Journal of Chromatography, 433 (1988) 81–94
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4405

QUANTITATIVE EVALUATION OF COLLAGEN FRACTIONS SEPARATED BY ACRYLAMIDE GEL ELECTROPHORESIS: ITS APPLICATION FOR COLLAGEN TYPING IN NORMAL AND PATHOLOGICAL TISSUES

G. BELLON* and J.P. BOREL

Laboratory of Biochemistry, URA 84 CNRS, Faculty of Medicine, 51 Rue Cognacq Jay, F 51095 Reims (France)

(First received June 21st, 1988; revised manuscript received July 27th, 1988)

SUMMARY

A method for the quantitation of collagen chains or cyanogen bromide peptides separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis is described. After electrophoresis, the bands, slightly stained by Coomassie brilliant blue, are cut and hydrolysed in 6 *M* hydrochloric acid. Proline and hydroxyproline are measured by a fluorometric procedure after thin-layer separation. When the method is applied to the fractions solubilized by pepsin digestion, it provides a measurement of type I, III, IV and V collagens. When it is applied to cyanogen bromide peptides, its permits the calculation of the proportions of type I and III collagens. Applied to polycystic kidney, this method indicates a significant increase of type I and a limited increase of type IV collagen in this abnormal tissue compared with normal kidney.

INTRODUCTION

The quantitation of the various types of collagen in tissues is made difficult by two different drawbacks: (i) ensuring complete extraction of the collagens and (ii) staining and scanning the acrylamide gels when the separation is performed by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE).

The second problem has been tackled in many ways. Goldberg and Fuller [1] recommended 2-methoxy-2,4-diphenyl-3(2*H*)-furanone (MDPF), a fluorescent marker for proteins; Laurent et al [2], Hanson and Bentley [3], Kirk et al. [4] and Chan and Cole [5] used Coomassie brilliant blue R 250 to stain specific peptides; Szymanowicz et al. [6] described a semi-quantitative method for typing collagen in human arteries using Coomassie brilliant blue G 250 and Armendariz-Borunda and Rojkind [7] described a quantitative method for collagen typing in human liver, based on the densitometric analysis of pepsin-solubilized collagen

0378-4347/88/$03.50

stained with Sirius red. However, in all these methods, the results are largely dependent on the molar intensity of the colour obtained with peptides or proteins, on losses sustained during staining and destaining procedures [3,8], on the time of incubation with the dye, on the temperature used for destaining [9] and in particular on the fidelity of PAGE separation.

In our laboratory, we set up a sensitive and reproducible method for the evaluation of picomole amounts of proline (Pro) and hydroxyproline (Hyp) isomers by derivatization of these amino acids with a fluorogenic compound, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), followed by thin-layer chromatography (TLC) of the fluorescent derivatives [10–12]. We have applied this fluorometric technique to the quantitation of Pro and Hyp liberated by hydrochloric acid hydrolysis from every fraction of collagen separated by SDS-PAGE. We have used the method for quantitating α-chains and/or CB-peptides obtained from various preparations of purified collagens and from extracts of several organs. This method was found to be far more reliable than those using the quantitation of dyes bound to collagen in PAGE.

EXPERIMENTAL

Chemicals and materials

All chemicals were of analytical grade and, if their origin is not specifically mentioned, were purchased from Prolabo (Paris, France). Triethylamine, hydrochloric acid and cyanogen bromide (CNBr) were bought from Merck (Darmstadt, F.R.G.). NBD-Cl was purchased from Aldrich (Beerse, Belgium) and purified according to the method of Bisker et al. [10]. *o*-Phthalaldehyde and 2-mercaptoethanol were obtained from Sigma (St. Louis, MO, U.S.A.). Pro and 4-Hyp were from Calbiochem (San Diego, CA, U.S.A.). 3-Hyp was prepared in the laboratory [13]. SDS, acrylamide, methylene bisacrylamide and tetramethylenediamine (TEMED) were purchased from BDH (Poole, U.K.). Coomassie brilliant blue G 250 was bought from Serva (Paris, France). L-[U-^{14}C]Pro (260 mCi/mmol) was obtained from CEA (Saclay, France). TLC was performed on 20 cm$\times$20 cm silica gel plates (thickness 0.2 mm), layered on a glass sheet for fluorometric determinations (Merck No. 5721) and on an aluminium foil for radioactivity measurements (Merck No. 5553). Pepsin A grade (2500 U/mg) was bought from Millipore (Freehold, NJ, U.S.A.). Normal human kidneys were obtained at autopsy carried out within 12 h after death. Three human polycystic kidneys were obtained following surgical interventions. The material was stored at $-80°$C until the time of analysis.

Preparation of collagen standards

Collagen standards were prepared from the chorio-amniotic membranes of human placenta, according to the usual techniques of pepsinization [14–16]. Calf skin type V collagen was prepared according to Laurain et al. [17]. Type I collagen from lathyritic rat skin was kindldy provided by Dr. R.W. Glanville (Max Planck Institut, Munich, F.R.G.).

