Journal of Chromatography, 431 (1988) 474–476
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4319

Letter to the Editor

Use of the Pharmacia Phast System for sodium dodecyl sulphate polyacrylamide gel electrophoresis of non-globular proteins: application to collagens

Sir,

Among the different components of connective tissues, twelve collagen types have been already described [1,2]. Each collagen molecule contains at least one triple helical domain consisting of three chains denoted α chains. These collagen α chains behave anomalously in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in comparison with globular proteins [3]. As the protocols proposed by the manufacturer concern globular proteins only, it appeared of interest to investigate the SDS-PAGE of rod-like non-globular macromolecules such as collagens in the new Phast System (Pharmacia, Uppsala, Sweden). The Phast System apparatus is a two-unit electrophoresis system, one being used for fast and high resolution separation and the other for development. We report the first separation of α chains from several collagen types by SDS-PAGE using the Phast System. We have obtained a good resolution for α chains in a short time with four times less sample than with classical vertical electrophoresis using the discontinuous system of Laemmli [4].

EXPERIMENTAL

Human type I and V collagens and bovine type IX and XI collagens, purified following established procedures [5,6], were purchased from Bioétika (Lyon, France).

SDS-PAGE was performed on PhastGel homogeneous media (7.5% polyacrylamide gels precast on to a rigid polyester film). Samples were dissolved at a concentration of 0.25 mg/ml in 100 m*M* Tris–HCl buffer (pH 8.0) containing 1.0 m*M* EDTA and 2.5% (w/v) SDS and denatured for 2 h at 40°C. Sample (0.5 μl per lane) was applied for 10 V h. The separation was achieved at 250 V, 10.0 mA and 3.0 W (limiting) for 100 V h at 15°C in the Phast System apparatus.

The gels were stained automatically with silver nitrate in the development unit of the Phast System following to the manufacturer's instructions.

0378-4347/88/$03.50

RESULTS AND DISCUSSION

Collagen chains exhibit lower electrophoretic mobility than globular proteins of similar molecular mass [3]. This anomalous behaviour is illustrated in Fig. 1. Immunoglobulins G (molecular mass ca. 160 000) migrated as a broad band with an electrophoretic mobility similar to that of α chains which had a molecular mass of 95 000 as measured by equilibrium sedimentation [7].

The type I collagen molecule consists of two α_1 (I) chains and one α_2 (I) chain. The two chains appeared well resolved, as shown in Fig. 1a. The dimers β_{11} [two α_1 (I) chains] and β_{12} [composed of one α_1 (I) chain and one α_2 (I) chain] were also clearly separated.

Type V collagen was chosen for this experiment because of its heterotrimer composition containing three genetically distinct chains, α_1 (V), α_2 (V) and α_3 (V), with close electrophoretic mobilities. SDS-PAGE with the Phast System allowed a good separation of the three chains (Fig. 1b and c). In particular, the α_3 (V) chain was distinguished from the α_1 (V) chain, which has a lower electrophoretic mobility. The efficiency of separation by SDS-PAGE with the Phast System has been confirmed for the three chains of type XI collagen (Fig. 1d and

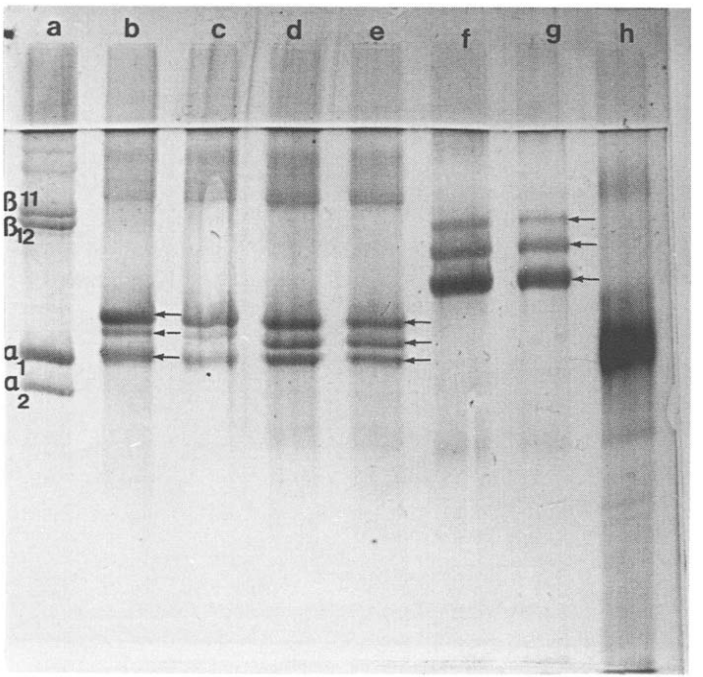

Fig. 1. SDS-PAGE of collagen samples. PhastGel homogeneous medium (7.5%), run for 30 min and stained with silver nitrate for 60 min. Lanes a, b, d and f; 125 ng of sample per lane. Lanes c, e, g and h: 62.5 ng of sample per lane. a, Human type I collagen; b and c, human type V collagen, from top to bottom (arrows) α_1 (V), α_3 (V) and α_2 (V) chains; d and e, bovine type XI collagen, from top to bottom (arrows) α_1 (XI), α_2 (XI) and α_3 (XI) chains; f and g, bovine type IX collagen, from top to bottom (arrows) X_1, X_2 and X_3 fragments; h, rabbit γ-globulins.

e) and the high molecular mass components of type IX collagen (molecular mass ranging from 125 000 to 180 000) (Fig. 1f and g).

In conclusion, we have established experimental conditions that allow a good resolution of collagen α chains by SDS-PAGE with the Phast System. One of the main advantages of this technique is its rapidity: collagen SDS-PAGE separations are completed in about 30 min compared with a minimum of 5 h in a classical apparatus. This technique seems to be of particular interest in improving immunoblotting experiments. First, the thickness of the gels (0.45 mm) may allow a complete transfer of collagens on to nitrocellulose membranes, and second, only a small amount of antibodies will be required because of the reduced dimensions of the gels (43 mm $\times$ 50 mm). Our results suggest that the Phast System apparatus may be used in a reliable way for the characterization of circulating collagen antibodies by immunoblotting in a number of clinical situations.

ACKNOWLEDGEMENTS

We thank D. Herbage (UA CNRS 244) and M. Manach (Pharmacia) for helpful discussions and O. Breton for skilful technical assistance.

Centre de Radioanalyse,
Institut Pasteur de Lyon,
13-15 Rue Domer,
69366 Lyon Cedex 07 (France)

S. RICARD-BLUM*
G. VILLE
D.J. HARTMANN

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(First received April 27th, 1988; revised manuscript received May 19th, 1988)