COLLAGEN COMPOSITION IN THE DUCTAL
INFILTRATING CARCINOMA OF HUMAN BREAST

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ABSTRACT
   Ductal infiltrating carcinoma of the breast is character-
ized by a remarkable amount of collagen fibrils surrounding
nests and cords of neoplastic cells. Sequential extractions
with pepsin release three classes of intact collagen chains,
which have been identified as α_1(I), α_2(I) and α_1(III) types.
   Alpha$_1$(I) is prominent among these classes.

INTRODUCTION
   Much evidence in the past few years has shown that colla-
gen is the product of one or probably several multigene
families. So far, at least nine different collagen chains
have been detected in mammals, with some degree of tissue
specifity. In normal conditions mesenchymal cells in differ-
ent connective tissues are responsive to regulatory mechan-
isms that control both the genetic type of collagen and the
amount of each chain synthesized. At present, little is known
about extracellular matrix of invasive tumours. In the ductal
infiltrating ("scirrhous") carcinoma of human breast the
stroma is deeply modified and has been the object of histol-
ogical and ultrastructural investigations (Lundmark, 1972;
Azzopardi and Laurini, 1974; Tremblay, 1976). So far, however,
nothing is known about its molecular composition and organ-
ization. In a first approach to this problem, we have under-
taken the characterization of collagen chains by means of
SDS-polyacrylamide gel electrophoresis, CM-cellulose chrom-
atography and amino acid analysis.

MATERIAL AND METHODS
   Fragments of tissue (from 1 to 1.5g) were obtained from
surgical specimens of human mammary carcinoma having hist-
ological properties of "scirrhous" type. Minced samples were
rinsed for 24hrs at 4°C with several changes of 0.05M Tris/
HCl, pH 7.4, 0.15M NaCl, in order to remove soluble non-colla-

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genous proteins. Collagen was extracted three times by
mild pepsin digestion as already described (Minafra et al.
1982). SDS-PAGE was performed in polyacrylamide gels (5%
acrylamide,0.13%bisacrylamide) containing 0.1% SDS and
4 M urea. Interrupted gel electrophoresis was performed
essentially as described by Sykes et al.(1976). Stained
gels were scanned at 560 nm in their linear range.

   For CM-cellulose chromatography 10-15 mg of lyophilized
collagen was solubilized and denatured at 42°C for 20' in
0.016 M K-acetate buffer,pH 4.8, containing 4 M urea.The
same was used as starting buffer on (cm 1x10) columns of
CMC (Whatman CM 32) packed and equilibrated at 42°C.
Collagen was eluted from the column with a linear gradi-
ent 0.-0.1 M NaCl,at 36 ml/hr in a total volume of 200 ml.

   Amino acid analysis was performed on a Carlo Erba Anal-
yzer by a two column system of sulfonated resins.

RESULTS

   Routine electron microscopy shows that mammary carci-
noma interstitium is mainly occupied by an atypically
increased amount of collagen fibrils surrounding cords
and nests of neoplastic cells. Details on the structural
aspects of collagen organization will be given separately.

   Sequential extraction with pepsin solubilizes a relevant
amount of intact collagen molecules from the tissue,rang-
ing from 50% to 75% in the different samples observed.

   A quantitative estimation of collagen chains released
by the pepsin digestion was performed by means of densit-
ometric scan of the SDS-PAGEs. Figure 1A shows the electro-
phoretic pattern of unfractionated collagen mixture,per-
formed with one hour interruption and "in situ" reduction
with mercaptoethanol. About 60% of the material applied
on the gel migrates as monomers; the remaining material
consists of dimers and trimers in the proportion of 18%
and 22% respectively. Under the reducing conditions de-
scribed, three classes of monomers are clearly resolved.