Type I collagen was labelled with [^{14}C]Pro and prepared from nine-day-old chick embryo according to Peterkofsky and Diegelmann [18].

Tissue preparations

Human placenta. The chorio-allantoic membranes of human placentas were treated by three steps of 0.5% (w/w) pepsin digestion. After centrifugation at 10 000 *g*, the supernatants were pooled and a crude mixture of collagen was precipitated from the 0.5 *M* acetic acid solution by addition of sodium chloride up to 1.2 *M*. The precipitate was dialysed against distilled water and lyophilized.

Calf skin and human kidney samples. Samples of gram amounts of these tissues were homogenized in a meat grinder, lyophilized and treated by limited pepsin digestion according to the same methods as human placenta.

Hydroxyproline evaluation in tissues

Samples of tissues or aliquots of acidic extracts were submitted to hydrolysis by 6 *M* hydrochloric acid, and their Hyp content was analysed as described below, in order to evaluate the respective amounts of total and solubilized collagen.

Cyanogen bromide digestion

Lyophilized samples of tissue of purified collagen were digested by CNBr according to ref. 19.

Acrylamide gel electrophoresis

SDS-PAGE of collagens was performed in cylindrical tubes with a stacking gel of 3.5% and a separation gel of 5% acrylamide in 0.05 *M* phosphate buffer (pH 7.2) containing 0.1% SDS, according to Hayashi and Nagai [20]. Electrophoresis of CNBr peptides was performed in gels of 12.5% acrylamide in Tris–HCl buffer containing 0.1% SDS as described by Laemmli [21]. After electrophoresis, the gels were stained using Coomassie brilliant blue G 250 for 30 min and destained in 10% acetic acid solution for two periods of 24 h each.

Hydroxyproline and proline quantitation

Pro and Hyp contained in the fractions separated by SDS-PAGE and localized by Coomassie brilliant blue staining were evaluated by a modification of a fluorometric technique already described [10]. The gels were sliced with a razor blade, and every stained zone was transferred to a test-tube to which 1 ml of 6 *M* hydrochloric acid was added. The tubes were sealed and hydrolysed for 24 h at 106 °C. The hydrolysates were evaporated to dryness under a stream of nitrogen. The residues were dissolved in distilled water at 65 °C and then passed through a 6.4 cm × 1.1 cm I.D. column of Dowex 50W-X2 (Bio-Rad) in the H^+ cycle. The amino acids were bound in these conditions and acrylamide was removed. The amino acids were then eluted by 15 ml of 2 *M* ammonium hydroxide solution, which was evaporated under a stream of nitrogen. The residue was treated by a NBD-Cl solution and chromatographed by TLC as described previously [10–12]. The plates were developed with the solvent system, acetone–toluene–methanol–triethylamine (40:40:15:5, v/v). The fluorescent spots were scanned in a Farrand spec-

trofluorometer equipped with a thin-layer attachment. Fluorescence was linear over the concentration range 2–400 pmol.

In the case of evaluation of radioactive [^{14}C]Pro and [^{14}C]Hyp, thin layers fixed to aluminium sheets were used: the spots were cut under UV light and counted in a Packard Tricarb scintillation counter Model 3400.

Statistical analysis

The significance of the results was tested by Student's t-test and linear regression analysis [22].

RESULTS

Patterns of collagen separation

Under the conditions we used for PAGE, we consistently obtained the patterns shown in Fig. 1A. The identity of each band was established with several control preparations of collagens (Fig. 1B). The α fraction contained three different α bands. The fastest, designed as $\alpha 2$, contained $\alpha 2$(I) chains. The following one, designed as $\alpha 1$, contained $\alpha 1$(I) and $\alpha 2$(V), if not reduced, as checked with samples of purified collagens. If the samples had been reduced prior to electrophoresis, the band $\alpha 1$ contained in addition $\alpha 1$(III) and the 100 000 (IV) component. The third one, designed as αB, contained $\alpha 1$(V) and $\alpha 3$(V) (if present in tissue), and possibly the 140 000 component of type IV collagen. There were β bands, and several γ bands that were collectively designated the γ fraction.

