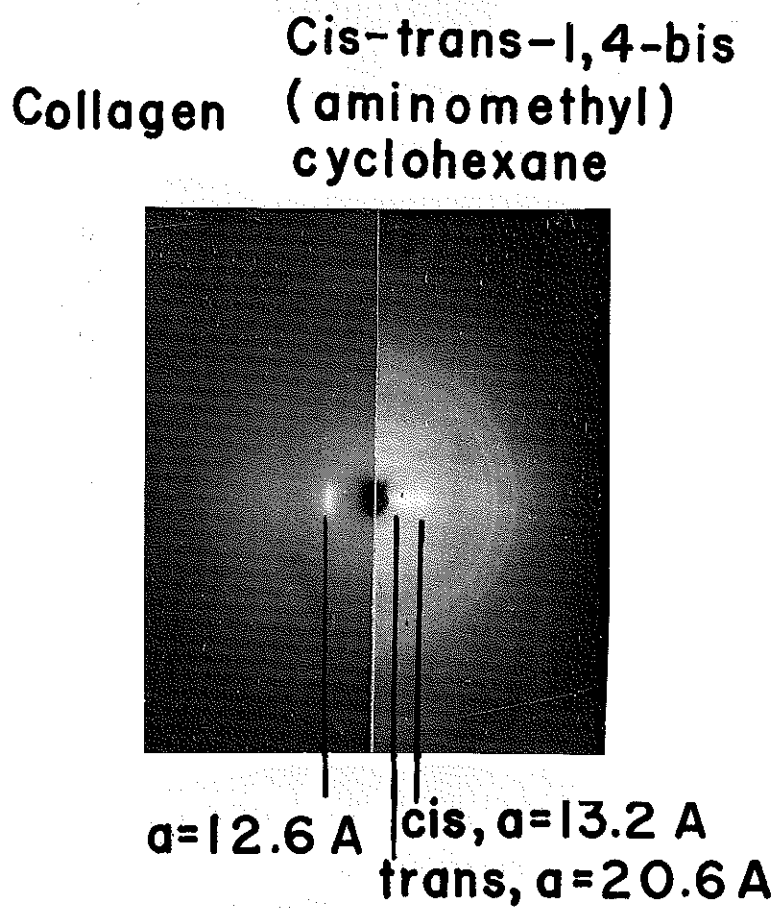

Fig. 1. X-ray diffraction pattern of cis-trans-1, 4-bis (aminomethyl) cyclohepane in collagen.

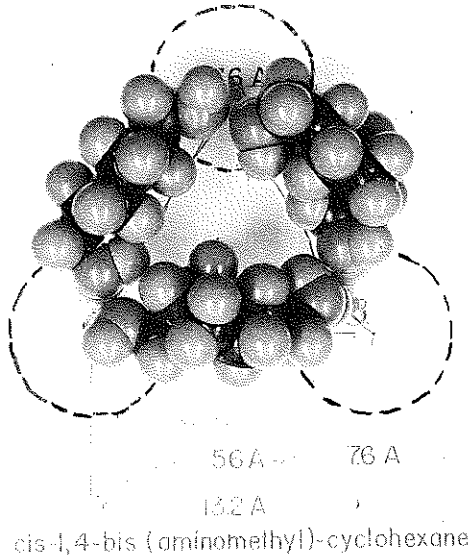

Fig. 2. Model of Cis-1,4-bis (aminomethyl)-cyclohexane in collagen.

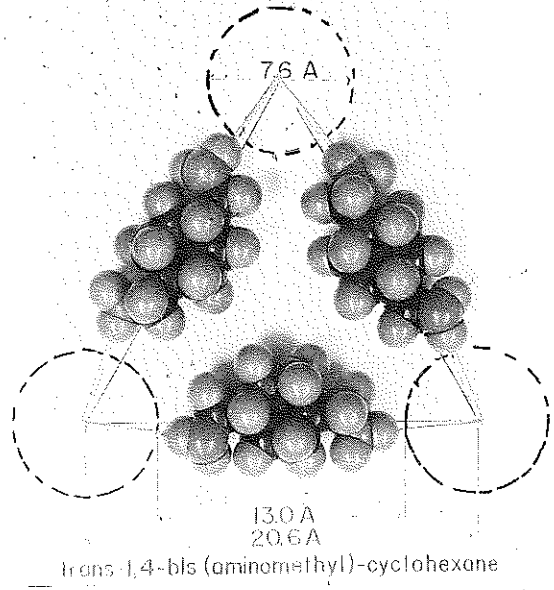

Fig. 3. Model of trans-1,4-bis (aminomethyl)- cyclohexane in collagen.