   More than 70% monomers are α_1(I) type chains,while α_2(I)
chains represent 12-13%(α_1/α_2 ratio being between 4 and
6 in different samples). The slow moving band before α_1(I)
displays electrophoretic mobility of α_1(III) and repres-
ents 12-15% of the total monomers. Supporting evidence
for the identification of this component was obtained by
comparing electrophoretic migration with and without "in
situ" reduction with mercaptoethanol. In the latter condi-

tion α_1 (III) chains are released in minimal amounts from
trimers and are barely separated from α_1 (I) chains (Sykes
et al. 1977). Figure 1B shows a densitometric scan of a
gel electrophoresis in non-reducing conditions, in which
the γ components represent about 38%, and α_1 (III) chains
appear as a small shoulder of α_1 (I). The α_1 (I) / α_2 ratio
does not appear modified.

Since the α_1 / α_2 ratio expected for normal type I col-
lagen is 2:1, the excess of α_1 (I) chains may reflect an
increased rate of α_1 (I) synthesis or an incomplete release
of α_2 from extensively cross-linked β_{12} dimers. If α_1 (I)
are assembled as homotrimers, they should be separated by
differential salt precipitation from normal type I mole-
cules. Collagen with chain composition α_1 (I)$_3$ is apparent-
ly found in minimal amounts in normal connective tissues
of adult mammals, while it has been detected in significant
proportions in embryonic and foetal tissues, in manipulat-
ed cell cultures and in some diseased tissues (Bornstein
and Sage, 1980, for review). Mayne et al. (1975) reported
that homotrimer has a greater solubility in salt solution
at neutral pH than normal type I collagen. However other
authors have reported that conditions allowing a complete
separation of homotrimer from type I, by means of salt
fractionation, are difficult to obtain (Narayanan and Page,
1976; Benja et al. 1977; Wohllebe and Carmichael, 1978).

We have tested the salting out behaviour of the carci-
noma collagen in neutral solvent by increasing NaCl concent-
ration from 0.4 M to 5.0 M. Preliminary results indicate
that the largest fraction containing almost exclusively
α_1 (I) and β_{11} dimers is obtained at low NaCl concentration
(between 0.4 and 0.6 M). When this fraction is removed by
centrifugation, the fraction which precipitates at 2.5 M
comprises normal type I and type III molecules (figures
2A and 2B).

In order to further characterize the α_1 (I) chains of "scir-
rhous" carcinoma an enriched fraction was purified by CM -
cellulose chromatography and the top of each peak was de-
salted and electrophoresed in parallel with calf skin type
I collagen (figg.3 and 4). The first chromatographic peak
comprises only one class of α_1 chains correspondent to the
α_1 of type I collagen; the intermediate peak contains dimers
and trimers, and the third a small amount of α_2 chains.

The amino acid analysis of CMC purified α_1 (I) chains
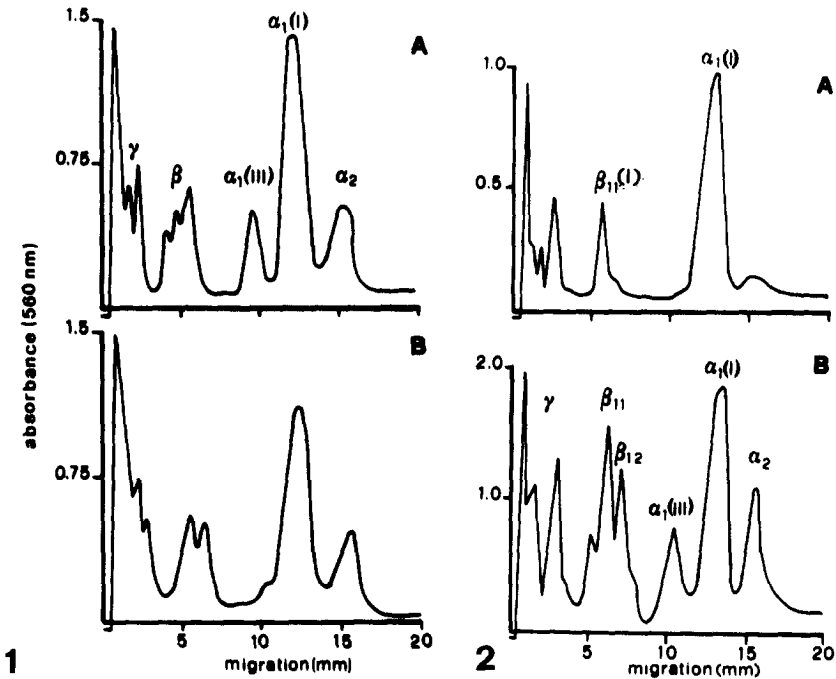shows a close similarity with correspondent chains of type I