Reliability of the method

In order to avoid misleading calculations based on the amount of Hyp in the collagen bands when collagen is under-hydroxylated, we measured in all experiments the sum of the concentrations of Pro and Hyp. We deposited known amounts of purified type I collagen and measured the amount of Pro+Hyp in every band. The total amount recovered in six different experiments averaged $94.6 \pm 5.1\%$ of the amount of Pro+Hyp in the deposited collagen. The smallest amount of Pro and Hyp that could be detected on a thin-layer plate, in the form of NBD derivatives, was 1 pmol [10]. This value corresponded to ca. 1 ng of collagen. Considering the sensitivity of Coomassie brilliant blue staining and the dilution introduced between the acrylamide gel stage and the fluorometric measurements, it can be determined that the lowest amount of collagen that could be analysed on the gel was 1 μg. There was a linear relationship between the amount of Pro+Hyp in $\alpha 2$ and $\alpha 1$ bands and the amount of collagen deposited (Fig. 2). In order to verify further the reproducibility, we submitted several samples of labelled collagen to PAGE and measured in parallel the radioactivities of every band and their concentrations of Pro+Hyp. As shown in Table I, the reproducibility in the case of fluorometric measurements was very satisfactory with coefficients of variation between 2.7 and 6%, depending on the chains. As shown in Fig. 3, there was a linear relationship between radioactivity and the Pro+Hyp content in the bands. The equation of the curve, determined by linear regression

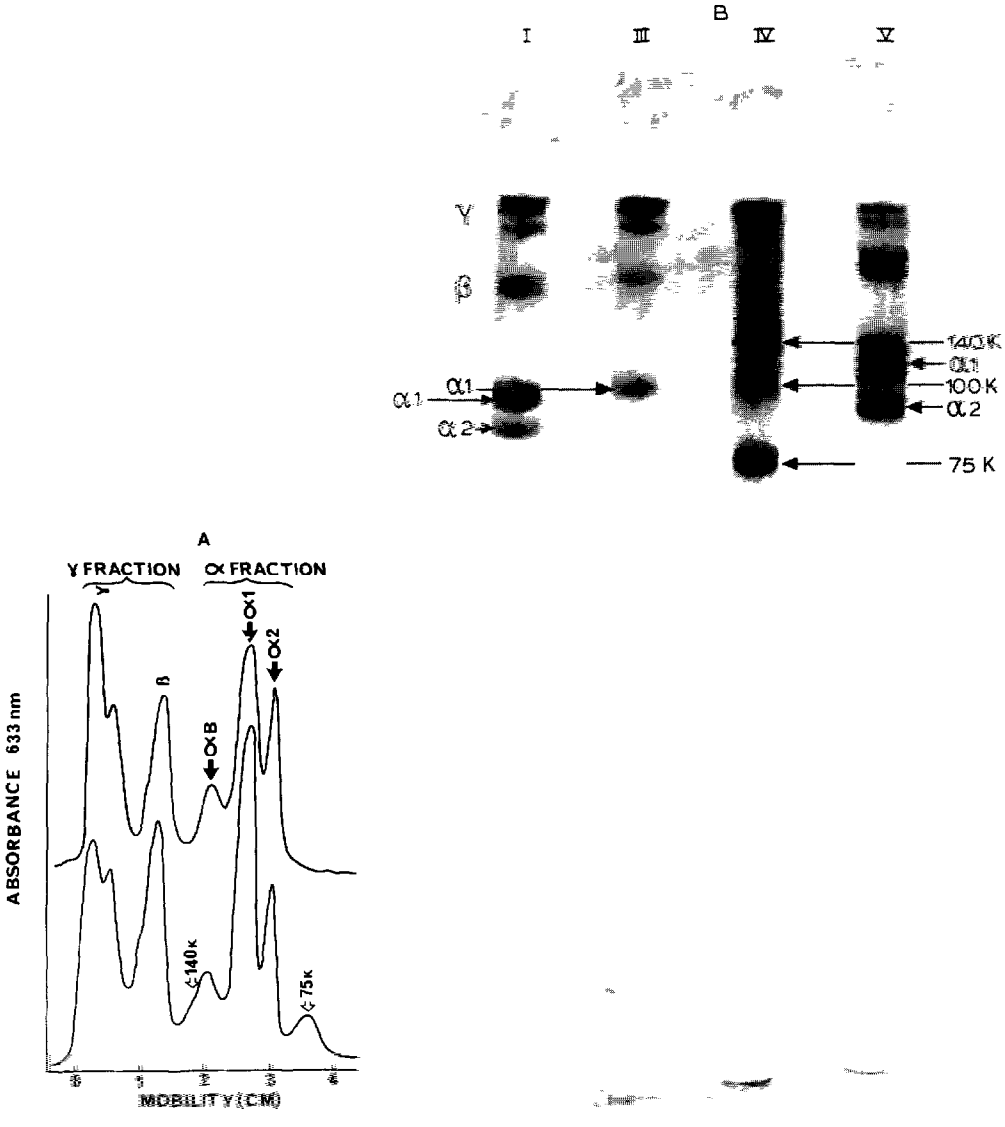

Fig. 1 (A) Densitometric profiles of Coomassie brilliant blue stained SDS-PAGE electropherogram obtained from pepsin digest of human placenta under unreduced conditions (upper profile) and reduced conditions (lower profile). (B) SDS-PAGE of purified type I, III, IV and V collagens from human placenta. Types III and IV were run after reduction by 2-mercaptoethanol. The positions of α chains are indicated by arrows.

analysis, was $y = 1.08x - 0.6$ with a correlation coefficient (r) of 0.996. The specific activity of Pro + Hyp was taken into account in the calculation.

