Molecular weight Heterogeneity of the α-chain sub-units of collagen

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The α-chain sub-units of rat and bovine collagen, previously believed to have the same molecular weights are shown to exhibit different mobilities on SDS-polyacrylamide gel electrophoresis corresponding to a molecular weight difference of about 6,000 Daltons. Comparison with the mobilities of proteins of accurately known molecular weight gave approximate molecular weights for the α 1 and α 2 chains in the expected region of 100,000.

Further heterogeneity in the molecular weight of the α components of other species is also apparent using this technique, the α 1 components of cod skin and dog fish skin having a molecular weight intermediate between mammalian α 1 and α 2.

It has been clearly demonstrated that the collagen molecule possesses a triple helical structure, both in the fibrous state and in solution, each of the three polypeptide chains having a polyproline type helix and the three helices wound together in a super helix (Ramachandran, 1968). Further studies have shown that most mammalian collagen contain two polypeptide chains (α components) that are chemically identical (α 1); whilst the third (α 2), although basically similar, is sufficiently different to permit separation by carboxymethyl cellulose chromatography (Piez, 1968). Some exceptions to this general structure have been reported; cod skin contains three non-identical α-chains (Piex, 1965) whilst mammalian cartilage (Miller and Matukas, 1970) and some primitive animals, such as the sea anemone (Pikkarainen et al., 1965; Nordwig and Hayduk, 1969) contain a single type of α-chain. Examination of the denatured form of acid-extracted collagen revealed, in addition to the single α chains, the presence of higher molecular weight components derived from crosslinking of the α chains, giving rise to α chain dimers (β components) and trimers (γ components) (Piez, 1968).

Molecular weight determinations of the isolated α 1 and α 2 components by equilibrium sedimentation showed them both to possess molecular weights of 98,000 ± 5,000 (Lewis and Piez, 1964). This molecular weight was supported by the compositional analysis of both the intact chain and the additive value of the molecular weights of the CNBr peptides derived from the α chain (Piez et al., 1968). The β components were shown to have molecular weights of 196,000 ± 10,000 (Lewis and Piez, 1964). The additive molecular weights of the three α chains agreed well with the values around 300,000 obtained for the intact tropocollagen molecule (Rice et al., 1964; Davison and Drake, 1966).

At the present time a variety of techniques are available for the determination of molecular weights of purified proteins: perhaps the most accurate being sedimentation equilibrium. Recent studies suggest that acrylamide gel electrophoresis incorporating the anionic detergent sodium dodecyl sulphate may become at least as accurate a method (Shapiro et al., 1967; Weber and Osborn, 1969), but more importantly in the present studies it is extremely effective in demonstrating small differences in molecular weight of components in a mixture. In this communication we report the application of SDS-acrylamide electrophoresis to the analysis of the subunits of collagen.

Methods and Results

Preparation of soluble collagen. Intact collagen fibres were obtained from rat tail tendon, calf achilles tendon, cod skin, dog fish skin, by cleaning the tissue free from adhering fat and muscular tissue, shredding in an MSE Ato-Mix homogenizer and washing with copious amounts of 0.9% NaCl, pH 7.4. Tropocollagen was extracted from all these tissues with 0.05% acetic acid and purified by precipitation with 0.9% NaCl, dissolved in acetic acid, centrifuged and the process repeated three times.

Isolation of α and β components. The components of denatured purified rat tail tendon and calf achilles tendon collagen were separated and isolated on CM-cellulose (Whatman CM-52) by using the procedure described by Piez et al., (1963).

SDS-acrylamide gel electrophoresis. The protein samples were incubated with 1% sodium dodecyl sulphate - 1% mercaptoethanol for 2 hr. at 37°, transfered to slots cut in a slab of 8% polyacrylamide gel, and the electrophoresis of the samples (10) was carried out for 2½ hr. at room temperature (4ma/cm^2). Both gel and electrode buffers were tris (50 mM) : boric acid (30 mM) buffer at pH 8.5 (cf. Koenig et al., 1970). The slab was sliced to remove the top and bottom faces and the centre section stained with Coomassie Blue (0.5% solution in 40% methanol : acetic acid (1M)) for 16 hrs. and then washed with the methanol:acetic acid until clear. A typical electrophoretogram is shown in Fig. 1.