Fig.1A. Densitometric scan of interrupted SDS-PAGE of to-
tal unfractionated collagen. Trimers and dimers repre-
sent 40% and monomers 60%; α_1(I): α_2 ratio is about 4;
α_1(III) chains are about 12% of total monomers. The posi-
tion of each chain was marked with standard collagen.
Fig.1B. SDS-PAGE of same material performed without "in
situ" reduction. α_1(III) chains are not completely relea-
sed from trimers and appear as a small shoulder of α_1(I).
Fig.2A. Collagen fraction precipitated at 0.6 M NaCl (pH
7.5). The amount of α_1(I) plus β_{11}(I) represents about
80% of the material applied on the gel; trimers are 18-
19%; α_2 is below the limit of a reliable quantitation.
Fig.2B. 2.5 M NaCl fraction precipitated from 0.6 M super-
natant. α_1(I): α_2 ratio is close to 2; α_1(III) are about
11% of total monomers.

collagen (Table I), with a small increase of lysyl hydro-
xylation with respect to type I adult collagen, and a
higher amount of hydrophobic residues (especially alani-
ne and proline) if compared with placental type I chains.
  The microheterogeneity in primary structure detected
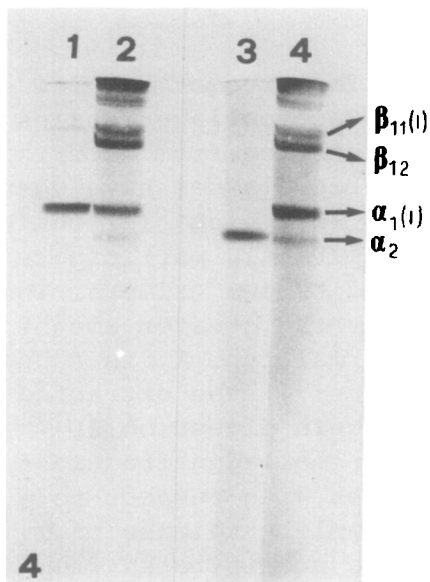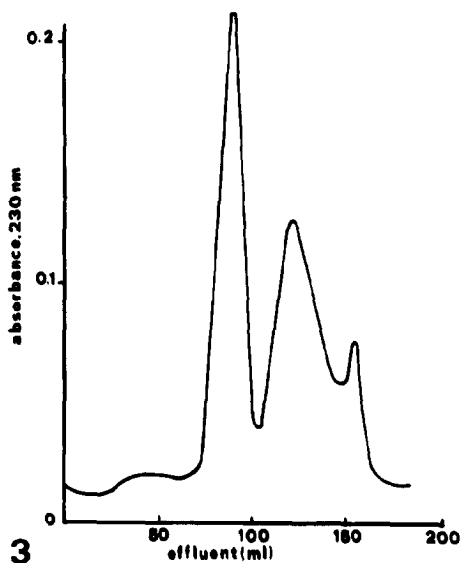in collagen α_1(I) chains of carcinoma with respect to

Fig.3. CM-cellulose chromatogram of homotrimer enriched fraction. First peak contains only one class of α_1 chains, intermediate peak comprises β and γ components and third peak α_2 chains.

Fig.4. Interrupted SDS-PAGE of CMC peak 1 (tube 1) and peak 3 (tube 3) run in parallel with type I calf skin collagen (tube 2 and 4).

typical type I chains, may enhance charge differences between molecules which contain or lack α_2 chains, leading to a different behaviour in solution.