Calculations of the proportions on purified samples of collagen

Table II shows the respective amounts of Pro + Hyp in the α, β and γ bands separated from six different preparations of purified collagens obtained by pepsin

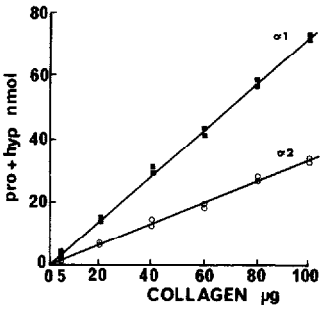

Fig. 2. Concentration of Pro + Hyp (expressed in nmol per band) found in the α chains separated by SDS-PAGE when the amount of deposited rat skin type I collagen varied from 5 to 100 μg.

TABLE I

RADIOACTIVITIES AND CONCENTRATIONS OF Pro AND Hyp HYDROLYZED FROM THE BANDS OBTAINED BY SDS-PAGE FROM A SAMPLE OF CHICK EMBRYO TYPE I [^{14}C]-COLLAGEN

Each value represents the mean ± S.D. of four determinations.

Chain	Radioactivity (cpm)		Amount (nmol)		Hydroxylation ratio* calculated from	
	Pro	Hyp	Pro	Hyp	Radioactivity	Amount
α1(I)	653±36	574±22	19.6±0.6	16.9±0.7	46.6	46.8
α2(I)	335±17	274±10	10.1±0.4	8.4±0.5	45.4	45.0
β	281±11	248±19	8.6±0.3	7.5±0.2	46.9	46.6
γ	248±12	216±11	7.4±0.2	6.6±0.2	46.5	47.1

* $\frac{\text{Hyp} \times 100}{\text{Pro} + \text{Hyp}}$

digestion. For each sample, the evaluations were performed on a non-reduced and on a reduced aliquot. Types I and III contained, on average, 65% of free α chains, and type V contained 85% of α chains.

Application to the evaluation of proportions of collagens in tissue samples

Table III shows the results obtained for several samples of tissues, compared with several mixtures of purified collagens deposited as controls in known proportions. Compared with normal human kidney, the proportion of type I collagen was significantly higher in polycystic kidney whereas that of type III collagen was lower. The significance of the results was $p < 0.05$.

The amounts of collagen types were calculated according to the following assumptions.

(1) The amount of type III was given by the difference in concentrations of Pro+Hyp in the α1 chain in gels obtained from reduced and unreduced samples.

(2) The amount of type V was calculated from the concentrations of Pro+Hyp

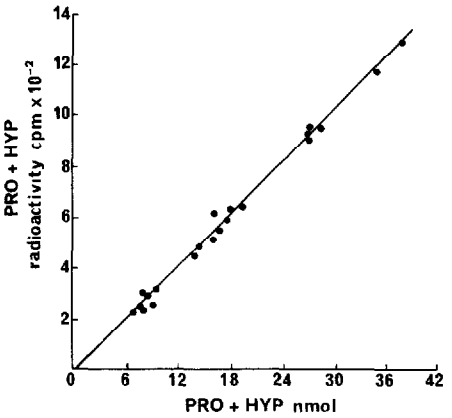

Fig. 3. Correlation between the concentration of Pro + Hyp and radioactivity in the α chains of ^{14}C-labelled type I collagen from chick embryo, separated by SDS-PAGE.

in the αB chain by assuming that there were two $\alpha1$(V) chains for one $\alpha2$(V) chain or either one $\alpha2$(V) or one $\alpha3$(V) chain for one $\alpha1$(V) chain.

(3) The amount of type I collagen was calculated from the concentration of Pro+Hyp in the $\alpha1$ band, obtained from unreduced preparations, by assuming that the $\alpha1$ band contained $\alpha1$(I) and $\alpha2$(V) and that the amount of $\alpha2$(V) was half that of αB bands. In these conditions, the concentration in $\alpha1$(I) was equal to $\alpha1-\frac{1}{2}\ \alpha$B and that of total type I collagen to $\frac{3}{2}\ (\alpha1-\frac{1}{2}\ \alpha$B). The concentrations of Pro+Hyp in the $\alpha2$ band were not used for the calculation, for reasons explained in the discussion. Fig. 4 shows that better correlations were obtained with our technique than with the densitometric method. By linear regression analysis we obtained with the fluorometric method the following equations: $y=0.99x+2.06$ ($r=0.998$) and $y=1.01x+0.22$ ($r=0.999$), respectively, for type I and V collagens, and with the densitometric method $y=1.16x+3.33$ ($r=0.996$) and $y=0.95x-3.68$ ($r=0.986$).