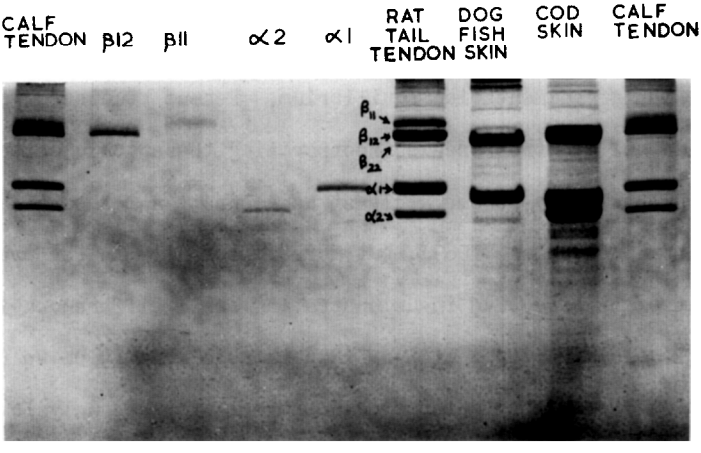

Fig. 1 Separation of the polypeptide chain sub-units of collagen by acrylamide gel electrophoresis in the presence of sodium dodecyl sulphate.

Molecular weight calibration. Proteins of well characterized sub-unit molecular weight were utilized as standards for calibration:- phosphorylase (92,500) pyruvate kinase (57,000) creatine kinase (41,000) triose phosphate isomerase (27,000) and myoglobin (17,200). A linear plot of the mobility against the log of the molecular weight similar to that of Weber and Osborn (1969) was obtained. The molecular weights of the α and β components of the collagen were then calculated from their mobilities using the calibration plot.

Discussion

It might have been anticipated that the application of the SDS-acrylamide technique to denatured collagen would reveal two primary bands, one due to the two α components, believed to be of identical molecular weight, and a second band due to the two β components. However, during examination of extracts from lathyritic porcine aorta by this technique an apparent separation of the two α components of collagen was observed. Analysis of purified collagen from other sources confirmed the identification of these bands as collagen sub-units and the separation of both α components. The ratio of the intensities of the bands indicated the component of highest mobility to be the α 2 chain, and this was confirmed by comparison with α 2 isolated from CM-cellulose columns. Similarly β_{12} had a higher mobility than β_{11} and their identities were also confirmed by comparison with isolated β components.

The electrophoretic mobility of SDS-protein complexes of some forty proteins has been shown to be proportional to the polypeptide chain molecular weight (Weber and Osborn, 1969). All proteins examined to date, from the highly basic histones to highly acidic proteins bind identical amounts of SDS and adopt similar conformations (Reynolds and Tanford, 1970), and under these conditions all protein specificity is lost and the mobility in the gel is a measure of the molecular size.

The α 1 and α 2 chains being so similar chemically are unlikely to bind different amounts of SDS, nor would it be expected that the SDS-protein complexes adopt different conformations. If then the difference in mobility of the α components is not due to conformational anomalies it would therefore appear to be due to a difference in molecular weight of the α 1 and α 2 chains. This dissimilarity of the α chains is again reflected in the mobilities of the β_{11} and β_{12} components (Fig.1).

From the calibration curve the α_2 chain was calculated to be smaller than the α 1 chain by about 6,000 Daltons. Equilibrium sedimentation

carried out independently on isolated $\alpha 1$ and $\alpha 2$ solutions (Lewis and Piez, 1964) would be unlikely to reveal a difference of 6,000 in 100,000 with any certainty. Using the gel technique, however, the $\alpha 1$ and $\alpha 2$ components are directly comparable.

To lend support to our contention that the variation in mobilities was not due to compositional differences resulting in the two chains adopting alternative conformations on reaction with SDS, we examined a number of α components from other phyla known to have widely divergent chemical compositions, particularly in their imino acid content.

Two α components of cod skin were again clearly separated, the $\alpha 2$ chain having approximately the same molecular weight as mammalian $\alpha 2$ but the '$\alpha 1$' component had a molecular weight intermediate between those of mammalian $\alpha 1$ and $\alpha 2$. No third α component could be detected, presumably the '$\alpha 1$' band contains two α components of identical molecular weight ($\alpha 1$ and $\alpha 3$). Dog fish skin collagen apparently contained a single component but isolated dog fish $\alpha 1$ and $\alpha 2$ were found to have identical mobilities whilst the mammalian α's clearly separate, thus supporting the proposal that the higher mobility of the $\alpha 2$ chain is indeed due to a lower molecular weight rather than a conformational difference.

Although the theoretical aspects of SDS gel electrophoresis are not clearly understood and it may be that some unforeseen anomalies occur with collagen, we feel that the use of this technique has indicated a difference in the molecular weights of the $\alpha 1$ and $\alpha 2$ chains of mammalian collagen. In addition we have inferred a heterogeneity of the molecular weights of the α chains of different phyla and application of the technique to a wider range of collagen may reveal further divergences.

The significance of the molecular weight difference of the α chains is not at present clear, but the presence of a shortened $\alpha 2$ chain must have important consequences in the fibrogenesis and crosslinking of the collagen molecules.

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