TABLE I - Aminoacid composition of α_1 (I) chains expressed as residues / 1000

3-hydroxyproline	n.d.	valine	15
4-hydroxyproline	102	methionine	4
aspartic ac.	43	isoleucine	8
threonine	16.5	leucine	19
serine	35	tyrosine	2
glutamic ac.	79	phenylalanine	10
proline	134.5	hydroxylysine	9
glycine	332	lysine	26
alanine	120	histidine	3
half-cystine	–	arginine	46

DISCUSSION

The present results demonstrate that three classes of intact collagen chains are released by mild pepsin digestion from "scirrhous" carcinoma of human breast.The three classes have been identified as α_1(I), α_1(III)and α_2(I). Alpha$_1$(I) chains are the major component and they apparently participate in the formation of both type I and type I trimers. The hypothesis that homotrimer accounts for the greater amount of α_1(I) than that required for normal type I, is supported by the following considerations: 1) the extracted material was resistent to limited pepsin digestion;2)the amount of β_{12} dimers in the different collagen fractions analyzed was never in so large excess with respect to β_{11} dimers, as to justify an incomplete release of α_2 chains; 3) only native molecules are precipitated from salt solution and at no time during collagen preparation was the material exposed to conditions facilitating the "in vitro" renaturation of any randomly coiled chains.

Such collagen composition appears atypical for mammary gland interstitium. Recently Wakimoto and Oka (1983) have shown that type I and type III collagen are the major species which accumulate during the differentiation of the mammary gland in organ culture. The production of a collagen species which is considered more "primitive" in the tumour may reflect an alteration of the mechanisms which regulate the amount and the kind of collagen produced by cells. If the fibroblasts induced by some carcinogenic factor, or the neoplastic cells themselves, are responsible for the extra amount of collagen present in the stroma of scirrhous carcina, is a question of remarkable biological interest which requires further investigation.

REFERENCES

Azzopardi,J.G. and Laurini,R.N.(1974) Elastosis in breast cancer. Cancer,33,174-183

Benja,P.D.,Padilla,S.R. and Nimni,M.E.(1977) The progeny of rabbit articular chondrocytes synthesize collagen types I and III and type I trimer, but not type II. Biochemistry,16,865-872

Bornstein,P. and Sage H.(1980) Structurally distinct collagen types. Ann.Rev.Biochem.,49,957-1003

Lundmark,C.(1972) Breast cancer and elastosis. Cancer,30, 1195-1201

Mayne,R.,Vail,M.S. and Miller,J. (1975)Analysis of changes
   in collagen biosynthesis that occur when chick chondrocy-
   tes are grown in 5'-bromo-2'-deoxyuridine. Proc.Nat.Acad.
   Sci.USA, 72,4511-4515

Minafra,S.,Pucci Minafra,I.,Sciortino,F.,Tomasino,R.M.(1982)
   Characterization of collagen from scirrhous carcinoma of
   the breast. Abstr.VIIIth Meeting of the Federation of Eur.
   Conn.Tissue Soc.p. 138

Narayanan,A.S. and Page,R.C. (1976) Byochemical characteri-
   zation of collagens synthesized by fibroblats derived from
   normal and diseased human gingiva. J.Biol.Chem.,251,5464-7

Sykes,B., Puddle,B.,Francis,M. and Smith,R. (1976) The esti-
   mation of two collagens from human dermis by interrupted
   gel electrophoresis. Biochem.Biophis.Res.Commun.,72,1472-8

Tremblay,G. (1976) Ultrastructure of elastosis ib scirrhous
   carcinoma of the breast. Cancer,37,307-316

Wakimoto,H. and Oka,T. (1983) Involvement of collagen forma-
   tion in the hormonally induced functional differentiation
   of mouse mammary gland in organ culture. J. Biol.Chem.,258
   3775-79

Wohllebe,M. and Carmichael,D.J. (1978) Type I trimer and ty-
   pe I collagen in neutral salt soluble lathyritic rat denti-
   ne. Eur.J.Biochem.,92,183-188