Characterization and evaluation of the proportions of various peptides by SDS-PAGE after CNBr treatment of collagen mixtures and tissues

CNBr digestion solubilized an average 95% of total collagen from tissues, such as kidney or placenta. Fig. 5A shows an SDS-PAGE densitometric scan of CNBr peptides from human placental collagen. The identity of every peak was established by comparison with CNBr peptides prepared from samples of pure collagen I, III, IV and V. After reduction, several low-molecular-mass peptides appeared whereas the amounts of larger ones decreased (Fig. 5B). When samples of tissues, such as normal or polycystic kidney or placenta, were submitted to direct CNBr digestion, it was not possible to detect the CNBr peptides originating from type V collagen. They were masked because the amounts of CNBr peptides formed from type I and III collagens were too high. In addition, many CNBr peptides, such as $\alpha1$(III)-CB-9, involved in the cross-linked domains, remained at the top of the gel with the high-molecular-mass material (Fig. 5C).

The proportions of type I and III collagens were deduced from the content in

TABLE II

EVALUATION OF Pro + Hyp IN BANDS SEPARATED BY SDS-PAGE OF PURIFIED COLLAGENS

The percentages of chains are calculated from the concentration of Pro + Hyp found in PAGE bands, assuming that the total of Pro + Hyp from α, β and γ chains is 100%.

Nature of purified collagen	Concentration of Pro + Hyp (nmol)						Percentage of α and $\beta+\gamma$ chains	
	75 000	140 000	$\alpha 2$	$\alpha 1$	β	γ	α	$\beta+\gamma$
Human placenta type I*	0	0	30.0 ± 1.4	65.7 ± 4.1	27.3 ± 1.2	24.4 ± 1.3	64.9	35.1
Rat skin type I	0	0	32.8	72.5	32.2	22.2	65.9	34.1
Human placenta type III*	0	0	0	84.7 ± 3.8	29.8 ± 1.3	14.2 ± 0.7	65.8	34.2
Human placenta type IV	35.1	66.3	0	0	0	0	-	-
Human placenta type V*	0	0	24.3 ± 1.1	52.3 ± 2.4	9.4 ± 0.3	4.1 ± 0.2	85	15
Calf skin type V	0	0	28.1	60.3	12.1	4	84.6	15.4

*Mean ± S.D. of four experiments.

TABLE III

CALCULATION OF THE PROPORTIONS OF COLLAGEN TYPES FROM THE CONCENTRATIONS OF Pro+Hyp FOUND IN α CHAINS SEPARATED BY SDS-PAGE FROM VARIOUS MIXTURES OF PURIFIED COLLAGENS AND FROM UNKNOWN SAMPLES OF TISSUES

Amount of collagen loaded on gels, 100 μg. Figures in brackets refer to the proportions of control collagens loaded on gels in the order I, III, V.

Deposited samples	Percentage of collagen types deduced from Pro+Hyp concentrations in α bands				Type I/type III ratio
	Type I	Type III	Type IV	Type V	
Control 1 (50:0:50)*	52.9±2.3	-	-	50.7±1.3	-
Control 2 (20:40:40)	18 8	38.6	-	40.9	0.49
Control 3 (60:20:20)	62.1	20.9	-	19.3	2.97
Control 4 (80:10:10)	81.3	10.5	-	11.3	7 74
Control 5 (90:5:5)	91.4	4.7	-	5.5	19.45
Human placenta*,**	51.5±1.2	34.1±1.6	3.2±0.5	10.5±1.0	1.51
Calf skin*,**	92.8±0.9	7.2±0.9	Not detected	Traces	12.89
Normal human kidney*,**	44.2±2.1	39.6±1.9	13 3±0.5	4.9±0.6	1.12
Polycystic kidney*,**	55.8±4.2	24.1±5.9	14.9±1.1	5.2±0 8	2.31

*Mean ± S.D. of four experiments.

**Total collagen is each gel is assumed to be 100%, and the different collagen types are calculated as relative percentages.

