THE SUBUNIT STRUCTURE OF CHLOROCRUORIN

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1. Introduction

There is disagreement about the size of the subunit of chlorocruorin. Results obtained from iron analysis and zero-time extrapolation of carboxypeptidase hydrolysis gave molecular weights of 34 000 and 37 000 [1,2].

SDS–Gel electrophoresis, however, gave molecular weights of 13 000–16 000 [3,4].

We have recently examined the subunit structure of chlorocruorin obtained from *Spirographis spallanzanii* by electrophoretic and chromatographic techniques.

The results obtained indicate the presence of two types of subunits.

2. Materials and methods

Chlorocruorin was prepared from hemolymph obtained from *Spirographis* collected from Palermo (Sicily).

Fresh hemolymph was diluted with sea water buffered with 0.1 M Tris–HCl, pH 8.1. The sample was centrifuged for 10 min at 10 000 × *g* and the resulting supernatant was centrifuged at 131 000 × *g* for 7.5 h. All centrifugations were carried out at 4°C. The precipitated chlorocruorin was redissolved in phosphate buffer, pH 7.0 and tested for purity by chromatography on Bio-gel A5m (1 × 150 cm) and by electrophoresis using 5% acrylamide gels [5].

Globin was prepared from chlorocruorin redissolved in distilled water with cold acid acetone (89 ml acetone + 1 ml concentrated HCl). Globin was either reduced and carboxymethylated [6] or oxidised with performic acid [7]. Electrophoresis of the alkylated globin was carried out in gels (T = 12.32%; C = 2.6%) containing 0.1% SDS. Samples for electrophoresis were prepared by the method of Weber and Osborn [8]. Electrophoresis was also performed on gels (T = 7.7%; C= 2.6%) in the presence of 8 M urea, at pH 2.3, 3.8, 4.1 and 9.4.

Protein bands were detected with Coomassie Blue.

Alkylated globin was chromatographed on Sephadex G-75 (1.5 × 90 cm) equilibrated with 0.1 M phosphate buffer, pH 7.0, containing 0.1% SDS. The protein was first incubated for 2 min at 100°C in buffer containing 1% SDS and then dialysed against 0.1% SDS.

3. Results

Purified chlorocruorin was found to be homogeneous by chromatography and electrophoresis.

SDS–Gel electrophoresis of the alkylated globin gave two main bands with a molecular weight of 15 800 and 16 100 respectively (figs 1 and 2). Faint dimeric and polymeric bands were also visible. Untreated globin gave several bands with molecular weights ranging from 16 000–150 000.

Gel filtration in SDS of the alkylated protein shows

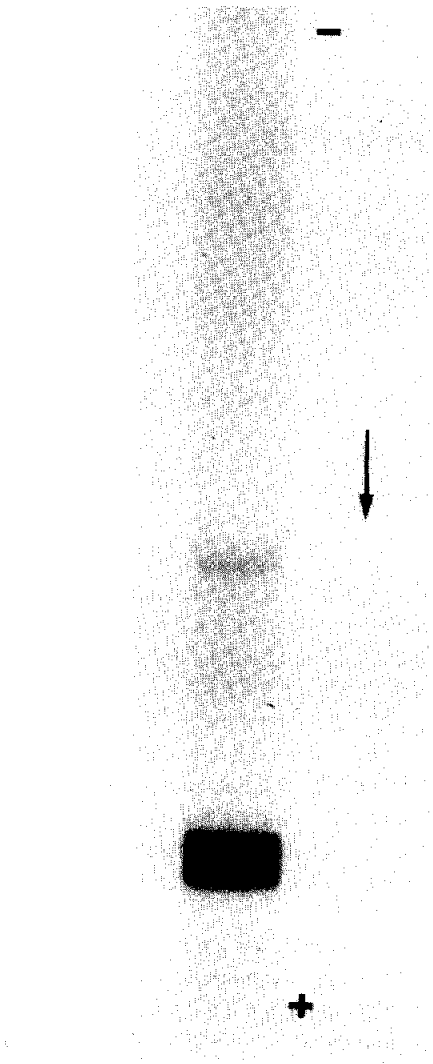

Fig.1. SDS–Gel electrophoresis of reduced and carboxymethylated *Spirographis* globin. Electrophoresis was carried out for 2.5 h at 3 mA/gel.

two components (fig.3). The first peak was found by SDS electrophoresis to contain dimeric and polymeric components, whilst the second peak was found to contain two subunits of molecular weight 15 800 and 16 100.

The separations obtained from electrophoresis of the alkylated globin in the presence of 8 M urea are shown in fig.4. At pH 2.3, two main components are visible whilst at higher pH values more components are visible.