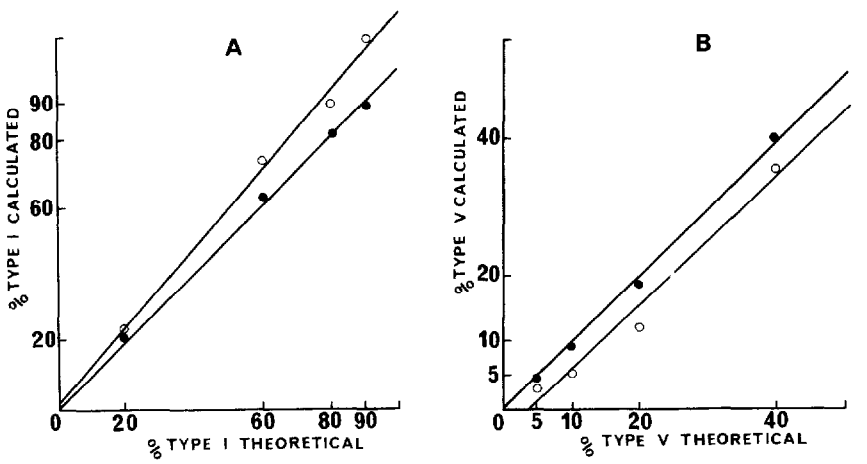

Fig. 4. Correlation between the percentage of collagen calculated from the densitometric (○) and the fluorometric (●) techniques and the percentage of collagen loaded on gels: (A) type I; (B) type V. The densitometric method was performed with an LKB 2202 Ultroscan laser densitometer on gel stained with Coomassie brilliant blue G 250, at 633 nm.

Hyp of the bands of two well separated CNBr peptides: α1(I)-CB-8 and α1(III)-CB-5. Two α1(I)-CB-8 peptides obtained from every type I molecule contained 29 Hyp residues each. An amount of 58 nmol of Hyp found in this peptide corresponded to 1 nmol of type I collagen in the initial mixture. Three α1(III)-CB-

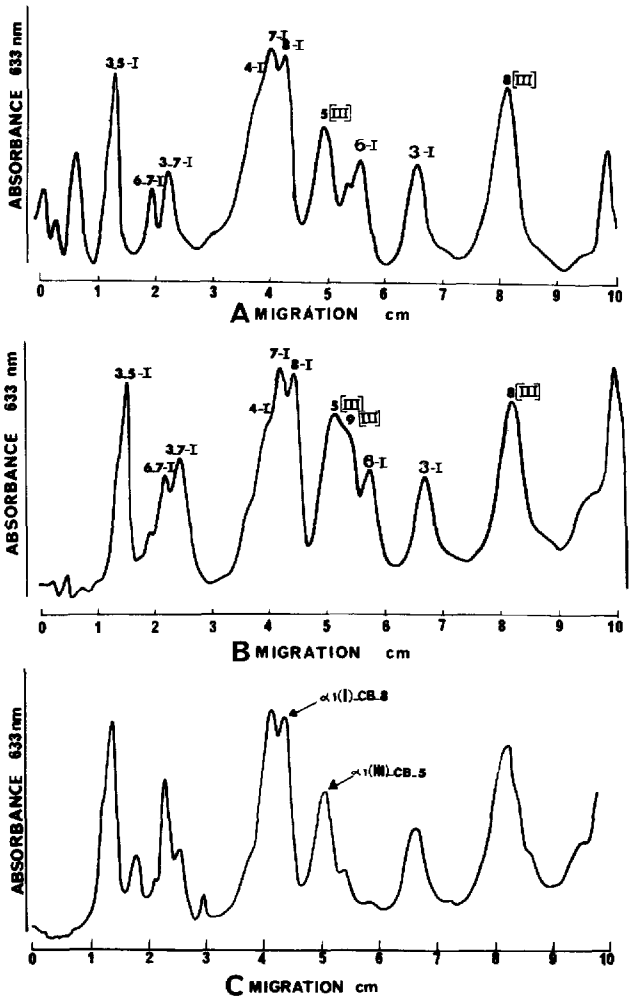

Fig. 5. Densitometric profiles of Coomassie brilliant blue stained SDS-PAGE electropherogram obtained from CNBr digest. (A) Unreduced pepsin-solubilized CNBr peptides from human placenta. (B) Reduced pepsin-solubilized CNBr peptides from human placenta (C) Unreduced solubilized CNBr peptides from human placenta. The peaks labelled 8-I [α1(I)-CB-8] and 5[III] [α1(III)-CB-5], respectively, were used in the quantitation of types I and III collagens.

TABLE IV

RATIO OF TYPE I COLLAGEN TO TYPE III COLLAGEN

Values are mean ± S D of four experiments.

Sample	Type I/type III ratio
Control 1, type I + III (2:1)	2.04 ± 0.09
Control 2, type I + III (1:1)	0.95 ± 0.04
Human placenta (direct treatment by CNBr)	1.62 ± 0.10
Human placenta (pepsin extract)	1.47 ± 0.11
Human normal kidney	1.26 ± 0.04
Human polycystic kidney	2.64 ± 0.70

5 peptides were obtained from every type III molecule. An amount of 90 nmol of Hyp found in this peptide corresponded to 1 nmol of type III collagen in the initial mixture. The results of the method are shown in Table IV.

DISCUSSION

The method reported in this paper comprises the SDS-PAGE electrophoresis of solubilized collagens. The proteins bands are stained by slight exposure to Coomassie brilliant blue G 250, then sliced manually, and the amounts of Pro and Hyp are evaluated in every band by fluorescence measurement after TLC separation. The amount of Hyp is proportional to the amount of deposited collagen, provided that the collagen is normally hydroxylated. The evaluation of Pro + Hyp in every band should overcome this uncertainty about hydroxylation.

This technique overcomes the problems of the staining of bands and the quantitation of the amount of bound dye. In addition, the use of standard collagens is not necessary. The only inaccuracies relate to the electrophoresis itself: is the fractionation quantitative, are the bands perfectly separated from each other, does any band contain only one type of α chain? A major advantage of the method is the evaluation of Pro + Hyp contained not only in the α band, but also in the β and γ bands. The evaluation of the α band proportion is never possible when estimating the amounts of bound dyes, but it is accurate when it depends on Pro + Hyp quantitation. It allows the estimation of percentages of α chains on the basis of total collagen. We found that the proportion of α chains solubilized by pepsin digestion appears to differ from one type of collagen to another by a factor of 20% (65% of α chains for types I and III and 85% for type V). These differences should be accounted for.

In order to assess the reproducibility of the technique, we compared the results of Pro + Hyp evaluation with the amounts of radioactivity found in every band after PAGE performed on labelled samples of collagen. We found a high correlation. On the other hand, when we compared the surfaces of the scanned peaks obtained by densitometry of stained bands, we found a poor correlation. This poor correlation is mainly due to the fact that the staining by Coomassie brilliant blue of α chains is not reproducible and depends on the collagen type. In our hands, the ratio of $\alpha 1$ to $\alpha 2$ bands obtained by densitometry averages 1.59, which is far from the expected ratio of 2, whereas the ratio obtained by our fluorometric method averages 2.1. Nevertheless, we use a method of calculation independent of the $\alpha 2$ chain.

The problem of quantifying type IV collagen remains difficult because of its low amounts in most tissues, its sensitivity to pepsin and its insolubility prior to reduction. The amount of type IV could be tentatively obtained by measuring the amount of Pro + Hyp in the γ fraction before and after reduction and deducing the amount of Pro + Hyp assigned to type III collagen.

The presence of bands of apparent molecular masses 140 000, 100 000 and 75 000 in PAGE obtained after reduction of samples seems to be relevant to their origin from type IV collagen. Unfortunately, they cannot be used for the calculation of the amount of type IV because pepsin digestion does not furnish repro-

ducible amounts of these bands. On the other hand, the 100 000 component, located in the $\alpha 1$ band, may artefactually increase the calculated amount of type III collagen. The only way to control this overestimation is to measure the proportions of type I and III collagens by the CNBr technique and to calculate the amount of type III from the amount of type I found by the pepsin method.

CNBr-solubilized samples

When samples of tissues were submitted to direct CNBr digestion, the best way to quantitate type I and III collagens was to measure Hyp in the marker peptides $\alpha 1$(I)-CB-8 and $\alpha 1$(III)-CB-5. The identities of the individual peaks were established by comparison with CNBr peptides from purified type I, III, IV and V collagens and with published data on their amino acid composition [23,24]. The $\alpha 1$(III)-CB-9 and $\alpha 1$(I)-CB-6 peptides could not be used because they were absent or greatly diminished in the CNBr-solubilized samples. They were involved in intermolecular cross-links [24,25]. In these conditions, they remained at the top of the gel associated with high-molecular-mass proteins [4,25,26]. We selected the $\alpha 1$(III)-CB-5 peptide for the quantitation of type III collagen because it is known not to be involved in covalent cross-linkages, it contains the highest proportion of Hyp [24] and it is much less subject to losses by diffusion than the $\alpha 1$(III)-CB-8 peptide [28]. On the other hand, the $\alpha 1$(I)-CB-8 peptide was used as the marker peptide of type I collagen. No correction was made for the presence of the partially cleaved $\alpha 1$(I)-CB-4-5-8-3 and $\alpha 1$(I)-CB-5-8-3 peptides [28]. As reported by Laurent et al. [2] the contribution of the $\alpha 1$(I)-CB-8 peptide to these bands would be less than 10% of the total. The $\alpha 1$(I)-CB-8 and α(III)-CB-5 peptides are reported to contain 29 and 30 Hyp residues, respectively [23,24,29].

The main problem with the use of CNBr fractionation was the disappearance of minor collagens from the CB peptides because their amounts were too low to be quantitated by this technique.

Use of these quantitation methods for polycystic kidney collagens

Polycystic kidney disease is a severe genetically determined pathological state, inducing a significant number of fatal renal insufficiencies. Most of the patients suffering from this disease eliminate in their urine an increased amount of 3-Hyp, suggesting a defect of the basement membrane collagen [30]. Our results show that the proportion of type IV collagen in polycystic kidney increases only to a small extent, but that the proportion of type I collagen increases largely at the expense of type III. These results are in agreement with those previously reported by Bellon [31], who used an enzyme-linked immunoabsorbent assay for CNBr peptides. Kidney type I collagen was shown by Fujiwara and Nagai [32] to contain a high proportion of 3-Hyp residues, so the urinary increase of 3-Hyp may well depend on this increase in kidney type I collagen. We verified (data not shown) that the amount of 3-Hyp in normal kidney type I collagen averages six residues per thousand. In the case of polycystic kidney, the amount of 3-Hyp increased to 8%.