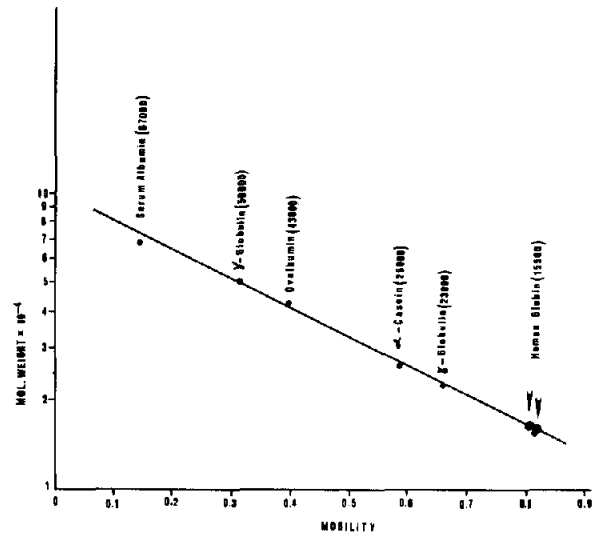

Fig.2. Molecular weight determination of reduced and carboxymethylated *Spirographis* globin by SDS–gel electrophoresis. Arrows indicate the position of the globin.

4. Discussion

Previous investigations on *Spirographis* chlorocruorin have indicated a subunit molecular weight of 34 000 and 37 000 [1,2]. The results obtained in the present investigation indicate a minimum molecular weight of around 16 000 for this chlorocruorin. This is in good agreement with data obtained for chlorocruorins from other species [3,4]. The high molecular weight obtained by other workers on *Spirographis* chlorocruorin can

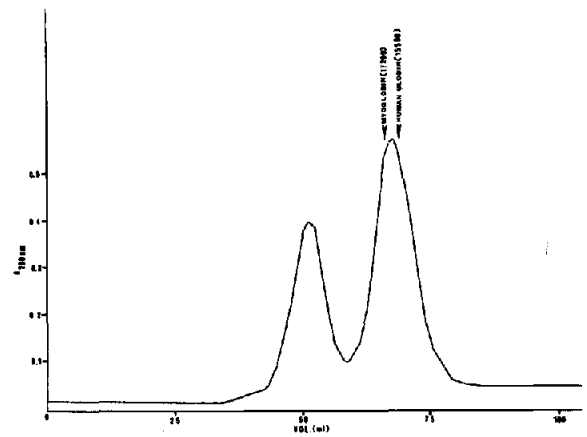

Fig.3. Elution pattern of reduced and carboxymethylated *Spirographis* globin on G 75 in 0.1% SDS.

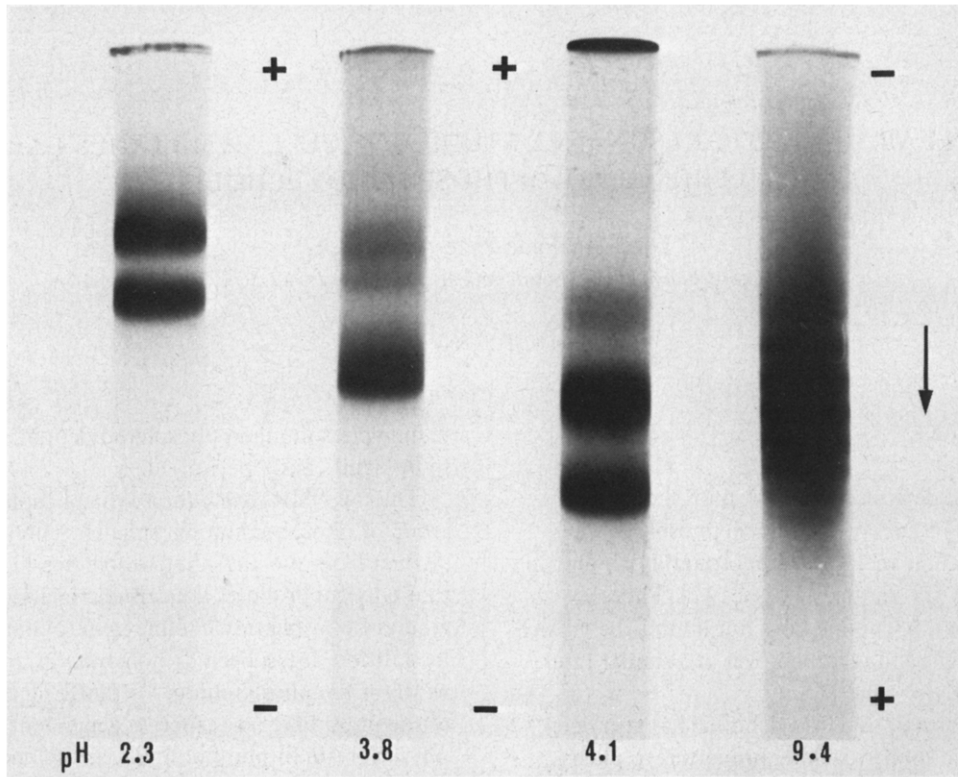

Fig.4. Gel electrophoresis of reduced and carboxymethylated *Spirographis* globin in the presence of 8 M urea at various pH values. At pH 2.3, 3.8, and 4.1 electrophoresis was carried out with reverse polarity.

probably be accounted by the limitation of the techniques used.

The presence of two subunits of around 16 000 molecular weight is also seen in *Mixicola* and *Hydroides* [3].

The protein is, therefore, either composed by two non-identical chains, or the presence of two components can also be due to anomalous SDS binding. In *Eudistylia* a single component is seen [4].

Electrophoresis in 8 M urea also suggests the presence of two polypeptide chains. Both these bands at pH values higher than 2.3 display several components, suggesting the presence of a distribution of globins in chlorocruorin.

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