CONCLUSION

The method of quantitation of Pro and Hyp in the collagenous bands after PAGE represents an improvement over the methods for quantitation of dyes. It gives more comprehensive results when applied to pepsin digest (amounts of types I, III, IV and V being roughly obtained) than when used for CNBr peptides (proportions of types I and III only being given). It could be easily applied either to two-dimensionnal PAGE, transforming this qualitative technique into a quantitation of the collagen proportions, or to the study of collagen metabolism in cultures. Applied to polycystic kidney, it can demonstrate an increase in the proportion of type I collagen.

ACKNOWLEDGEMENTS

We thank Professors J. Chanard and B. Lardenois for kindly providing the kidneys, and Mrs. M. Debref and Mr. S. Etienne for typing the manuscript.

REFERENCES

1 R.L. Goldberg and G.C. Fuller, Anal. Biochem., 90 (1978) 69.
2 G.J. Laurent, P. Cockerill, R.J. MacAnulthy and J.R. Hastings, Anal. Biochem., 113 (1981) 301.
3 A.N. Hanson and J.P. Bentley, Anal. Biochem., 130 (1983) 32.
4 J.M.E. Kirk, B.E. Heard, I Kerr, M. Turner-Warwick and G.J. Laurent, Collagen Related Res., 4 (1984) 169.
5 D Chan and W.G. Cole, Anal. Biochem., 139 (1984), 332.
6 A.G. Szymanowicz, G. Bellon, G. Laurain-Guillaume, T. Delvincourt, A. Randoux, T. Caulet and J.P. Borel, Artery, 10 (1982) 250.
7 J. Armendariz-Borunda and M. Rojkind, Coll. Rel. Res., 4 (1984) 35.
8 K.M. Reiser and J.A. Last, Anal. Biochem., 104 (1980) 87.
9 C.M. Wilson, Anal. Biochem., 96 (1979) 263.
10 A. Bisker, V. Pailler, A. Randoux and J.P. Borel, Anal. Biochem., 122 (1982) 52.
11 G. Bellon, A. Malgras, A. Randoux and J.P Borel, J. Chromatogr., 278 (1983) 167.
12 G. Bellon, R. Berg, F. Chastang, A. Malgras and J.P. Borel, Anal. Biochem., 137 (1984) 151.
13 A. Szymanowicz, O. Mercier, A. Randoux and J.P. Borel, Biochimie, 60 (1978) 499.
14 E H. Epstein, J. Biol. Chem., 249 (1974) 3225.
15 R.W. Glanville, A. Rauter and P.P. Fietzek. Eur. J. Biochem., 95 (1979) 383.
16 H Bentz, H.P. Bächinger, R.W. Glanville and K. Kühn, Eur. J. Biochem , 92 (1978) 563.
17 G. Laurain, T. Delvincourt and A.G. Szymanowicz, FEBS. Lett., 120 (1980) 44.
18 B Peterkofsky and R. Diegelmann, Biochemistry, 10 (1971) 988.
19 E.H. Epstein, R D. Scott, E.J. Miller and K.A. Piez, J. Biol. Chem., 246 (1971) 1718.
20 T. Hayashi and Y. Nagai, J. Biochem., 87 (1980) 803.
21 U.K. Laemmli, Nature (London), 227 (1970) 680.
22 G.W Snedecor and W.G. Cochran, Statistical Methods, The Iowa State University Press, Ames, IA, 1967.
23 E.M. Click and P. Bornstein, Biochemistry, 9 (1970) 4699.
24 E. Chung, E.M. Keele and E.J. Miller, Biochemistry, 13 (1974) 3459.
25 N.D. Light, Biochim. Biophys. Acta, 702 (1982) 30.
26 J. Rauterberg, Clin. Orthop., 97 (1973) 196.
27 P.G Scott, Connect. Tissue Res., 10 (1982) 217.
28 P.G. Scott and A. Veis, Connect. Tissue Res., 4 (1976) 107.
29 J M. Seyer, C. Mainardi and A.H. Kang, Biochemistry, 19 (1980) 1583.

30 J. Chanard, A. Szymanowicz, J.P. Brunois, O. Toupance, J.P. Melin, P. Birembaut, A. Randoux and J.P. Borel, Clin. Nephrol., 17 (1982) 64.
31 G. Bellon, Anal Biochem., 150 (1985) 188.
32 S. Fujiwara and Y. Nagai, J. Biochem., 89 (1981) 1397.